THE ADRENERGIC SYSTEM IN CARDIOVASCULAR PHYSIOLOGY AND PATHOPHYSIOLOGY

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THE ADRENERGIC SYSTEM IN CARDIOVASCULAR PHYSIOLOGY AND PATHOPHYSIOLOGY

2nd Edition

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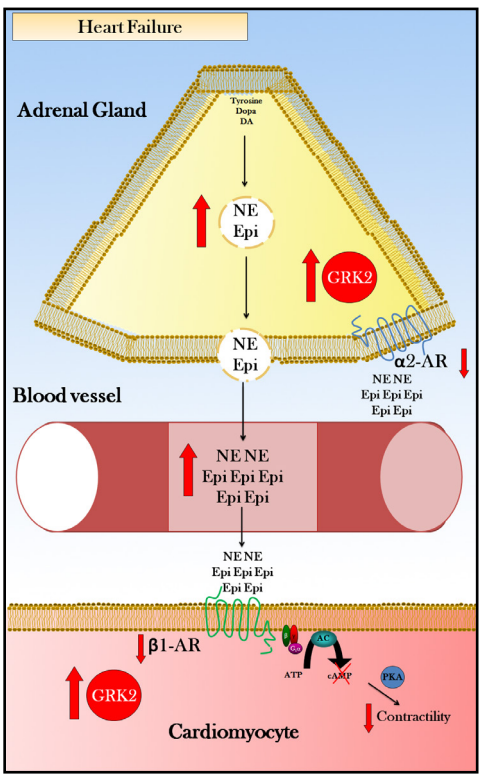

Representation of the pathophysiologic role of GRK2 in adrenal CA-production/secretion: Body's major source of CAs is the adrenal medulla, the central part of the adrenal gland, where the chromaffin cells secrete approximately 20% NEpi and 80% Epi.

Cardiovascular diseases pose an enormous clinical challenge, remaining the most common cause of death in the world. β-adrenoceptors play an important role on cardiac, vascular and/or endothelial function at a cellular level with relevant applications in several cardiovascular diseases, such as heart failure and hypertension. G protein–coupled receptors (GPCRs), including β-adrenergic receptors, constitute the most ubiquitous superfamily of plasma membrane receptors and represent the single most important type of therapeutic drug

target. Sympathetic nervous system hyperactivity, which characterizes several cardiovascular diseases, such as heart failure and hypertension, as well as physiological ageing, has been proved to exert in the long-term detrimental effects in a wide range of cardiovascular diseases. Acutely, sympathetic hyperactivity represents the response to an insult to the myocardium, aiming to compensate for decreased cardiac output. This process involves the activation of beta-adrenergic receptors by catecholamine with consequent heart rate and cardiac contractility increase. However, long-term exposure of the heart to elevated norepinephrine and epinephrine levels, originating from sympathetic nerve endings and chromaffin cells of the adrenal gland, results in further progressive deterioration in cardiac structure and function. At the molecular level, sustained sympathetic nervous system hyperactivity is responsible for several alterations including altered beta-adrenergic receptor signaling and function (down-regulation/desensitization). Moreover, the detrimental effects of catecholamine affect also the function of different cell types including, but not limited to, endothelial cells, fibroblasts and smooth muscle cells. Thus, the success of beta-blocker therapy is due, at least in part, to the protection of the heart and the vasculature from the noxious effects of augmented catecholamine levels. The research topic aimed to support the progress towards understanding the role of sympathetic nervous system under physiological conditions, and the contribution of its hyperactivity in the pathogenesis and progression of cardiovascular diseases.

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EDITORIAL
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The adrenergic system in cardiovascular pathophysiology: a translational science point of view

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Keywords: GRK2, heart failure, sympathetic nervous system, beta-blockers, beta-adrenoceptors, functional recovery, exercise training

Heart failure (HF) is one of the leading causes for mortality and morbidity worldwide. Despite advantages in the management and treatment of this syndrome, nowadays, it is estimated that 50% of HF patients die within 5 years from diagnosis (McMurray et al., 2012). Thus, a better understanding of the molecular mechanisms underlying structural, functional, neuro-hormonal, and metabolic alterations of the failing heart is necessary for the identification of new therapeutic targets and strategies. The adrenergic system is crucial for cardiac function, and even more critical in diseased states characterized by elevated sympathetic nervous system (SNS) hyperactivity (Lymperopoulos et al., 2013). SNS hyperactivity is a salient characteristic of chronic HF and causes cardiac up-regulation of G protein-coupled receptor kinase 2 (GRK2), which in turn induces beta-adrenergic receptor dysregulation in the heart (Rengo et al., 2009, 2012).

The present Research Topic aims to present some of the more relevant and recent acquisitions on the molecular abnormalities of the adrenergic system occurring in HF. Dr. Lymperopoulos has reported the molecular mechanisms of regulation of SNS in HF patho-physiology, discussing their therapeutic implications for the failing heart (Lymperopoulos, 2013). The importance of SNS hyperactivity as a main therapeutic target in HF, represents the rationale for the use of beta-blockers, as discussed by Drs. Barrese and Taglialatela. These authors also reported the molecular bases explaining the differences in response to beta-blocker therapy among HF patients (Barrese and Taglialatela, 2013). Dr. Ferrara and collaborators showed the molecular similarities between physiological aging and HF. Both these conditions are characterized by SNS hyperactivity and cardiac beta-adrenergic receptor signaling dysfunction, and this may help to explain why HF is more frequent and its manifestation more severe in the elderly patients (Ferrara et al., 2014). The interconnections between adrenergic system and cardiac metabolism, oxidative stress and nitric oxide signaling have also been discussed in this Research Topic. It is known from several years that the adrenergic system has a profound effect on the regulation of cardiac metabolism. In this regard, Ciccarelli et al. reported the most updated discoveries in the molecular mechanisms involved in the interactions between adrenergic system hyperactivity and metabolic abnormalities, such as insulin resistance and altered glucose metabolism (Ciccarelli et al., 2013). The effects of beta-adrenoceptors on Reactive Oxygen Species generation are described by Corbi et al.; these authors reported also a fascinating hypothesis of the involvement of sirtuins on beta-adrenergic receptors signaling with a potential role in HF pathophysiology (Corbi et al., 2013). Dr. Conti and collaborators reported the mechanisms of the crosstalk between nitric oxide and beta-adrenergic receptor system, in particular in the control of endothelial function and vascular tone (Conti et al., 2013).

Evidences accumulated over the past 20 years support the pathogenic key role of cardiac GRK2 levels/activity in determining HF-related beta-adrenergic receptor dysfunction and cardiac inotropic reserve reduction. All these data indicate GRK2 inhibition, via gene therapy, as a new HF therapeutic approach that has been shown to be compatible and, in some models, also synergistic to beta-blockers. Cannavo et al. provide a contemporary update of this field by describing the therapeutic potentialities of this approach and its beneficial effects not only on beta-adrenergic receptor signaling, but also on cardiac metabolism, apoptosis, and mitochondrial dysfunction (Cannavo et al., 2013). De Lucia and collaborators extended the therapeutic potentialities of GRK2 inhibition to the adrenal glands and to the control of HF-related SNS outflow (de Lucia et al., 2014). Since its first demonstration by Lymperopoulos et al. (2007), GRK2 appeared to be critical in the regulation of adrenal alfa2 adrenergic receptor function, extending also to other organs the therapeutic benefits of GRK2 inhibition in HF. Finally, Dr. Leosco in his review explained the molecular mechanisms involved in the beneficial effects of exercise training in curbing SNS hyperactivity and beta-adrenergic receptor dysfunction observed in HF. A crucial role seems to be played by the ability of physical activity to reduce GRK2 levels both in the heart and in the adrenal medulla, with relevant effects on cardiovascular function (Leosco et al., 2013).

The overall Research Topic indicates that the great advances achieved in the last decades in understanding the molecular alterations involved in the pathophysiology of HF are opening new opportunities for the treatment of this syndrome and, potentially, their future application to the clinical practice might result to further improvements of patient care. Moreover, the interesting new findings, discussed herein, will hopefully stimulate further research on these arguments.

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β-adrenergic receptor responsiveness in aging heart and clinical implications

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Elderly healthy individuals have a reduced exercise tolerance and a decreased left ventricle inotropic reserve related to increased vascular afterload, arterial-ventricular load mismatching, physical deconditioning and impaired autonomic regulation (the so called "β-adrenergic desensitization"). Adrenergic responsiveness is altered with aging and the age-related changes are limited to the β-adrenergic receptor density reduction and to the β-adrenoceptor-G-protein(s)-adenylyl cyclase system abnormalities, while the type and level of abnormalities change with species and tissues. Epidemiological studies have shown an high incidence and prevalence of heart failure in the elderly and a great body of evidence correlate the changes of β-adrenergic system with heart failure pathogenesis. In particular it is well known that: (a) levels of cathecolamines are directly correlated with mortality and functional status in heart failure, (b) β_1-adrenergic receptor subtype is down-regulated in heart failure, (c) heart failure-dependent cardiac adrenergic responsiveness reduction is related to changes in G proteins activity. In this review we focus on the cardiovascular β-adrenergic changes involvement in the aging process and on similarities and differences between aging heart and heart failure.

Keywords: β-adrenergic receptors, β-adrenoceptor desensitization, β-adrenoceptor down-regulation, G-protein coupled receptor kinase, aging heart, failing heart, exercise

INTRODUCTION

Epidemiological studies reveal an high incidence and prevalence of heart failure in the elderly (Roger et al., 2011). In chronic heart failure substantial and characteristic changes occur in the cardiac structure and function and these modifications are not very different from those observed in the aging heart (Shioi and Inuzuka, 2012). The peculiar age-related cardiac structural changes are represented by an increase in cardiomyocyte size and in myocardial thickness (Scholtz et al., 1988; Olivetti et al., 1991), which are able to affect the contractile efficiency of the left ventricle. These changes are associated with increased cardiac fibrosis and vascular stiffening. However, epidemiological and autopsy-based studies, performed in subjects free from coronary artery disease and hypertension, have demonstrated no significant age-related changes in cardiac mass in elderly female and a decrease in left ventricular mass in elderly male compared to young male (Hess et al., 2002; Khouri et al., 2005) ("cardiac sarcopenia"). Nevertheless, ageing is not associated with impaired systolic cardiac function at rest, as demonstrated by echocardiographic and radionuclide studies performed in normotensive healthy subjects (Khouri et al., 2005). Differently, ageing is related to diastolic left ventricle function with increased prevalence of diastolic heart failure. It is well known that in healthy elderly there is a reduction in left ventricle inotropic reserve and exercise tolerance. Reduced inotropic cardiac reserve is thought to be related to increased vascular afterload, arterial-ventricular load mismatching, physical deconditioning and impaired autonomic regulation (so called "β-adrenergic desensitization"). It is interesting to point out the similarity observed in terms of hemodynamic profile under adrenergic challenge between younger β-blocked subjects and healthy elderly subjects without β-blocker treatment (Fleg et al., 1994). Adrenergic receptors activation by catecholamines is the most important regulatory mechanism of cardiovascular performance. Adrenergic receptor agonists, as well as exercise, stimulate the adrenergic system increasing heart rate, myocardial contractility and relaxation, reducing left ventricular afterload and redistributing blood flow to skeletal muscle. Anyway, adrenergic responsiveness is altered with aging (White et al., 1994). In fact, both animal and human studies indicate a decline in heart rate, cardiac contractility, cardiac output and ejection fraction in response to β-adrenergic stimulation and exercise (Rinaldi et al., 2006; Corbi et al., 2012a,b). Part of the age-related decline in β-adrenergic responsiveness has been attributed to a general decrease in cardiac contractility. However, several observations indicate a crucial role of reduced β-adrenergic receptor density and some defects involving the adenylyl cyclase cascade beyond β-receptor levels (Ferrara et al., 1995, 2005; Freedman et al., 1995). The age-associated reduction in maximal heart rate during high levels of exercise are in relationship with a reduced β-adrenergic responsivity despite an increase in circulating levels of catecholamines (Corbi et al., 2013a). Aging is associated with elevated neuro-hormonal activation, and characterized by elevated plasma norepinephrine and epinephrine circulating levels, due to increased spillover from tissues (including the heart) and

reduced plasma clearance of catecholamine (Ng et al., 1993; Esler et al., 1995). The "β-adrenergic desensitization," at least in part, is due to the reduction of β-adrenergic receptor plasma membrane density described in hearts of both senescent animals and elderly humans (White et al., 1994; Xiao et al., 1998). The β-adrenergic receptors are members of the G-protein-coupled receptor family, which acts by coupling with guanine nucleotide binding proteins, and the age-induced decrease in β-adrenoceptor responsiveness is characterized at the molecular level by decreased activation of adenylyl cyclase and reduced production of cAMP. Beside β-adrenergic receptor down-regulation, another crucial age-related alteration of this signaling pathway seems to be the coupling of the β-adrenergic receptor to adenylyl cyclase via the G_s protein, which leads to a reduction in the ability to increase cAMP and to activate protein kinases. Some studies have also reported an increase in $G_{\alpha i}$ activity as a possible additional mechanism in "β-adrenergic desensitization." Moreover, the reduction in the efficacy of cardiac β-adrenoceptor stimulation with aging could be also related to other mechanisms, such as the upregulation of G protein–coupled receptor kinases (Rengo et al., 2012a), whereas the role of these kinases in aging heart is controversial. From an overall data analysis on the role of aging in β-adrenoceptor regulation in human and animal hearts it is possible to conclude that the reduced response to β-agonists is common to all species and all cardiac tissues investigated. Moreover, the age-related changes are limited to β-adrenoceptor-G-protein (s)-adenylyl cyclase system abnormalities, while the type and level of abnormalities change with species and tissues. These differences could explain the inconsistency in results obtained in different experimental models of aged heart. Interestingly, several evidence suggest that the β-adrenergic receptor system plays an important role also in heart failure pathogenesis. In fact, it is well known that (a) the levels of cathecolamines are directly correlated with mortality and functional status in heart failure, (b) cardiac β-receptors, in particular β_1 subtype, are downregulated in heart failure and (c) heart failure-dependent cardiac adrenergic responsiveness reduction is related to adrenoreceptor kinases and $G_{\alpha i}$ increased activities.

AIMS

This review focuses on (a) the development of knowledge on aging heart over the years, (b) the changes involving the sympathetic system in relationship to the cardiovascular aging in different species, (c) the clinical implications of changes in β-adrenergic mechanisms in the aging heart and d) the similarity between aging and failing heart.

β-ADRENERGIC SIGNALING IN THE HEART AT MOLECULAR LEVEL

For the first time the existence of β-adrenergic receptors (β-AR) was described in 1948 by Alquist (1948). At the present three subtypes of β-AR: β_1-AR, β_2-AR, β_3-AR have been recognized. A fourth subtype has been proposed and investigations have been recently clarified its functioning and localization (Lewis et al., 2004). At the beginning it was thought that only β_1-AR subtype was expressed in the cardiac cells. However, many studies provided evidence that both heart β_1-AR subtype and β_2-AR subtype (Lemoine and Kaumann, 1991; Altschuld et al., 1995; Lonardo et al., 2005), coexist in humans as well as in animals. In the human heart approximately 80% of the β-AR subtype expressed belong to the β_1-AR, followed by 20% of the β_2-AR subtype (Lakatta and Levy, 2003). It is important to underline that this β-AR expression proportion has been observed in the non-failing young, but not in elderly human heart.

Modern molecular biology techniques and radio-ligand binding studies have shown that major expression and main contribution in the contractile functioning of cardiac cells belong to the β_1-AR subtype (Benovic et al., 1991; Borea et al., 1992). In humans, as well as in other animals with relative big body weight like sheep, dogs or cats, the β_1-AR and β_2-AR are both significantly present, while in other small animals like rats or guinea-pigs the presence of β_2-AR is undetectable. Some other studies found the presence of β_2-AR in rat hearts but not localized in ventricular myocytes (Buxton and Bruton, 1985). Even the different specie-dependent contribution of β-AR and the difficulties to find a perfect experimental model, it is well known that in all different species the general mechanical pathway is always related to adenylyl cyclase (AC) activation, cyclic AMP (c-AMP) formation, Protein Kinase A (PKA) activation and G-Protein Coupled Receptor Kinase (GRK) activation. β-AR are members of the G-Protein Coupled Receptors (GPCRs) family which acts by coupling with Guanine nucleotide binding proteins (Rengo et al., 2012a). β_1-AR subtype is coupled to the stimulatory G protein (G_s). G_s protein is a heterotrimetric protein made up of α, β, and γ subunits. The presence of β-AR agonists induces the dissociation of G_s protein in two subunits: α subunit and β-γ subunit. The primary effect of this dissociation is the activation of AC that catalyzes the conversion of ATP to c-AMP, a second intracellular messenger, and induces the activation of c-AMP dependent PKA. Serine and threonine residues of many regulatory proteins are phosphorylated by PKA. These regulatory proteins include: β-AR themselves, myofilament proteins (troponine I and C protein), membrane proteins (phospholamban—PLB, L-type Ca^{++} channels, Sarcoplasmatic Reticulum—SR, Ca^{++}/ATPase inhibitory protein). The stimulation of β-AR modifies not only the cardiac excitation and contraction but also other cellular functions such as gene transcription and growth, and can induce death. An important role for the above mentioned functions has played by the activation of Mitogenic-Activated Protein Kinase (MAPK). Moreover, these kinases are thought to be implicated in the regulation of several vital cellular processes, including differentiation, proliferation, growth, and death (Van Biesen et al., 1995). Ultra Violet light, osmotic stress and heat shock can activate MAPK signaling cascades and GPCRs play a pivotal role in the regulation of MAPKs, particularly of the extracellular signal-regulated kinase ($ERK_{1/2}$) MAPK. One major pathway of GPCRs-mediated activation of MAPKs is dependent on "transactivation" of a group of receptor tyrosine kinases, such as epidermal growth factor and insulin-like growth factor. Additionally the activation of p38 MAPK, also called "stress-activated protein kinase," is associated with the initial signs of cardiac hypertrophy in response to "*in vivo*" pressure overload or ischemic/reperfusion injury (Bogoyevitch et al., 1996; Wang et al., 2013).

The β-AR stimulation induced by catecholamines is also responsible for the Ca^{++} influx, that by itself triggers a potential release of Ca^{++} from SR, acting on the ryanodine receptors.

The intracellular Ca^{++} release activates contractile proteins, finalizing the muscular contraction (positive inotropic effect). Then, intracellular Ca^{++} is removed from the cytoplasm by the SR-Ca^{++}/ATPase pump and the Na^{+}/Ca^{++} exchange. The further acceleration of Ca^{++} removing leads to the muscle relaxation. The maximum velocity of relaxation is defined as positive lusitropic effect. As a result of PKA activation, the β-AR stimulation triggers the G-Protein Coupled Receptor Kinase family, like GRK2. GRKs are a family of serine/threonine protein kinases that phosphorylates GPCRs only when the receptors are in the activated (agonist-bound) state. When β-ARs are stimulated by agonists, the β-γ subunits G interact with GRK_2 bringing the kinase from the intracellular to the transmembranic localization, phosphorylating the β-ARs, becoming target for binding of β-Arrestin proteins. The β-Arrestin bounds to these receptors and prevents their further coupling to the G-protein, reducing the level of functional receptors, inducing the internalization of receptors and, as final result, their decreased density and desensitization (Freedman et al., 1995).

"*In vitro*" studies showed further mechanisms induced by stimulation of β_1-AR. For example, persistent stimulation of β_1-AR is able to activate Calmoduline Dipendent Kinase II, without the implication of PKA pathway. This mechanism induces cardiomyocyte hypertrophy and could explain the well-known relationship between adrenergic stimulation and cardiac hypertrophy[23–24] (Ramirez et al., 1997; Morisco et al., 2000). In addition to the cardiac effects, β_1-ARs regulate the release of renin, the activation of Renin-Angiotensine-Aldosteron (RAA)-system and the lipolysis.

β_2-AR, one of the first receptors identified, belongs to the GPCRs family and plays an important role in the cardiovascular and respiratory physiology. Its main effects are related to vasodilatation and bronchodilatation (Corbi et al., 2013b). In addition β_2-AR is responsible for glycogenolysis (Corbi et al., 2002) and relaxation of uterine muscle. Despite similarities β_1-AR and β_2-AR present different signaling pathways. The β_2-AR is coupled to the G_s protein and to the G_i protein too (dual coupling of β_2AR to G_s and G_i protein). There is also evidence that β_2-AR signaling is coupled to an independent pathway like the Na^{+}/H^{+} exchanged regulatory factor (Hall et al., 1998).

The effects of β_2-AR G_s stimulation are not identical to them obtained from β_1-AR stimulation. However, similarly to β_1-AR, β_2-AR-G_s stimulation increases the c-AMP and PKA activity. Recent studies have demonstrated that the effect of β_2-AR c-AMP/PKA stimulation is limited to the subsurface membrane of the L-type Ca^{++} channels without cellular signal transmission. As a result of this mechanism it is observed a positive inotropic effect without influence on the intracellular Ca^{++} transient decay time, changes in myofilaments sensitivity to Ca^{++} and increase SR Ca^{++} uptake. Obviously β_2-AR stimulation does not affect the relaxation time (lusitropic effect) as β_1-AR does (Kuschel et al., 1999; Xiao et al., 1999).

Surprisingly the Ca^{++} influx, the PKA and the c-AMP levels apparently do not show significant association after β_2-AR stimulation in studies using adult rat and canine models. Even if the reason of this dissociation remains unclear, the role of c-AMP modulation in the contractility of cardiac muscle is well established. In heart animal models forskolin induces c-AMP levels augmentation increasing the inotropic effect. Moreover, it has well demonstrated that the Ca^{++} influx mechanism is exclusively mediated by c-AMP pathway (Xiao et al., 1994).

On the other hand, the $G_{\alpha i}$ protein subunit inhibits the adenylcyclase enzyme activity. The β_2-AR G_i signaling inhibits the c-AMP synthesis and has negative effects on the PKA activation. Persistent activation of β_2-AR-$G_{\beta-\gamma i}$ signaling activates in turn the phosphoinositol3- Kinase (PI3-K), an important downstream messenger that triggers the antiapoptotic factor Akt and seems to have a cardioprotective role. (Zhu et al., 2001; Cannavo et al., 2013).

The model of β_2-AR dual coupling of to multiple G protein (G_i and G_s protein), is not well clarified. Several evidence indicate that β_2-AR–G_i signaling compartmentalizes the β_2-AR-G_s–c-AMP signaling. Disrupting the G_i functioning by a potent G_i inhibitor like Pertussis Toxine (PTX) induces an enhance in the phosphorylation of PLB and an increased inotropic effect after β_2-AR stimulation. In this occasion the β_2-AR signaling is comparative to the β_1-AR signaling[31] (Xiao et al., 1995).

The β_2-AR phosphorylation by PKA and GRK_2 switches the β_2-AR receptor coupling from G_s to G_i. As demonstrated in several studies, the β_2-AR-G_i coupling is suppressed after GRK_2 activity inhibition. In a near future, it may be possible to prevent important structural changes, like myocardial stiffness, reactive fibrosis and remodeling present in the aging and failing heart, modifying the GRK_2 and the G_i activated or inhibited status.

Another candidate mechanism, underling the compartmentalization of β_2-AR–G_s-c-AMP-PKA signaling in response to the β_2-AR-Gi coupling, is the structural restriction of PKA diffusion by muscle specific protein A kinase anchory proteins (AKAP) (Enns et al., 2009). The phosphorylation of AKAP plays multiple roles including: ions influx, contraction, transcription of different genes, phosphorylation of multiple intracellular targets in cardiac myocytes including the L-type Ca^{++} channel in the sarcolemma, the ryanodine receptor (RyR_2), and phospholamban in the SR. Deficiencies in this pathway have been linked to cardiomyopathy in humans, due to reduced phosphorylation of downstream targets such as cardiac troponin (McConnachie et al., 2006). Moreover, in genetically manipulated models it is obtained an increased positive inotropic effect after disrupting the APAK kinase anchory protein (Marshall, 1995; Spindler et al., 2013).

AGE-INDUCED CHANGES IN THE β-AR SIGNALING

Adrenergic signaling is a very important for cardiovascular physiology. In conditions involving physical or psychological stress high levels of cathecolamines like norepinephrine and epinephrine are released from the adrenal medulla. It is well known that the action of cathecolamines is mediated by adrenergic receptors and the effects on cardiovascular system include: increased heart rate and myocardial contractility force and relaxation, increased cardiac output, reduced left ventricular afterload, a diversion of blood flow from the skin and splanchnic vessels to those supplying skeletal muscles, bronchial dilatation and a decline in metabolic activity (Young and Landsberg, 1998). Generally the age-related decrease in β-adrenoceptor response has

been explained by a mechanism called "β-adrenoceptor desensitization." It is a process characterized by β-AR molecular changes: phosphorylation of receptor structures enhanced by an agonist-receptor bind state, that induces the reduction of receptors density and their internalization. This process is well-described also in the heart failure (Rengo et al., 2012a). By aging a post-synaptic "β-adrenoceptor desensitization" responsible for a reduction in the autonomic modulation of the cardiac system, especially during physical exercise (Ehsani, 1987; Scarpace et al., 1994), is observed. During exercise the stroke volume increase is similar in young and older, but the mechanism of this increase is different. The elderly tends to augment stroke volume more through cardiac dilatation with an end-diastolic volume increase, while the young shows an increase in the ejection fraction without cardiac dilatation. Moreover, during exercise the older has a lower increase in heart rate and a greater raise in blood pressure (Ferrara et al., 2006; Corbi et al., 2012b). In particular in the aging human heart the maintaining of cardiac output during exercise is supported more heavily by the Franck-Starling mechanism and less by sympathetic stimulation demonstrating the presence of an age-related change in the adrenergic modulation. It is interesting to point out the similarity observed in terms of hemodynamic profile between younger β-blocked subjects and healthy elderly without β-blockers treatment in human models without HF (Fleg et al., 1994). Younger β-blocked subjects showed during exercise reduced heart rate and contractility index, and increased left ventricular volume, in short terms they apparently "looked older" but the results of β-AR blockade were greater in the young subjects compared to older ones, suggesting that β-AR responsiveness reduces with aging.

Interestingly, Leosco et al. (2007) demonstrated that exercise training alone as well as metoprolol alone or in combined therapy (exercise + metoprolol) improved the β-AR signaling in the aged heart suggesting a similar effect on β-AR signaling of chronic treatment with β-blockers and chronic exercise training. Furthermore they found an increased β-AR density inducing a reversible level of β-AR desensitization. The overall reduction in cardiac reserve is responsible for the decreased exercise tolerance and for the impairment in cardiac response at exercise in terms of reduced ejection fraction at peak and heart rate responsiveness during dynamic exercise. The responsibility of this response could be related to increased vascular afterload, arterial-ventricular load mismatching, reduced intrinsic myocardial contractility, physical deconditioning and impaired autonomic regulation (so called "β-adrenergic desensitization"). The mechanism of this impaired autonomic regulation induced by age is not completely clarified, although it is hypothesized that the increase in cathecolamines levels plays an important role in the "β-adrenergic desensitization" in aging and failing heart. In both conditions, as a result of a reduced plasma clearance and an increased spillover from the tissues, the level of circulating cathecolamines further rises. This prolonged cathecolamines action seems to be also related to the age-dependent reduction of the cathecolamines re-uptake transporter localized in the sympathetic nerve terminals (Leineweber et al., 2002). However, controversial opinions exist in the present literature about the changes in the systemic norepinephrine levels with age and the relationship between the age-related increased levels of cathecolamines and the suggested mechanisms of age-related "β-adrenergic desensitization" (Folkow and Svanborg, 1993; Esler et al., 1995).

The age-related "β-adrenergic desensitization," a possible adaptive mechanism, has been observed in both animals and humans. An age-related effect on the maximum contractility response to isoproterenol has been demonstrated in arterially perfused interventricular septa from adult and senescent rats (Froehlich et al., 1978), and a reduced inotropic response of aged myocardium to catecholamines has been found in superfused trabeculae (Jiang et al., 1993). At molecular level, in particular, the decrease in β-adrenoceptor responsiveness has been related to changes in G-proteins and kinases activity even if differences in the level and extent of these changes exist among different species. Concerning the β-AR density, the first studies performed in circulating lymphocytes did not show any important age-related changes in β-AR density (Abrass and Scapace, 1981; Landmann et al., 1981). Also other data obtained from young and old rats did not report any changes in β-AR number (Bohm et al., 1993) did not find any changes in β-AR number but noticed a G_i increased content. This study hypothesized that $G_{i\alpha}$ might serve as a age-related regulator of cardiac AC activity in the absence of β-adrenoceptor changes. Interestingly Gudmundsdottir et al. (1991) showed that dietary fat and age modified the density of Ca^{++} channels and reduced the β -AR number in the rats.

Cerbai et al. (1995) confirmed that β_2-AR as well as β_1-AR are both functionally present in rat hearts, but only the β_1-AR density was reduced with aging. In human aging heart White et al. (1994) found that the β_1-AR down regulation mechanism was linked to the reduced number of β_1-AR in a high affinity agonist binding state. In our experience, using a model of myocytes obtained from human failing (donors) and non-failing hearts (small biopsies from elderly and young patients undergoing coronary vein graft with preserve ventricular function), the contractile response to β-adrenoceptor stimulation has been found to be strongly reduced in single myocytes from failing human ventricle, but a part of this reduction was statistically related to the patient's age (Dobson et al., 1990; Davies et al., 1996). At beginning, it was thought that the desensitization mechanism of β-AR in the elderly is related to the increased levels of adenosine because its clear antiadrenergic action in the heart, as suggested by studies performed in guinea pig aging hearts. However, utilizing single isolated left ventricular cardiac myocytes from hearts of animals at different age, other studies showed that the age-related contractility impairment during β-adrenergic stimulation was confined to the β-adrenergic pathway with reduced net production of c-AMP, that could not be explained by the increased adenosine stimulation (Ferrara et al., 1995).

Ferrara et al. (1995), in a study performed on myocytes isolated from the young and old guinea-pigs, observed that there was a pronounced age-related decrease in contractility of myocytes after both isoproterenol and high Ca^{++} concentration stimulation. However, the Ca^{++} influence was less significant concluding that a general diminished contractility of single myocytes occurs during the physiological aging and these effects were more marked for β-AR stimulation than for high Ca^{++}, suggesting a specific lesion in the AC related pathway. In a further

investigation, using the same experimental model, the same authors (Ferrara et al., 1997a) studied the role of the inhibitory G-proteins (G_i) in the decline of contractility related to β-stimulated activity. In these experiments they found that β-AR number was decreased by 27% in senescent animals and the $G_{i\alpha}$ activity, detected by PTX-catalyzed ADP ribosylation, was significantly increased in aged animals, while immunodetectable level of $G_{i\alpha}$ was not modified. The authors concluded that increased $G_{i\alpha}$ activity contributes significantly to the decreased response to β-AR stimulation in myocytes in aged guinea-pigs.

In different species (rats) Xiao et al. (1998) found a clear age-related reduction in contractility in response to β-AR subtype stimulation associated to a non-selective decrease in the density of both β-AR subtypes and a reduction in membrane AC response to either β_1-AR or β_2-AR stimulation. Moreover, the activity of β-AR kinase GRK_5, and of G_i did not significantly change with aging, suggesting that the marked decreased response to β-ARs stimulation with aging in rat ventricular myocytes was linked to a decrease in both β-AR subtypes densities and a reduction in membrane AC activity, while neither GRKs nor G_i proteins appear to play a role in this mechanism. This apparent contrast with the results from Ferrara et al. (1997a) could be referred to the different animal models used for the experiments.

As in the failing heart, the role of chronic sympathetic drive in the β-AR desensitization in the aging heart can be hypothesized. Because the role of GRKs in the aging heart could be defined controversial same studies have been planned to focus on the role of GRKs in the aged cardiovascular system.

Schutzer et al. (2001) examined the correlation of GRKs level to age-related modifications in aorta of old rats. In particular they studied the age-related changes in distribution of GRK subtypes 2, 3, or 5 and β-arrestin (cytoplasmic vs. crude membrane preparations), and demonstrated that GRKs are implicated in the reduction of β-AR-mediated vasorelaxation with advancing age, suggesting a strong evidence that increased GRK activity plays an important role in cardiovascular physiology of aging. However, it is important to underline that in this study was not performed detection of GRKs in the heart tissues as it was performed in the previous studies mentioned above, explaining this controversial results. On this basis Leosco et al. (2003), studying the effects of exercise, found a reduced β-AR expression in aged rat carotid arteries, and the exercise itself restored the age-associated blunted β-AR responsiveness. This could explain with the age-related reduced adaptation of cardiovascular system to different stressors.

For the first time Leinweber et al. (2003) studied the possible GRKs activity alterations related to aging in humans. The study evaluated the cytosolic and membranous levels of GRKs in right atria from children with congenital heart disease undergoing cardiac surgery, elderly with coronary heart disease but not suffering from heart failure undergoing coronary artery by-pass grafting, and from a small group of elderly patients with heart failure also undergoing cardiac surgery. The main result was that neither cytosolic nor membranous GRKs activity were modified in elderly compared to children, while, as confirmed in previous studies (Ungerer et al., 1993; Ping et al., 1997; Vinge et al., 2001; Rockman et al., 2002; Iaccarino et al., 2005) there was a notable up-regulation in the GRKs activity in the failing heart. The reason because the β-AR desensitization in the aging heart is not associated to GRKs up-regulation is not well clarified at present. It can be hypothesized that the GRKs levels are more affected by acute triggers as in heart failure happens and not influenced by a gradual chronic "aging" process of β-adrenergic drive, or at least during this life-long process GRKs levels are associated to other adaptive mechanism that may influence their up-regulation. In **Table 1** the main general age-related changes in β-adrenergic signaling are described.

CLINICAL IMPLICATIONS OF ALTERATIONS IN β-ADRENOCEPTOR MECHANISMS IN THE AGING HEART. RELATIONSHIP WITH HEART FAILURE

Heart failure and associated clinical implication induced by alterations in β-adrenoceptor mechanisms are a central problem for elderly population (Ferrara et al., 1997b). Epidemiological studies showed high prevalence and incidence of heart failure in the elderly (Corbi et al., 2008; Roger et al., 2011) due also to an increased longevity. The incidence doubles with each decade of life and prevalence rises almost 10% of those older than 80 years (Cacciatore et al., 1998). About 50% of all heart failure cases is found in patients older than 70 (Roger et al., 2011). Although nowadays an important decrease in the mortality for heart disease, cardiovascular disease is the most frequent single cause of death in the elderly population. The prognosis of heart failure among elderly is poor with a 4 year survival of only around 50% (Cacciatore et al., 1998) heart failure is also one of the most important causes of comorbidity and hospitalization rising health costs.

It is important to underline that heart failure in the elderly appears when cardiovascular structural and functional age-related changes are already evident. Moreover, the age-associated changes in cardiovascular structure/function are involved in the increased risk for heart failure in older people. An age-dependent increase in left ventricular wall thickness in men and woman without hypertension has been described in the Framingham Heart Study and Baltimore Longitudinal Study on Aging (Ho et al., 1993). These modifications in structure of heart are characterized by an increase in cardiomyocyte size and myocardial thickness modifying the contractile efficiency and increasing left ventricular mass (Olivetti et al., 1991; Khouri et al., 2005). As a result of age-related chronic stress, the myocardium can undergo cardiomyocyte death including necrosis, apoptosis or autophagy. This process induces initially a compensatory remodeling characterized by the alterations of extracellular matrix (ECM) composition involving the synthesis of myofibroblasts, the degradation of collagen through TGF-B signaling. These alterations lead to the hypertrophy of the remaining cells and to pathologic remodeling, with consequent reactive fibrosis that increases cardiac stiffness and reduces the cardiac compliance (Boyle et al., 2011).

It has been reported that the process of cardiac aging, as well as the progressive heart failure, is also characterized by the impaired Ca^{++} reuptake and the decreased SR Ca^{++} storage. In some studies this decline has been explained by the modification of $SERCA_2$ protein. This impaired Ca^{++} reuptake is responsible for the delays in the ventricular relaxation (Sucharov et al., 2006). The myocardial remodeling, myocardial stiffening and the

Table 1 | Cardiovascular system: the main general age-related changes in β-adrenergic signaling.

Author	Constituent	Species	Changes
Abrass and Scapace, 1981	β-AR density	Human (circulating lymphocytes)	Unchanged
Landmann et al., 1981	β-AR density	Human (circulating lymphocytes)	Unchanged
Gudmundsdottir et al., 1991	β-AR density	Rat (heart)	Decreased
Bohm et al., 1993	β-AR density	Rat (heart)	Unchanged
Bohm et al., 1993	G_i content	Rat (heart)	Increased
White et al., 1994	β_1-AR density	Human (heart)	Decreased
Bazan et al., 1994	β-AR density	Rat (heart)	Unchanged
Cerbai et al., 1995	β-AR density	Rat (heart)	Decreased
Ferrara et al., 1995	β-AR density	Guinea-pig (heart)	Decreased
Ferrara et al., 1997a,b	G_i content	Guinea-pig (heart)	Unchanged
Ferrara et al., 1997a,b	G_i activity	Giunea-pig (heart)	Increased
Xiao et al., 1998	$\beta_{1,\,2}$-AR density	Rat (heart)	Decreased
Xiao et al., 1998	G_i activity	Rat (heart)	Unchanged
Schutzer et al., 2001	β-AR	Rat (aorta)	Decreased
Schutzer et al., 2001	GRK_2, GRK_3, β-arrestin	Rat (aorta)	Increased
Leinweber et al., 2003	GRK	Human (heart)	Unchanged

delayed ventricular relaxation compromise the left ventricular filling in early diastole. In order to maintain an adequate left ventricular filling in elderly the atrial contraction (A wave) is further increased leading to the atrial hypertrophy increasing so the risk for atrial fibrillation. However, these data have not been confirmed utilizing other epidemiological and autopsy-based studies (**?**) in subjects free from coronary artery disease and hypertension. In fact, these studies have demonstrated no significant changes in cardiac mass in women and a decrease in left ventricular mass in men. Based on these and other studies it has been shown that there is no significant changes in left ventricular mass in the elderly women, but there is a reduction of cardiac mass in men (cardiac sarcopenia). Regarding to this Lin et al. (2008) demonstrated, combining experimental and mathematical models, in mice an age-related cardiac sarcopenia and that LV remodeling due to increased end diastolic pressure could be an underlying mechanism for age-related LV dysfunction. Senescence is characterized by a reduction of myocytes cell number. In order to maintain the cardiac function, some compensatory mechanisms are induced such as: increase of individual myoctye size, increase of intracellular glycogen storage and reactive fibrosis. This changes induce a depressed cardiac function in elderly. Moreover, asymmetric increase in the intraventricular septum does not influence the total cardiac mass. Nevertheless these changes do not modify significantly the systolic cardiac function at rest as demonstrated by echocardiographic and radionuclide studies in normotensive healthy population (Hess et al., 2002; Khouri et al., 2005). Instead the pattern of diastolic function changes with aging and plays a pivotal role in increased prevalence of diastolic heart failure in the elderly. There are many similarities between the aging and failing heart. For example, morphologically myocytes hypertrophy and cardiac fibrosis occur in both aging and failing heart that exhibit decreased diastolic function and increased ventricular mass. In both there is a decreased functional reserve and defective cardiac energetics. The age-related changes in β-AR behavior are quite similar to those related to failing heart and these changes may be induced by the compensatory adrenergic drive activation. The stimulation of adrenergic receptors by catecholamines is the most important regulatory mechanism for cardiovascular performance. It is well known that the levels of cathecolamines are directly correlated with mortality and functional class of heart failure (Cohn et al., 1984). The cardiac β-receptors, in particular the β_1 subtype, are downregulated in heart failure and the heart failure-associated reduced cardiac adrenergic responsiveness are related to an increase in $G_{\alpha i}$ activity and in activity of the adrenoreceptor kinases. Failing heart-associated β-AR down-regulation seems to effect the β_1-AR but not the β_2-AR density and this abnormality is also related to GRK_2 and G_i up-regulation (Bristow et al., 1986). Physical performance as well as other stressors stimulate the adrenergic system increasing heart rate, myocardial contractility and relaxation, reducing left ventricular afterload and redistributing blood to working muscle. As above described, this pattern modifies during aging. A possible explanation of this behavior is in relationship with the chronic and gradual sympathetic hyperactivity. The association of failing to aging heart physiologic changes is not important only for the worldwide health care system but also for explaining the worst of quality of life in these patients. For these reasons since about 30 years the research studies about the relationship between adrenergic system and heart failure are interestingly increasing. In the early 1982, Bristow et al. (1982) studying the myocardium contractility "*in vivo*," examined the β-AR pathway in failing hearts obtained from human subjects undergoing cardiac-transplantation. It was noticed an important reduction in the contractile response to isoproterenol in these "*in vivo*" cardiac cells and also an evident reduction of the β-AR density. The same author (Bristow et al., 1986) after a couple of years, developed a radiolig and biopsy method for identifying β-AR in human ventricular myocardium. This method it is thought to be useful in the direct analyses in the β-AR density

and in the study of down-regulation. The development of this new method opened a new window in the molecular pharmacology of adrenergic system. Brodde et al. (1989) concluded that the β-AR density level reduction is associated to the severity of congestive heart failure, and in valvular heart failure exist a decrease in either β_1-AR and β_2-AR density. β-AR desensitization in the failing heart is proved from many authors, but this is not the one and only mechanism involved in a complex disease like heart failure. Feldman et al. (1988) observed the increased activity of α G40 complex in the human failing heart and considered as a possible new marker for the severity of failing heart. Fu et al. (1992) also concluded that the role of G_i protein is crucial in the failing heart but it was observed an increase in functional activity rather than in its amount. In 1997 Ping et al. (1997) during the pacing induced congestive heart failure (CHF) examined the alteration of β-ARs, AC activity and GRKs. They concluded that in the advanced stages of CHF, β-ARs and AC are down-regulated while there is an increase in the GRKs levels since the early stages of CHF. Ungerer et al. (1993) studying the involvement of β-AR in human heart failure models found for the first time an inverse correlation between β-AR density and β-ARKs. The β-AR density is reduced but also the remaining receptors are less effective. Initially the catecholamines released from sympathetic nerves try to restore the cardiac functioning by acting on the β-AR. In long term the enhanced sympathetic drive causes the β-AR desensitization, At least part of this adaptative mechanism should protect heart from further sympathetic activation, but the overall cardiac performance is depressed.

The genetic engineering and the need to establish a quite similar model of human disease have driven the scientific research to develop transgenic animal models. These model are produced by using DNA microinjections in animals or by using another methodology like the gene knock-out models. In 1996 Rockman et al. (1996) in order to investigate the β-AR alteration and contribution in heart failure, used a model of transgenic mice with overexpression of β-ARK_1 inhibitors or β_2-AR overexpression. It resulted that overexpressed β-ARK_1 inhibitors increased the myocardial contractility and prevented the development of heart failure. A couple of years later Harding et al. (2001) developed a transgenic mice model overexpressing β-ARK_1 inhibitor (β-ARK_{1CT}) and calsequestrin protein. In this model a treatment with β-blockers led to an improvement of cardiac contractile functioning and an longer survival.

The development of animal surgery techniques also helped to obtain similar rat models of heart failure. Vinge et al. (2001) studied the involvement of GRKs in postinfarction heart failure model in rats undergoing ligation of left coronary artery. They concluded that GRK_2 and β-$arrestin_1$ are primary regulators in the endothelial function in the heart failure, while the GRK_3 and GRK_5 play a very important role in the cardiac myocyte functioning. The increased levels of $GRK_2$was related to the infarction induction suggesting that this protein may precipitate the development of acute heart failure. Iaccarino et al. (2005) found an inverse correlation between the β-ARs expression and the levels of GRK_2. This inverse correlation can be explained by the phsophorylation of β-ARs by GRKs and furthermore their internalization and desensitization process. Interestingly the level of $GRK_2$is associated to

Table 2 | Failing heart: the main general changes in β-adrenergic signaling.

Author	Constituent	Species	Changes
Bristow et al., 1982	β-AR density	Human (heart)	Decreased
Bristow et al., 1986	β_1-AR density	Human (heart)	Decreased
Brodde et al., 1989	$\beta_{1,\ 2}$-AR density	Human (heart	Decreased
Feldman et al., 1988	α G_{40} activity	Human (heart)	Increased
Ungerer et al., 1993	β-ARK	Human (heart)	Up-regulated
Fu et al., 1992	G_i	Human (heart)	Increased
Ping et al., 1997	β-AR	Human (heart)	Down-regulated
Ping et al., 1997	AC	Human (heart)	Down-regulated
Ping et al., 1997	GRKs	Human (heart)	Increased
Rockman et al., 1996	β-ARK_1 Inhibitors (overexpression)	Transgenic mouse (heart)	Increased contractility
Harding et al., 2001	β-ARKct (overexpression)	Transgenic mouse (heart)	Increased contractility
Vinge et al., 2001	β-$ARRESTIN_1$	Rat (heart)	Increased
Vinge et al., 2001	GRK_2	Rat (heart)	Increased
Vinge et al., 2001	GRK_5	Rat (heart)	Increased
Iaccarino et al., 2005	GRK2	Human (heart)	Up-regulated
Lymperopoulos et al., 2007	α_2-AR	Rat (adrenal medulla)	Down-regulated
Lymperopoulos et al., 2007	α_2-AR	Transgenic mouse (adrenal medulla)	Down-regulated
Lymperopoulos et al., 2007	GRK_2	Rat (adrenal medulla)	Up-regulated
Lymperopoulos et al., 2007	GRK_2	Transgenic mouse (adrenal medulla)	Up-regulated
Rengo et al., 2012a,b,c	α_2-AR density	Rat (adrenal medulla)	Down-regulated
Rengo et al., 2012a,b,c	GRK_2	Rat (adrenal medulla)	Increased
Rengo et al., 2014	GRK_2	Human (lymphocytes)	Increase

the degree of heart failure, suggesting an importance of GRKs as a heart failure progression biomarker. In 2007, for the first time, Lymperopoulos et al. (2007) studied the involvement of adrenal signaling in the pathophysiology of heart failure. The study was performed in two different models, in transgenic mice with cardiac overexpression of SR calcium binding protein calsequestrin (CSQ) and rat model undergoing myocardial infarction. It was noticed an α_2-AR down-regulation independent of species and a GRK_2 up-regulation. In 2012, in a post-infarction heart failure rat model Rengo et al. (2012b) studied the influence of adrenergic blockade in the in GRK_2 and α_2-AR adrenal medulla dysregulation. It has been confirmed that there is an up-regulation of adrenal medulla GRK_2 and a down-regulation of α_2-AR and that their blockade with β-blockers normalizes the level of GRK_2. The same author (Rengo et al., 2012c) in 2012 investigated the role of β_2-AR in a post-myocardial infarction heart failure rat model. This model underwent an adenoviral-mediated overexpression of β_2-AR after 4 weeks of surgery procedure. It was observed that the overexpression of β_2-AR improves the angiogenesis process and enhanced the coronary reserve and myocardial blood flow. This pro-angiogenic characteristic of β_2-AR was associated to the activation of pro-angiogenic vascular endothelial growth factor, protein kinase B, endothelial nitric oxide synthase VEGF/PKB/eNOS pathway.

Recently returning to the well-known method of detection GRKs levels from the lymphocytes, Rengo et al. (2014) performed a prospective study in a group of heart failure patients who underwent exercise training. It was shown for the first time that the subjects after physical performance presented reduced levels of GRKs and also predicted survival. In **Table 2** the main general failing heart-related changes in β-adrenergic signaling are described.

CONCLUSIONS

Cardiovascular diseases are the most common cause of death in elderly population. Increased longevity is associated to an increased heart failure morbidity with poor prognosis among elderly. For this reasons it is very important to understand and clarify the pathophysiological mechanisms that underlay the aging heart process. From the present literature it seems that "β-adrenoceptor desensitization/down-regulation" is a general and common mechanism which explains age- and heart failure-related decrease in β-adrenoceptor response to agonists. In particular, in both aging and failing hearts the decrease in β-adrenoceptor responsiveness is related to changes in G-proteins and kinases activity, although there are differences in the level and the extent of these changes among different studied species. These molecular alterations are responsible for the most structural and functional changes in aging heart. The improved understanding of this mechanism could help in the future to develop new therapy approach and ameliorate life quality among elderly population.

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Adrenergic receptors and metabolism: role in development of cardiovascular disease

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Activation of the adrenergic system has a profound effects on metabolism. Increased circulating catecholamine and activation of the different adrenergic receptors deployed in the various organs produce important metabolic responses which include: (1) increased lipolysis and elevated levels of fatty acids in plasma, (2) increased gluconeogenesis by the liver to provide substrate for the brain, and (3) moderate inhibition of insulin release by the pancreas to conserve glucose and to shift fuel metabolism of muscle in the direction of fatty acid oxidation. These physiological responses, typical of the stress conditions, are demonstrated to be detrimental for the functioning of different organs like the cardiac muscle when they become chronic. Indeed, a common feature of many pathological conditions involving over-activation of the adrenergic system is the development of metabolic alterations which can include insulin resistance, altered glucose and lipid metabolism and mitochondrial dysfunction. These patterns are involved with a variably extent among the different pathologies, however, they are in general strictly correlated to the level of activation of the adrenergic system. Here we will review the effects of the different adrenergic receptors subtypes on the metabolic variation observed in important disease like Heart Failure.

Keywords: cardiac metabolism, beta adrenergic system, heart failure, GRKs, mitochondria

INTRODUCTION

Heart function relies to a great extent on cardiac muscle oxidative metabolism. Given the high mitochondrial content of this tissue, cardiac muscle generates ATP almost exclusively (about 90%) through oxidative phosphorylation (Stanley et al., 2005) by using different metabolic substrates. Indeed, cardiac muscle possesses a metabolic flexibility or plasticity, allowing it to maintain its function during stressful conditions. In the adult heart the major pathway for ATP production is fatty acid oxidation while the relative contribution of glucose increases during stress or injury, such as exercise or ischemia (Bing et al., 1954; Wisneski et al., 1987). Thus, it is not surprising that an impairment of cardiac muscle energy metabolism represents an important risk factor for the development of cardiac diseases (Stanley et al., 2005; Neubauer, 2007). Indeed, under pathological conditions, the heart exhibits a severe malfunction of different metabolic pathways, such as the tricarboxylic acid (TCA) cycle and b-oxidation (Stanley et al., 2005; Neubauer, 2007). This metabolic remodeling is characterized by a lower oxidative capacity, contractile dysfunction and cardiac muscle insulin resistance (Stanley et al., 2005; Neubauer, 2007). Different therapeutic strategies have been undertaken to modulate metabolic pathways in the failing heart, though it remains controversial whether targeting glucose vs. fatty acid metabolism individually or combined represents a better approach to improve metabolic flexibility and cardiac function (Kolwicz and Tian, 2009; Ardehali et al., 2012). Surely, the importance of a preserved metabolism includes not only an efficient energy supply needed for myocardium to accomplish his contractile function but also protection against oxidative stress (Neubauer, 2007), which is involved in the remodeling and progression of the disease.

CARDIAC METABOLIC DYSFUNCTION DURING HF

Animal models of HF have evidenced excessive uptake and myocardial free fatty acid (FFA) accumulation with reduced glucose utilization (Lommi et al., 1998; Taylor et al., 2001) and in both animal models of HF and in human disease these metabolic alterations reduce myocardial oxygen efficiency and lead to a depletion of intracellular ATP (Neubauer et al., 1992; Taylor et al., 2001). The importance of a preserved glucose cardiac utilization may be related to its higher efficiency in terms of ATP production and molecule of oxygen consumed, with consequently reduced oxygen wastage and reactive oxygen species (ROS) production, as compared to FFA. Insulin receptor signaling is critically involved in increasing glucose uptake in the myocardium and cardiac insulin resistance contributes to the development of left ventricular (LV) dysfunction by reducing cardiac efficiency through metabolic shift toward fatty acids utilization (Peterson et al., 2004b). Indeed, a profound state of insulin resistance has been found in the hearts of *ob/ob* mice and the ability of these

hearts to modulate substrate utilization in response to insulin and changes is altered (Mazumder et al., 2004). Accordingly, normalization of cardiac metabolism by overexpressing a human GLUT4 transgene in mice with cardiac insulin resistance recovered the altered cardiac function observed in these animals (Belke et al., 2000; Semeniuk et al., 2002). Therefore, these studies indicate that cardiac insulin resistance reduces the metabolic efficiency of the heart, which leads to a contractile dysfunction. Moreover, insulin resistance is a known and recognized phenomenon leading to HF (Boudina et al., 2009) as seen in positron emission tomographic (PET) studies showing that the failing human myocardium has reduced glucose uptake in favor of FFA uptake (Witteles et al., 2004). Several hypotheses have been proposed to explain the association between altered cardiac metabolism, insulin resistance, and HF, and among these there is a strong correlation with neurohormonal activation (Kostis and Sanders, 2005; Zucker, 2006), which increases plasma FFA levels, inhibits insulin receptor signaling and causes the loss of myocyte glucose uptake (Opie and Sack, 2002). As known neurohormonal activation includes over-activation of the adrenergic and RAAS system, and we will here specifically focus on the adrenergic mechanisms that underlie altered myocardial metabolism and insulin resistance in HF.

SYMPATHETIC NERVOUS SYSTEM AND CARDIAC METABOLISM: ROLE OF THE β ADRENERGIC RECEPTORS AND RELATED SIGNALING

The Sympathetic Nervous System (SNS) is maladaptively activated in response to a chronic reduction in cardiac output and it is characterized by an increased secretion and reduced cardiac catecholamine reuptake (Eisenhofer et al., 1996). The effects of the catecholamine incretion on the cardiac metabolism are mediated by both central and peripheral mechanisms. For example, increased catecholamines have directly detrimental effects on the heart, which cause marked enzyme loss as an index of diffuse myocardial damage, and substantial oxygen-wastage even in the absence of FFA in the perfusate (Opie et al., 1979). Furthermore, norepinephrine promotes both coronary vasoconstriction and increased plasma FFA levels (Paolisso et al., 1991), which further promote oxygen-wastage (Sasaoka et al., 2006). Infusion studies in volunteers support a role for increased norepinephrine levels in HF as a cause of elevated plasma FFA (Sasaoka et al., 2006). In turn, FFAs reciprocally augment SNS activity, at least in normal controls. In human skeletal muscle, a dose-response relationship exists between plasma FFA (Santomauro et al., 1999; Peterson et al., 2004a; Banerjee and Peterson, 2007) and defects in insulin signaling (Belfort et al., 2005). This may in part be caused by FFA-mediated activation of protein kinase C, which phosphorylates insulin receptors and results in reduced capillary opening and reduced myocyte glucose import (Itani et al., 2002; Wagenmakers et al., 2006). Moreover, also locally activated SNS appear to be relevant in the altered cardiac metabolism. With use of PET with a norepinephrine analog and 18F-fluorodeoxyglucose, myocardial segments with LV dysfunction have reduced presynaptic norepinephrine reuptake and myocardial glucose uptake in relation to less impaired myocardial segments in the same patients (Mongillo et al., 2007). Thus, after control for confounding variables, altered metabolism and IR directly relate to local SNS activity. As known, the adverse effects of the SNS on the heart are mediated by the adrenergic receptors (AR), however, extensive research has indicated that the various subtypes, in particular β_1 and β_2 ARs, are differently involved in pathophysiology of HF and so it is likely to be the same for modifications of cardiac metabolism observed during disease. Indeed, β_1- and β_2-AR regulate different signal pathways, resulting in different outcomes on cardiac function. Stimulation of β_1- and β_2-adrenoceptors can induce the activation of the stimulatory G protein ($G_{\alpha s}$)/adenylylcyclase (AC)/cAMP/cAMP-dependent protein kinase A (PKA) signaling pathway, which consequently leads to the phosphorylation of several target proteins within the cardiac myocyte, such as phospholamban, L-type calcium channel and troponin I (Woo and Xiao, 2012). Nonetheless, this signal pathway is the main mechanism by which β_1- rather than β_2 adrenoceptors regulates cardiac contractility/relaxation and rate (Baruscotti et al., 2010; Woo and Xiao, 2012). In contrast, the β_2 AR has been shown to regulate an alternative signaling pathway through activation of the inhibitory G protein ($G_{\alpha i}$) and the heterodimer formed by the β and γ subunits of the G protein ($G_{\beta\gamma}$) (Zhu et al., 2011). Besides the inhibition of AC, the main signal pathway regulated by β_2-AR through $G_{\alpha i}/G_{\beta\gamma}$ appears to be the phosphatidylinositol-3kinase (PI3K) signaling cascade, although other proteins such as the AMP-dependent protein kinase (AMPK), mammalian target of rapamycin (mTOR) and extracellular signal-regulated kinase1and 2 (ERK1/2) have recently been proposed as novel targets of β_2-AR (Zhang et al., 2011). About the effects of adrenergic system on metabolism, it is known that sustained beta adrenergic stimulation induce insulin resistance (Cipolletta et al., 2009) and in this context the β_2 adrenergic receptor appears to have a main role in overall glucose homeostasis by acting on pancreatic islet hormone secretion, liver and muscle glucose transport metabolism (Usui et al., 2005; Shahid and Hussain, 2007; Garcia-Guerra et al., 2010). At cardiac level several studies have raised the possibility of using selective β_2-agonists as potential modulators of cardiac muscle energy metabolism. Short- and long-term stimulation of the β_2-AR has been associated with the modulation of fatty acid and glucose metabolism (Philipson, 2002). Indeed, acute treatment of myocytes *in vitro* or skeletal muscle *ex vivo* with β_2 agonists induces a significant increase in glucose uptake, reaching comparable levels to insulin stimulation (Nevzorova et al., 2002, 2006; Ngala et al., 2008). A putative mechanism for these evidence would involve the activation of PI3K and its downstream signal pathway (Zhu et al., 2001; Jo et al., 2002; Perez-Schindler et al., 2011; Zhang et al., 2011) and in particular the phosphorylation and inactivation of TBC1D4 (also known as Akt substrate of 160 kDa, AS160) by Akt (Sakamoto and Holman, 2008). TBC1D4 inhibit the translocation of the glucose transporter type4 (GLUT4) from intracellular vesicles to the plasmamembrane, hence an increase in TBC1D4 phosphorylation would consequently enhance glucose uptake (Sakamoto and Holman, 2008). Moreover, TBC1D4 is also targeted by AMPK, which represent a key mechanism in the regulation of insulin-independent glucose uptake (Sakamoto and Holman, 2008; Maarbjerg et al., 2011). Consistent with the potential role of β_2-AR in glucose metabolism, high levels of AMPK phosphorylation and activity in response to β_2-AR stimulation

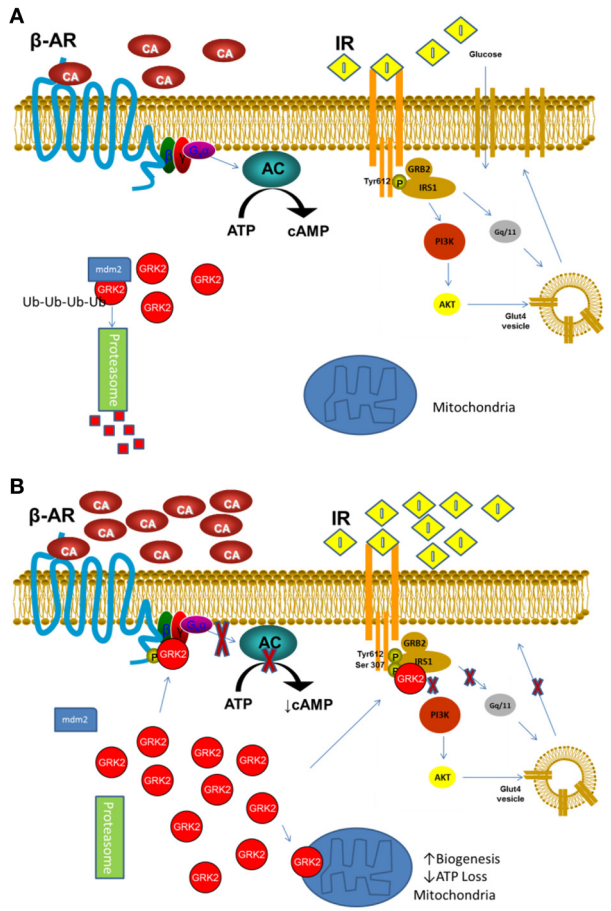

FIGURE 1 | (A) In basal or low stimulation conditions GRK2 is mostly cytosolic and its level are tightly regulated by mdm2 ubiquitination and degradation through the Proteasome. **(B)** Following acute or excessive stimulation by catecholamines or Insulin or hypoxia, GRK2 detaches from mdm2 and quickly accumulates in the cell, moving to membranes where desensitizes β-AR and IR. Moreover, it can translocates to the mitochondria, reducing ATP loss and inducing mitochondria biogenesis (β-AR, β Adrenergic Receptor; IR, Insulin Receptor; CA, Catecholamine; I, Insulin).

(Li et al., 2010; Perez-Schindler et al., 2011) have been found in cardiomyocytes, potentially as consequence of an increase in the AMP/ATP ratio, or activation of upstream AMPK kinases (Hardie et al., 2012). Different results, however, have been found in presence of an enhanced β_2-AR signaling by means of stable overexpression in non-cardiac cells, where, *in vitro* studies shows impaired glucose extraction later on insulin stimulation (Cipolletta et al., 2009). Moreover "*in vivo*" studies demonstrated a greater efficiency of carvedilol, a non-selective β-AR antagonist, in ameliorating myocardial insulin sensitivity and glucose extraction in animal model of HF as compared to metoprolol, a selective β_1-AR antagonist, indicating that antagonism of β_2AR positively regulates cardiac glucose metabolism in an animal model HF (Nikolaidis et al., 2006). These conflicting results may rely on the different models used for studies and/or to differences in acute and chronic stimulation of the β_2AR. While acute activation of the receptor can favor glucose uptake by increasing GLUT4 translocation to the plasmamembrane, chronic adrenergic stimulation, as seen during HF, would be rather detrimental by up or down regulation of specific intracellular molecules. Recent discoveries have found in the kinase of the G protein coupled receptor type 2 (GRK2) a potential molecular link between chronic adrenergic stimulation and development of altered myocardial metabolism observed during HF (Ciccarelli et al., 2011). So far, the implications of GRK2 upregulation during HF have been exclusively considered for the detrimental effects on βAR signaling and cardiac inotropism (Koch et al., 1995), however, studies in non-cardiac cells have shown ability to interact with different substrates belonging to different cellular pathways such as insulin signaling, and, of note, to mediate insulin resistance produced by enhanced β_2-AR receptor signaling (Cipolletta et al., 2009). In the failing heart GRK2 is upregulated (Iaccarino et al., 2005) and affects myocardial glucose uptake at the early stages of the disease, before cardiac dilation and reduced function are evident, indicating that metabolic modifications are relevant in the progression of HF and to be the molecular link between over-activation of the adrenergic system and the altered glucose uptake during HF (Ciccarelli et al., 2011). Recent studies have also indicated that GRK2 is also able to localize into mitochondria, but this role in the global cellular physiology is not completely clear. Indeed, different cellular models have shown different behaviors of GRK2 when it localizes into mitochondria. Specifically, Fusco et al. have indicated that GRK2 is protective for the cell by increasing ATP production and promoting mitogenesis (Fusco et al., 2012). As known, GRK2 shuttles from cytosol to plasmamembrane, by anchoring to free Gβγ through its pleckstrin homology and binding domains within the carboxyl terminus, following both catecholamine and insulin stimulation. Similarly, ischemia causes acute cellular and mitochondrial accumulation of GRK2 (Fusco et al., 2012), an effect reverted by oxygen restoration, but, surprisingly it preserves ATP loss and induces mitochondria biogenesis after ischemia/reperfusion, indicating a protective effect of GRK2 for mitochondria after acute stress.

These data are supported by the importance of GRK2 for embryonic cardiac development (Jaber et al., 1996) and, in adult life, by the early eccentric dilation of the heart and the vascular inflammation induced by selective GRK2 removal in the myocardium and endothelium, respectively (Brunn et al., 2006; Ciccarelli et al., 2013). On the contrary, Chen et al. have also shown that elevated level of GRK2 activates a pro-death signal through its localization into mitochondria during stress conditions (Chen et al., 2013).

This discrepancy could be related to the model and timing used in different studies, however, in a synthetic view of the latest GRK2 studies, we could advance the idea that the inhibitory property of GRK2 could be useful in acute phase of stress/stimulation, when the cytosolic molecule shuttles to the plasmamembrane and mitochondria, setting cellular activity in a resting phase and less susceptible to the stress and/or excess of signaling. However, in a perpetuating situation, the increased activity/level of GRK2 and chronic reduction of β adrenergic and insulin signaling becomes detrimental for cell function and survival (**Figures 1A,B**). Further studies will be needed to evaluate this hypothesis; nevertheless, several reports already indicate GRK2 as

a potential target for treatment of both altered metabolism and contractility during HF.

CONCLUSIONS AND PERSPECTIVES

Modification of cardiac metabolism is a fascinating field of investigation for development of new strategies for treatment of myocardial dysfunction. This consideration is based on the evidence that an efficient metabolic supply is needed for the single cardiomyocyte to accomplish its contractile function and efficient utilization is also important to counteract oxygen wastage and increased ROS on which relies progression of the disease. Current available compounds acting on the βARs seem to be effective for these goals, however, identification of specific target, as GRK2, clearly involved in regulation of cardiac contractility, remodeling and metabolism would prepare the ground for a more efficient therapy aimed to ameliorate patients prognosis and outcome.

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Adrenergic signaling and oxidative stress: a role for sirtuins?

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The adrenergic system plays a central role in stress signaling and stress is often associated with increased production of ROS. However, ROS overproduction generates oxidative stress, that occurs in response to several stressors. β-adrenergic signaling is markedly attenuated in conditions such as heart failure, with downregulation and desensitization of the receptors and their uncoupling from adenylyl cyclase. Transgenic activation of β2-adrenoceptor leads to elevation of NADPH oxidase activity, with greater ROS production and p38MAPK phosphorylation. Inhibition of NADPH oxidase or ROS significantly reduced the p38MAPK signaling cascade. Chronic β2-adrenoceptor activation is associated with greater cardiac dilatation and dysfunction, augmented pro-inflammatory and profibrotic signaling, while antioxidant treatment protected hearts against these abnormalities, indicating ROS production to be central to the detrimental signaling of β2-adrenoceptors. It has been demonstrated that sirtuins are involved in modulating the cellular stress response directly by deacetylation of some factors. Sirt1 increases cellular stress resistance, by an increased insulin sensitivity, a decreased circulating free fatty acids and insulin-like growth factor (IGF-1), an increased activity of AMPK, increased activity of PGC-1a, and increased mitochondrial number. Sirt1 acts by involving signaling molecules such P-I-3-kinase-Akt, MAPK and p38-MAPK-β. βAR stimulation antagonizes the protective effect of the AKT pathway through inhibiting induction of Hif-1α and Sirt1 genes, key elements in cell survival. More studies are needed to better clarify the involvement of sirtuins in the β-adrenergic response and, overall, to better define the mechanisms by which tools such as exercise training are able to counteract the oxidative stress, by both activation of sirtuins and inhibition of GRK2 in many cardiovascular conditions and can be used to prevent or treat diseases such as heart failure.

Keywords: oxidative stress, sirtuins, GRK2, β-adrenergic system, exercise training, heart failure, reactive oxygen species

INTRODUCTION

The sympathetic adrenergic system plays a central role in stress signaling and stress is often associated with increased production of reactive oxygen species (ROS).

ROS production is the result of several mechanisms, including generation during oxidative phosphorylation in the mitochondria as a product of normal cellular aerobic metabolism (Davies, 1995; Ide et al., 1999). Thus, the major process from which the body derives sufficient energy can also result in the production of ROS (Ide et al., 1999). The balance between the production of ROS and the activation of the antioxidant defense system is crucial for the human physiology and the control of cellular homeostasis. ROS play an important role in signaling processes, but their overproduction generates oxidative stress. In fact, ROS can regulate cellular functions, e.g., during immune and inflammatory processes (Remacle et al., 1995), in turn their overproduction causes damage to cellular constituents, including DNA, proteins, and lipids, especially when occurs with insufficient antioxidant enzyme activity (Varma, 1991).

In several cellular signaling pathways (Nishida et al., 2000), ROS act as second messengers downstream of specific ligands, including Transforming Growth Factor-β1 (TGF-β1), Platelet-Derived Growth Factor (PDGF), Fibroblast Growth Factor-2 (FGF-2), endothelin (Thannickal and Fanburg, 2000; Sawyer et al., 2002; Griendling and FitzGerald, 2003; Machida et al., 2003) and they are also involved in modulating the activity of specific transcription factors, such as Nuclear Factor-kB (NF-kB) and Activator Protein–1 (AP-1) (Hsu et al., 2000; Hirotani et al., 2002; Turpaev, 2002; Wu et al., 2002; Sabri et al., 2003; Rengo et al., 2013a).

Elevated ROS have also been implicated in the development and sustainment of several chronic degenerative diseases (i.e.,

cancer, diabetes, neurodegenerative and cardiovascular conditions) and in the mechanism of senescence and aging (Knight, 2000; Dröge, 2002; Westerheide et al., 2009; Marciano et al., 2012; Paolillo et al., 2013; Rengo et al., 2013b), and it has been suggested that they also contribute to adverse myocardial remodeling and the progression to heart failure (Sawyer et al., 2002; Seddon et al., 2007). However, relatively little is known about the type of ROS involved (e.g., superoxide, hydrogen peroxide, peroxynitrite), their specific role in mediating myocyte hypertrophy, apoptosis, fibrosis that participate in myocardial remodeling (Sawyer et al., 2002; Mann and Bristow, 2005; Seddon et al., 2007), and in relationship to the overall progression to myocardial failure.

The sympathetic adrenergic system plays a central role in ability to rapidly respond to various types of threats. One important target of adrenergic stimulation is the heart, where activation of β-adrenergic receptors causes increases in heart rate (chronotropy), relaxation speed (lusitropy) and contractility (inotropy) (Andersson et al., 2011).

Increased adrenergic drive is a major factor influencing the development of pathological cardiac hypertrophy, a stage which precedes overt heart failure. Whereas it is well known that heart failure, a highly prevalent syndrome, is characterized by both increased ROS production and β-adrenergic hyperactivity, still few evidence are available on the relationship between β-adrenergic system and oxidative stress.

Recently it has been discovered that a family of enzymes consists of NAD^+-dependent histone/protein deacetylases, called Sirtuins, represents pivotal regulator of redox cellular status.

In mammalian cells SIRT1 appears to control the cellular response to stress by regulating the family of Forkhead transcriptional factors (FOXOs) (Brunet et al., 2004) and directly deacetylating the Heat Shock Factor (HSF1) and thus regulating Heat Shock Proteins (HSPs) expression (Westerheide et al., 2009; Corbi et al., 2012a).

This review is aimed to focus on the relationship between adrenergic system activity and oxidative stress, with a light on the possible implications of sirtuins in the regulation of this mechanism.

OXIDATIVE STRESS IN THE CARDIOVASCULAR SYSTEM

Several *in vitro* and *in vivo* studies have demonstrated ROS activation in the cardiovascular system in response to various stressors and in the failing heart (Ide et al., 1999; Cesselli et al., 2001; Wallace, 2001; Sawyer et al., 2002; Sabri et al., 2003; Scortegagna et al., 2003; Suematsu et al., 2003), and animal studies have also suggested that antioxidants and ROS defense pathways can ameliorate ROS-mediated cardiac abnormalities (Chen et al., 1996; Yen et al., 1996; Ho et al., 1998; Conrad et al., 2004; Giordano, 2005).

The ROS oxide (O2-), nitric oxide (NO), hydroxyl (OH-), and peroxynitrite (ONOO-) are molecules characterized by the presence of unpaired electrons that are highly reactive with cysteine residues in the catalytic center of cellular enzymes, thus making them excellent signal transducers (Finkel, 1999).

ROS have been linked to key pathologic processes such as cardiac hypertrophy (Nakamura et al., 1998) cardiomyocyte apoptosis (von Harsdorf et al., 1999), ischemia-reperfusion (Zweier et al., 1989) and heart failure itself (Ide et al., 1999). But also oxidant overproduction occurs in response to several stressors, including chemicals, drugs, pollutants, high-caloric diets, and exercise (Kohen and Nyska, 2002). Physical exercise can increase oxidative stress, eventually causing a perturbation of homeostasis that is dependent on training specificity (Conti et al., 2012a) and workload (Conti et al., 2013), but in turn it is also able to counterbalance the deleterious effects of ROS by activation of several antioxidant systems, such as Super Oxide Dismutases (SODs), HSPs and catalase (Corbi et al., 2012a,b). The mechanisms by which ROS mediate these different biologic responses are not fully understood, but in many cases involve activation of specific redox-sensitive signaling molecules. Three important candidates for downstream effectors are p38 Mitogen-Activated Protein Kinase (p38MAPK) and c-Jun Kinase (JNK), members of the stress-activated kinase family, and the cell survival kinase Akt (Griendling et al., 2000).

Angiotensin II, Tumor Necrosis Factor alpha (TNF-α) and norepinephrine are neurohormones implicated in the development of cardiac hypertrophy and progression to end-stage human heart failure (Packer, 1998). There is currently evidence that at least some hypertrophic effects induced by these agents are mediated through ROS.

In vivo, under physiologic conditions, O2- is predominantly inactivated by SODs, which are present in high concentrations in mitochondria (MnSOD), cytosol (Cu/Zn SOD), or plasma membrane/extracellular spaces, and consequently the formation of ONOO- is minimal.

During physiological and pathological conditions, including aging, SODs convert O2- to hydrogen peroxide (H_2O_2), which has a longer half-life, can diffuse longer distances than O2-, and is able to influence signaling events at more distant sites. In fact H_2O_2 can regulate the activity of several enzymes essential for Ca^{2+} release, growth, or apoptosis (phospholipases A2, C, and D, Src kinase, p38MAPK, JNK and Akt/PKB) (Griendling et al., 2000).

It has been demonstrated that the expression and activity of the SOD system is modified in aging, with reduced cell ability to counteract the oxidant molecules, and consequent weak resistance to ROS accumulation (Rinaldi et al., 2006). Obviously, cytotypes with limited replication ability, such as brain and heart, are particularly vulnerable to this phenomenon, suggesting that it could explain, at least in part, high prevalence of cardiovascular and neurological disorders in elderly people (Navarro-Arévalo et al., 1999). In fact, it is widely known that oxidative stress and reduced antioxidant defense have negative effects on cardiac structure and function (Singal et al., 1988) and they are also involved in lipid membrane oxidation and other heart age-related conditions (Corbi et al., 2012b).

HSPs are another system of cellular defense against oxidative stress. These "stress-induced proteins" are ubiquitous and highly conserved chaperones, important in the folding of new synthesized or damaged proteins. Moreover, HSPs mediate mitochondrial protection against oxidative stress and some of those, such as HSP70, have been associated with myocardial protection. Martin et al. (Martin et al., 1997) showed an increased

survival in HSP70-transfected cardiomyocytes and consequent increased expression of the HSP70 enzyme against ischemic cardiac damage.

The metabolism of H_2O_2 is tightly regulated by the cellular glutathione peroxidases, which scavenge H_2O_2 (glutathione-dependent) or catalase (glutathione-independent) (Sorescu and Griendling, 2002). By converting H_2O_2 into water, catalase constitutes a primary antioxidant defense system and could protect cells from ROS and its deleterious consequences on diseases.

Recently it has been demonstrated that catalase protected cardiac mitochondrial aconitase enzyme from oxidative damage (Schriner et al., 2005) and overexpression of catalase targeted to mitochondria protects mice from cardiac aging, providing direct evidence for the role of mitochondrial ROS in the aging of this vital organ (Dai et al., 2009).

In fact, accumulation of oxidative damage has also been considered responsible of many different aspects of the aged heart. It has been found that cardiac fibrosis and size of myocytes increase with aging, while the number of myocytes decreases and ventricular hypertrophy is almost a constant finding in the aging rat heart (Anversa et al., 1986; Klima et al., 1990; Besse et al., 1994a). Hearts of old rats are characterized by reduced antioxidant defenses, such as SODs and Hsp 70 (Rinaldi et al., 2006).

Moreover, the oxidative stress with abnormalities in mitochondrial function, calcium (Ca^{2+}) handling, electrolytes alterations, hormones, and cardioprotective signaling have all been proposed as potentially implicated in the aging process (Besse et al., 1994b). In particular, regarding the effects of electrolytes changes implicated in the regulation of myocardial function, it has been demonstrated that magnesium (Mg^{2+}) interferes on failed cardiac contractility (Corbi et al., 2008) by modifying sarcoplasmic reticular Ca^{2+} transport systems with a calcium antagonism mechanism based on competition between Mg^{2+} and Ca^{2+} for the same binding sites on key myocardial contractile proteins, such as troponin C, myosin, and actin (Koss and Grubbs, 1994) that could explain the opposite effects of Mg^{2+} and Ca^{2+} on myocardial contractility (Kawano, 1998). Ca^{2+} overload can be induced by direct effect of ROS on Ca^{2+} handling proteins or indirectly, by inducing membrane lipid peroxidation (Valko et al., 2007).

SIRTUINS AND OXIDATIVE STRESS IN THE CARDIOVASCULAR SYSTEM

Another important mechanism involved in cellular redox regulation is represented by family of sirtuins, a cluster of seven homologous proteins regulating cellular biology and metabolism through deacetylation of histones and other cellular factors such as NFkB, HSF1, p53, FOXOs, and Peroxisome Proliferator-Activated Receptor Gamma Coactivator (PGC-1). By promoting deacetylation, sirtuins can either promote or inhibit the activity of several protein targets (Finkel et al., 2009; Haigis and Sinclair, 2010; Guarente, 2011).

SIRT1 and SIRT6 can deacetylate specific lysines on histone tails to promote transcriptional silencing. SIRT1 also deacetylates many non-histone proteins such as p53, FOXOs, Nuclear Receptor Corepressor (SMRT/NCOR), and PGC-1alpha (Finkel et al., 2009; Haigis and Sinclair, 2010; Guarente, 2011). SIRT3 targets mitochondrial enzymes involved in metabolism, ROS detoxification, and mitochondrial function including Long Chain Acyl coe-enzyme A Dehydrogenase (LCAD), Isocitrate Dehydrogenase 2 (IDH2), SOD2, and cyclophilin D (Hafner et al., 2010; Zhong and Mostoslavsky, 2011). Other enzymatic reactions catalyzed by selected sirtuins are the transfer of an ADP-ribosyl group from NAD^+ to an acceptor protein (SIRT1, SIRT4, and SIRT6) (Finkel et al., 2009; Haigis and Sinclair, 2010; Guarente, 2011), or the demalonylation and desuccinylation of modified proteins (SIRT5) (Du et al., 2011). However, the biological relevance of these reactions is only beginning to be unveiled (Oellerich and Potente, 2012).

In particular, SIRT1, the human homologous of the family, is involved in many functions of human physiology, including DNA repair, cell cycle regulation, apoptosis, gene expression, and aging (Grubisha et al., 2005). By FOXO3 acetylation and/or phosphorylation oxidative stress induces arrangement of SIRT1-FOXO3a, complex indispensable for cell cycle arrest and induction of DNA repair (Brunet et al., 2004). In turn, SIRT1 can modulate the cellular stress response directly deacetylating some proteins and regulating their expression (Porcu and Chiarugi, 2005). In fact, SIRT1 modulates the threshold of cell death in the setting of exogenous stress, including oxidative damage, interacting with p53, inhibits Bax-induced apoptosis by deacetylation of Ku70, and regulation of other targets linked to cell death (Cohen et al., 2004) and cellular antioxidant activity (such as Mn-SOD and catalase) (Corbi et al., 2012b).

Moreover, SIRT1 protects against endothelial dysfunction by preventing stress-induced premature senescence, thereby modulating the progression of cardiovascular diseases (Ota et al., 2007; Li et al., 2011; Nadtochiy et al., 2011; Stein and Matter, 2011), and it plays an essential role in mediating the survival of cardiac myocytes under stress *in vitro* (Alcendor et al., 2004; Pillai et al., 2005).

It has been observed that overexpression of Sirt1 reduces expression of the Angiotensin II Type 1 Receptor (AT1R) (Sunagawa, 2008) and this inhibition seems to prevent endothelial dysfunction of cerebral arterioles (Arrick et al., 2008; Miyazaki et al., 2008).

Although there are fewer studies of the other sirtuins, the importance of SIRT3 for cardiac function has been demonstrated by some authors. SIRT3 is expressed abundantly in the heart, and has been reported to play a protective role against hypertrophy, acting at different levels. SIRT3 overexpression blocks hypertrophy both *in vitro* and *in vivo*, whereas $SIRT3^{-/-}$ mice exhibit enhanced susceptibility to hypertrophy (Sundaresan et al., 2009), likely indirectly protecting against cardiac hypertrophy by specifically control of ROS levels. Moreover, SIRT3 attenuates Hypoxia-Inducible Factor 1-alpha (HIF-1α) activity indirectly by controlling intracellular ROS (Finley et al., 2011), suggesting a central regulatory function of sirtuins in the cellular response to hypoxia (Oellerich and Potente, 2012).

More recently Cardus et al. demonstrated that the presence of SIRT6 in endothelial cells protects from telomere and genomic DNA damage, thus preventing a decrease in replicative capacity and the onset of premature senescence. These findings suggest that SIRT1 and SIRT6 collaborate at different levels to maintain

endothelial homeostasis, with SIRT6 regulating chromatin functions and DNA repair, and SIRT1 intracellular signaling networks (Cardus et al., 2013).

Finally SIRT7 seems to be an essential regulator of tissue homeostasis in the heart through its interaction with p53. Sirt7-deficient primary cardiomyocytes show an approximately 200% increase in basal apoptosis, and a significantly reduced resistance to oxidative and genotoxic stress (Vakhrusheva et al., 2008; Corbi et al., 2013).

Because the sirtuins activity depends on NAD^+ availability it has been suggested that their enzymatic activity is directly linked to the energy and cellular redox status via the NAD^+/NADH ratio. Among the seven sirtuins, SIRT1 and SIRT3 are crucially involved in regulation of cardiomyocyte energy metabolism, production of ROS and signaling relevant to cell death/survival (Tanno et al., 2012) playing different roles in regulation of energy production and oxidative stress. Hearts consume large amounts of O_2 and yield high levels of ROS in the mitochondria. In addition, various extracellular factors, such as angiotensin II and Tumor Necrosis Factor-alpha (TNF-alpha), induce ROS formation and promote cardiomyocyte death together with the mitochondrial ROS (Giordano, 2005).

It has been demonstrated that MnSOD is required for normal biological function of tissues. In fact, Li et al showed that MnSOD$^{-/-}$ homozygous mutant mice die within the first 10 days of life with a dilated cardiomyopathy, accumulation of lipid in liver and skeletal muscle, and metabolic acidosis, and these findings were related to a severe reduction in succinate dehydrogenase (complex II) and aconitase (a TCA cycle enzyme) activities in the heart (Li et al., 1995).

Furthermore, Loch et al found that MnSOD$^{+/-}$ mice displayed a decrease in fraction shortening and ejection fraction and an increase in left ventricular internal diameter in systole, and developed heart hypertrophy with accompanying fibrosis and necrosis, demonstrating that lifelong reduction of MnSOD activity has a negative effect on normal heart function (Loch et al., 2009).

Both SIRT1 and SIRT3 up-regulate Mn-SOD expression through different mechanisms, such as HIF-2a (Dioum et al., 2009) and/or FOXO4 (van der Horst et al., 2004) for SIRT1 and FOXO3a for SIRT3 (Sundaresan et al., 2009). Sundaresan et al. (2008) demonstrated that overexpression of both nuclear and mitochondrial SIRT3 protected cardiomyocytes from genotoxic stress and oxidant stress.

However, sirtuins adopt several other different tools to counterbalance the oxidative stress.

For instance, Alcendor et al. showed that overexpression of either Sirt1 or constitutively active FoxO1a in cultured cardiac myocytes stimulated expression of catalase, suggesting that FoxO1a plays an important role in mediating Sirt1-induced upregulation of catalase, which may in part mediate suppression of myocardial damage caused by oxidative stress (Alcendor et al., 2007).

SIRT3 also increases activity of other ROS-detoxifying enzymes indirectly. SIRT3 deacetylates and activates IDH2 and glutamate dehydrogenase in murine liver (Alcendor et al., 2007; Lombard et al., 2007), both of which produce NADPH in the mitochondria. NADPH in turn is required for glutathione reductase to convert oxidized glutathione to reduced glutathione, which is a crucial cofactor for mitochondrial glutathione peroxidase to scavenge ROS.

Shinmura et al. (2011) demonstrated that treatment of cardiomyocytes with resveratrol, an activator of SIRT1 and SIRT3, decreased ROS production and improved cell survival after hypoxia/reoxygenation without increasing the expression level of MnSOD protein.

Recently, mitochondrial ALdehyde DeHydrogenase 2 (ALDH2) has been identified as a novel target of SIRT3 (Schlicker et al., 2008; Lu et al., 2011). Excessive ROS in stressed hearts triggers lipid peroxidation and accumulation of reactive aldehydes, which in turn impairs mitochondrial function and induces cell damage. ALDH2 removes the aldehydes reducing the toxicity (Chen et al., 2010). Then, SIRT3-mediated ALDH2 activation could be another mechanism that mitigates cardiomyocyte damage induced by ROS, resulting in cardioprotection (Tanno et al., 2012).

β ADRENERGIC SYSTEM AND OXIDATIVE STRESS IN CARDIOVASCULAR SYSTEM

It is well established that β-adrenoceptor (βAR) activation stimulates adenylyl cyclase activity through the participation of G proteins and promotes the formation of cAMP in the myocardium (Stiles et al., 1984; Bristow et al., 1990; Bohm, 1995; Chakraborti et al., 2000). The elevated level of cAMP increases the intracellular concentration of Ca^{2+} in cardiomyocytes on protein kinase A (PKA) mediated phosphorylation of different Ca^{2+}-handling proteins in the membrane and produces the positive inotropic effect in the heart (Stiles et al., 1984; Bristow et al., 1990; Bohm, 1995; Chakraborti et al., 2000). This βAR-mediated signal transduction mechanism not only regulates the contractile activity of the healthy heart, but it is also considered to provide critical support for the maintenance of cardiac function during the development of heart failure (Bristow et al., 1990; Bohm, 1995; Post et al., 1999; Chakraborti et al., 2000; Sethi et al., 2007; Cannavo et al., 2013a).

In failing hearts, elevated sympathetic activity initially compensates for decreased cardiac contractility. βAR-mediated signaling is markedly attenuated in heart failure subjects, owing to the downregulation and desensitization of the receptors and their uncoupling from adenylyl cyclase (Rockman et al., 2002; Di Lisa et al., 2011; Rengo et al., 2012a; Femminella et al., 2013).

Many different mechanisms are implicated in the genesis of heart failure. Effects of high levels of insulin on the cardiovascular function are well studied. In a model of isolated rats papillary muscles, it was demonstrated that insulin-induced modulation of contractility is calcium independent and that insulin leads to a supersensitization on the β1-adrenoceptors (β1-AR) (Ferrara et al., 2005). At the same time, elevated plasma free fat acid levels have a stimulatory effect on sympathetic nervous system, as showed by decreased QTc interval after weight loss (Corbi et al., 2002; Bianco et al., 2013).

One of the pathophysiological mechanisms involved in the genesis of heart failure is represented by a persistent β1-AR stimulation, that evokes a multitude of cardiac toxic effects, including

myocyte apoptosis and hypertrophy, as showed *in vivo* on rodent hearts and *in vitro* on cultured cardiomyocytes (Ferrara et al., 1997; Zheng et al., 2005; Cannavo et al., 2013b).

In particular it has been demonstrated a β1-AR downregulation and desensitization due apparently to overt and sustained stimulation, with largely unaltered β2-adrenoceptors (β2-AR) (Molenaar et al., 2007; Feldman et al., 2008; Rengo et al., 2012b), leading to an increased β2:β1 ratio.

β2-AR play an important role in the regulation of the angiogenic response in HF, as showed by the evidence that β2-AR overexpression was associated with a markedly increased capillary and arteriolar length density and enhanced *in vivo* myocardial blood flow and coronary reserve (Rengo et al., 2012b) and β-blockade promotes cardiac angiogenesis in heart failure via activation of VEGF signaling pathway (Rengo et al., 2013a).

G protein-coupled receptor kinases (GRKs) regulate numerous G Protein-Coupled Receptors (GPCR) by phosphorylating the intracellular domain of the active receptor, resulting in receptor desensitization and internalization. GRKs also regulate GPCR trafficking in a phosphorylation independent manner via direct protein-protein interactions (Evron et al., 2012).

GPCR are seven-transmembrane receptors that transmit a wide range of extracellular stimuli into cells, regulating the majority of biological processes. Upon agonist stimulation, GPCR activate G proteins, which exchange bound GDP for GTP, leading to the dissociation of the G protein into activated Gα and Gβγ subunits. This dissociation promotes downstream signaling through specific effector proteins and second messengers (Pierce et al., 2002; Takeda et al., 2002).

GRK2 is the most ubiquitous member of the GRK family. GRK2 rapidly phosphorylate GPCR upon agonist stimulation and facilitate the binding of arrestins to the phosphorylated receptors, leading to uncoupling of the receptor from the G protein (Pitcher et al., 1998a). This process, known as receptor desensitization, is the loss of receptor responsiveness upon prolonged stimulation. GPCR that are known substrates of GRK2 include the β2-AR, the chemokine receptors CCR2b and CCR5, the Platelet Activating Factor Receptor, and the neurokinin-1 receptor for substance P (Pitcher et al., 1998a; Lombardi et al., 2002).

GRK2 binds PhosphoInositide 3-Kinase (PI3K) and recruits it to the cell surface upon ligand stimulation of the βAR (Naga Prasad et al., 2001). This interaction has been shown to be important for βAR endocytosis, most likely via enhanced recruitment of AP2 to the receptor (Naga Prasad et al., 2002; Salazar et al., 2013).

Blocking the interaction of GRK2 with PI3K improves contractile function during heart failure by reversing βAR desensitization abnormalities and restoring βAR signaling (Perrino et al., 2005; Evron et al., 2012; Rengo et al., 2012c).

Interestingly, changes in GRK2 levels have been reported in a number of disease states. In human GRK2 levels are increased in myocardial tissue during heart failure (Ungerer et al., 1994; Rengo et al., 2013a,b,c), myocardial infarction (Santulli et al., 2011) and hypertension (Gros et al., 1997; Santulli et al., 2013).

Production of ROS has been detected in several cells stimulated with cytokines, peptide growth factors, and agonists of GPCRs (Thannickal and Fanburg, 2000). During inflammatory processes, lymphocytes are exposed to H_2O_2 and other ROS that are derived from activated macrophages and neutrophils as a first line of defense against invading pathogens. Further downstream, ROS regulate transcription factors, including NF-kB (Schreck et al., 1991).

Some authors showed that exposure of lymphocytes to oxidative stress results in a decrease in cellular GRK2 protein levels. ROS produced by activated macrophages and neutrophils can alter the activity of lymphocytes. Exposure of lymphocytes to ROS results in increased intracellular calcium level, rapid tyrosine phosphorylation of a variety of proteins (Schieven et al., 1993), and activation of transcription factors such as NF-kB (Schreck et al., 1991; Lombardi et al., 2002) (**Figure 1**).

Oxidative stress activates several other kinase signaling pathways, such as Protein Kinase C (PKC), MAPK, and PI3K. Activated PKC can phosphorylate GRK2, with increased kinase activity (Chuang et al., 1995). Interestingly, inhibition of PKC does not affect basal GRK2 levels nor does it interfere with the H_2O_2-induced decrease in cellular GRK2. In addition, specific inhibitors of MAPK or PI3K do not have any effect on H_2O_2-induced decreases in GRK2 protein (Lombardi et al., 2002).

Previous reports showed that GRK2 is the predominant GPCR kinase involved in agonist-induced receptor sequestration of the β2-AR. Moreover, studies in transfected cell systems suggest that changes in the intracellular level of GRK2 alter the rate and extent of sequestration of the β2-AR (Ferguson et al., 1996; Penela et al., 1998; Lombardi et al., 2002).

A growing body of evidence has shown that GRK2 is capable of phosphorylating non-receptor substrates. GRK2 is a microtubule-associated kinase that directly phosphorylates tubulin following βAR stimulation (Pitcher et al., 1998b; Yoshida et al., 2003), suggesting a functional link between GRK2 and the cytoskeleton. Accordingly, GRK2 levels can affect agonist-induced βAR internalization in a mechanism involving microtubule stability (Vroon et al., 2007). GRK2-mediated phosphorylation of the membrane-cytoskeleton linkers, radixin (Kahsai et al., 2010) and ezrin (Cant and Pitcher, 2005), provides another indication for this functional link to the cytoskeleton.

Another important target of GRK2 kinase activity is the Insulin Receptor Substrate 1 (IRIS).

It has been reported that increased GRK2 levels mediate insulin resistance in myoblasts and adipocytes via a mechanism which involves sequestration of Gαq and IRIS (Usui et al., 2005; Garcia-Guerra et al., 2010). Interestingly, GRK2 directly phosphorylates IRIS in cardiomyocytes, a process that negatively affects cardiac glucose uptake and insulin sensitivity following ischemic injury and ultimately leads to the development of heart failure (Ciccarelli et al., 2011; Evron et al., 2012).

In fact, GRK2, also known as βAR kinase 1 (βARK1), provides a link between altered vascular/tissue physiology in insulin resistance and impaired IRIS signaling. GRK2 can interfere directly with Gαq/11-mediated signaling via its regulator of G protein signaling domain/GAP activity (Usui et al., 2004). Increased plasma concentration of the vasoconstrictive ET-1 polypeptide is associated with insulin resistance and/or hypertension (Kohno et al., 1990), which is, in turn, promoted by direct and indirect (sympathoadrenal and angiotensin II dependent) effects of compensatory hyperinsulinaemia to cause sodium retention (Yatabe

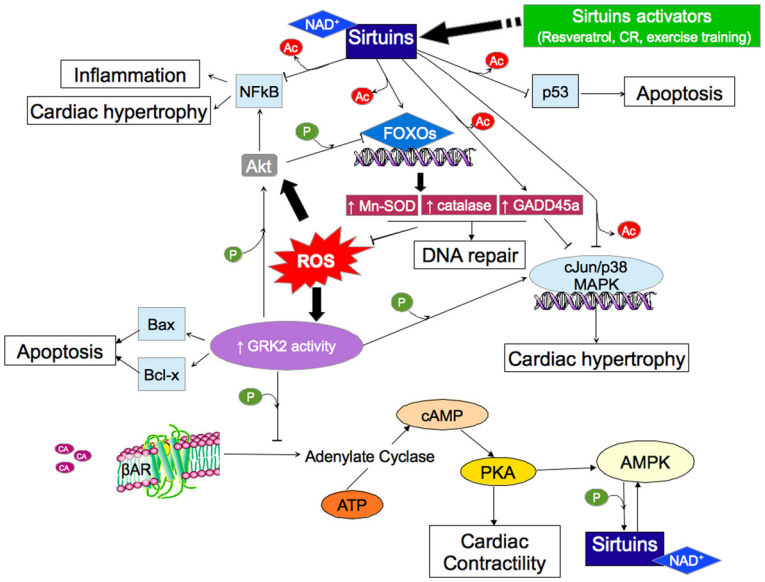

FIGURE 1 | Cellular response to oxidative stress mediating by β-adrenergic response and sirtuins involvement. The ROS induce GRK2 hyperactivity that determines desensitization and internalization of βARs with induction of cardiac hypertrophy (via AKT/NFkB pathway), apoptosis and senescence (by inhibition of FOXOs). Sirtuins (and their activators) are able to counteract these actions by direct effects on different molecules. ROS, reactive oxygen species; CR, caloric restriction; Ac, acetyl; P, phosphoryl. ↓ activation; ⊥ inhibition.

et al., 2010). The correlation between excessive β-adrenergic activity and insulin resistance has long been noted (Deibert and DeFronzo, 1980). While tissue GRK2 levels have been correlated with plasma norepinephrine/epinephrine levels (Cho et al., 1999), GRK2 can be upregulated in cultured cells by chronic insulin (Garcia-Guerra et al., 2010), potentially as a result of PI3K-dependent stabilization of GRK2 (Salcedo et al., 2006). Thus, both local/circulating GPCR ligands associated with insulin resistance/hyperinsulinaemia, and insulin itself, contribute to the high GRK2 levels observed in insulin-resistant rodent/human tissues (Garcia-Guerra et al., 2010; Copps and White, 2012).

These findings demonstrate that lowering GRK2 in myocytes after ischemic injury will contribute to restore cardiac metabolism and prevent the development of subsequent heart failure (Evron et al., 2012).

Moreover, during heart failure GRK2 is up-regulated in the adrenal medulla, causing α2-adrenoceptor dysfunction and catecholamine hypersecretion. By decreasing GRK2 levels in the adrenal gland, β-blocker treatment appears to restore adrenal α2-AR density and signaling at the plasma membrane and catecholamine feedback inhibition, reducing sympathetic overdrive in chronic heart failure (Rengo et al., 2012c).

Thus, the favorable effects of GRK2 inhibition in cardiac disease can be ascribed not only to the direct improvement of adrenergic response but also to more complex interactions among different and specific systems involved in the pathophysiological response to myocardial injury (Rengo et al., 2012a).

Also it is well known that oxidative stress represents an underlying mechanism involved in insulin resistance development. The evidence that reactive nitrogen and oxygen species generation occurs when endothelial cells respond to high glucose (Garcia Soriano et al., 2001) suggests another link between oxidative stress and β-adrenergic activity in the involvement of many cardiovascular conditions.

ROS may change the functioning of GPCRs during disease processes via the calpain-dependent regulation of cellular GRK2 levels (Lombardi et al., 2002).

Moreover, *in vitro* studies have revealed several non-classical signaling molecules utilized by β2-AR, including β-arrestin 1 (Drake et al., 2008; Gong et al., 2008; Tilley et al., 2009), p38MAPK (Gong et al., 2008; McAlees and Sanders, 2009) and ROS (Yin et al., 2006; Gong et al., 2008). Transgenic activation of β2-AR in cardiomyocytes leads to a sustained elevation of NADPH oxidase activity, which is accompanied by a greater ROS production as well as phosphorylation of p38MAPK. Inhibition of NADPH oxidase or ROS significantly reduced the p38MAPK signaling cascade. Chronic β2-AR activation *in vivo* is associated with greater extent of cardiac dilatation and dysfunction as well as augmented pro-inflammatory and profibrotic signaling, while antioxidant treatment protected hearts against these abnormalities, indicating ROS production to be central to the detrimental signaling of β2-AR. These findings highlight that the coupling of β2-AR with NADPH oxidase derived ROS/p38 MAPK is pivotal to the adverse signaling mechanism, and thus forms a potential therapeutic target (Xu et al., 2011).

More recently Chen et al. (2013) have been demonstrated that GRK2 localizes to heart mitochondria and it was an absolute requirement for prodeath signaling after oxidative and ischemic stress. Specifically, mitochondrial targeting of GRK2 in myocytes after ischemic injury promotes prodeath signaling because mitochondrial accumulation of GRK2 in myocytes increases after oxidative stress and it is dependent on ERK-mediated

phosphorylation of GRK2, with subsequent movement to mitochondria dependent on binding of phosphorylated GRK2 to Hsp90. Then the authors suggested that blocking this mechanism led to cardioprotection.

β-ADRENERGIC SYSTEM, OXIDATIVE STRESS AND SIRTUINS

It has been demonstrated that sirtuins, NAD^+/NADH deacetylases, are involved in modulating the cellular stress response directly by deacetylation of some factors that are also implicated in endothelial function control (Tang et al., 2012; Conti et al., 2013).

Sirt1 extends the lifespan of many organisms by increasing cellular stress resistance (Brunet et al., 2004; Alcendor et al., 2007), by an increase insulin sensitivity, a decrease circulating free fatty acids and insulin-like growth factor (IGF-1), an increased activity of the energy-sensing enzyme, AMP-activated Protein Kinase (AMPK), increased activity of Peroxisome proliferator activated receptor-gamma coactivator-alpha (PGC-1a), and increased mitochondrial number (Opie and Lecour, 2007). The requirement of NAD^+ for Sirt1 activity implies that Sirt1 effectiveness depends on the cellular metabolic state (Conti et al., 2012a). Moreover, Sirt1 acts by involving signaling molecules such phosphatidyl-inositol-3-phosphate-kinase (PI3K)-Akt, MAPK (Bezstarosti et al., 2006) and p38-MAPK-β (Das et al., 2006) (**Figure 1**).

SIRT1 has been demonstrated to be localized predominantly in the nucleus or cytoplasm depending on the cell type. SIRT1 shuttles between the two cellular compartments in response to cellular stress in C2C12 cells and cardiomyocytes (Tanno et al., 2010), and during differentiation in neural precursor cells (Hisahara et al., 2008).

The nucleo-cytoplasmic shuttling is regulated by nuclear localization signals and nuclear export signals in the aminoacid sequences of SIRT1. PI3K/Akt- and JNK1- mediated phosphorylation of SIRT1 induces its nuclear translocation (Tanno et al., 2007; Nasrin et al., 2009). Nuclear localization of SIRT1 seems to be necessary for its protective function in cardiomyocytes (Tanno et al., 2007, 2010) whereas the biological significance of cytoplasmic SIRT1 remains to be determined. It has been demonstrated that resveratrol, a SIRT1 activator, improves insulin sensitivity in diet-induced obesity in mice (Baur et al., 2006; Lagouge et al., 2006). Sun et al. (2007) found that SIRT1 repressed protein phosphatase 1B (PTP1B) and thereby increased the level of insulin receptor phosphorylation, improving insulin sensitivity both in C2C12 myotubes and in high fat-fed mice.

Recently it has been demonstrated that βAR stimulation antagonizes the protective effect of the Akt pathway that is mediated by both insulin and hypoxia preconditioning, through inhibiting their induction of Hif-1α and Sirt1 gene, which are key elements in cell survival (Rane et al., 2010).

Akt overexpression in mice suppressed autophagy, which was associated with cardiac hypertrophy, interstitial fibrosis and contractile dysfunction (Hua et al., 2011). SIRT1 regulates autophagy by interacting with and deacetylating autophagy-related proteins Atg5, Atg7, and Atg8 (Lee et al., 2008). Recently, Hariharan et al. (2010) demonstrated that SIRT1 was required for starvation-induced autophagy in cardiomyocytes, in which SIRT1-mediated deacetylation of FOXO1 and subsequent activation of Rab7 plays a role.

Furthermore, FOXO1 was indispensable for maintenance of cardiac function after starvation, suggesting that autophagy induced by activation of the SIRT1-FOXO1 axis is an important adaptive mechanism in the failing heart (Tanno et al., 2012). Moreover, recently it has been demonstrated that reduced SERCA2a protein level, ventricular dysfunction, ventricular dilatation and mortality in a mouse model of type-1 diabetes were nearly normalized by treatment with resveratrol in a SIRT1-dependent manner (Sulaiman et al., 2010; Tanno et al., 2012).

The presence of high levels of norepinephrine has been considered as a pathological marker of heart failure (Tavares et al., 2008). Another demonstration of the relationship between adrenergic system and sirtuins is represented by the evidence that resveratrol prevents norepinephrine induced hypertrophy in adult rat cardiomyocytes, by activating NO-AMPK pathway (Thandapilly et al., 2011). Thandapilly et al. (2011) proposed that norepinephrine binds with the β-adrenergic receptor on the cardiac cell membrane, the sarcolemma, and activates phospholipase C resulting in the formation of 1,2-diacylglycerol (DAG) and inositol triphosphate (IP3). In turn, DAG stimulates cytosolic protein kinase activity resulting in increased protein synthesis leading to the development of cardiac hypertrophy (Eskildsen-Helmond et al., 1997).

In addition, resveratrol restored sirtuin activity, and thereby improve cardiac function in rats with diabetic cardiomyopathy (Sulaiman et al., 2010). Breen et al. (2008) studied the interaction between AMPK and sirtuin in resveratrol mediated signaling in skeletal muscle cells. In this study increased skeletal muscle glucose uptake was observed upon resveratrol treatment which was mediated by the sirtuin-AMPK dependent pathway (Breen et al., 2008). Moreover, it has been also demonstrated that resveratrol prevented cardiomyocyte hypertrophy by restoring the impaired AMPK activity in phenylephrine exposed cardiomyocytes as well as in SHR rats (Chan et al., 2008; Dolinsky et al., 2009) suggesting an important role for AMPK in mediating resveratrol effects.

Some authors (Dolinsky et al., 2009; Thandapilly et al., 2010) have recently reported that resveratrol prevented the development of pathological cardiac hypertrophy in genetically hypertensive rats without any effect on blood pressure, which is considered a pathological stimulus for the development of hypertrophy (Thandapilly et al., 2010, 2011).

The antioxidant activities of sirtuins are well known. SIRT3 blocks the cardiac hypertrophic response through activation of Foxo-dependent antioxidants, MnSOD and catalase, as well as suppressing ROS-mediated Ras activation and the downstream MAPK/ERK and PI3K/Akt signaling pathways (Sundaresan et al., 2009) (**Figure 1**). In particular, SIRT1 and SIRT3 appear to share similar ROS-accumulating end-point targets that cause cardiac hypertrophy. All of these findings support the hypothesis that use and development of sirtuin-specific activators and inhibitors may help further dissect the collaborative functions of SIRT1 and SIRT3 in the heart.

Less is known about the physiological role of SIRT7 in the heart. SIRT7 is a nuclear protein that associates with rDNA and

interacts with RNA (Ford et al., 2006). It is not clear whether SIRT7 exhibits NAD^+-dependent deacetylase activity, but reports suggest that it does respond to metabolic conditions by stimulating ribosomal biogenesis in dividing cells (Michishita et al., 2005) and it regulates heart cell death and damage by inhibiting p53, Ras, and Akt signaling pathways (Vakhrusheva et al., 2008). In fact, SIRT7-deficient mice develop heart hypertrophy and inflammatory cardiomyopathy, which is characterized by extensive fibrosis (Vakhrusheva et al., 2008). However, the molecular details explaining how SIRT7 targets these pathways remains unclear (Schug and Li, 2010).

Recently it has been proposed that β-adrenergic activation of the cAMP/PKA pathway rapidly increases SIRT1 activity in a NAD^+ independent fashion. This mechanism enables SIRT1 to respond swiftly to the changing metabolic needs of the organism in settings of environmental stress. Cantó and Auwerx suggested that SIRT1 acts as a metabolic effector, synchronizing metabolic pathways with nutrient availability (Cantó and Auwerx, 2012). The molecular mechanism by which NAD^+ regulates SIRT1 catalytic activity, however, is still not fully understood. In a low energy state, SIRT1 deacetylates and increases the activity of PGC-1a, leading to transcriptional upregulation of genes involved in lipid catabolism and mitochondrial biogenesis (Rodgers et al., 2005; Lagouge et al., 2006; Gerhart-Hines et al., 2007). Current understanding of the regulation of this process has emphasized a role for AMPK signaling in controlling the abundance of the SIRT1 substrate NAD^+. The elevated AMP/ATP ratio during energy deficiency triggers phosphorylation of PGC-1a by AMPK, which primes PGC-1a for SIRT1-dependent deacetylation (and activation) (Cantó et al., 2009).

AMPK also increases the concentration of intracellular NAD^+, further fueling SIRT1 deacetylase activity. However, both of these processes occur over the course of several hours, too long to permit rapid response to acute changes in energy stress.

SIRT1 activity has been reported previously to be regulated by post-translational modifications, such as phosphorylation by JNK. But the fact that the residues targeted by this pathway do not reside in the catalytic domain and are not conserved evolutionarily indicates this is an unlikely mechanism to regulate the well-conserved metabolic functions of SIRT1 (Cantó and Auwerx, 2012). Given the evidence supporting a function for SIRT1 in bioenergetics stress, Gerhart-Hines et al. (2011) hypothesized that stress-induced β-adrenergic signaling might regulate SIRT1 activity. Activation of the βAR increases intracellular cAMP concentration and activates PKA and its downstream effectors. The authors demonstrated that each component of the βAR-cAMP-PKA axis is essential to SIRT1 deacetylation of PGC-1a. In U2OS cells, treatment with β-adrenergic agonists (epinephrine and clenbuterol) or cAMP mimetics (forskolin and 8-BrcAMP) led to potent dose-dependent deacetylation of PGC-1a within 30 min. Forskolin-induced PGC-1a deacetylation was dependent on both PKA and SIRT1, but this effect was abolished by genetic deletion of SIRT1 in mouse embryonic fibroblasts. Furthermore, reduction of SIRT1 expression prevented forskolin-mediated upregulation of the PGC-1a target genes ERRa and PDK4 (Gerhart-Hines et al., 2011).

The rapidity with which SIRT1 transduced cAMP/PKA signals suggested that SIRT1 might be a direct target for PKA phosphorylation. Using mass spectrometry, the authors identified a residue on SIRT1 in the catalytic domain that was uniquely phosphorylated in response to forskolin treatment. They showed that Serine 434 (S434) phosphorylation was dependent on cAMP/PKA signaling and was rapidly reversed by removal of cAMP mimetic (forskolin). Furthermore, the authors showed that S434 phosphorylation was essential for the forskolin-induced increase in intrinsic SIRT1 enzymatic activity (Gerhart-Hines et al., 2011). Importantly, in all experiments, total NAD^+ content was unchanged by cAMP/PKA signaling, indicating that cAMP-mediated deacetylation of PGC-1a was independent of NAD^+ regulation of SIRT1. Gerhart-Hines et al. depicts SIRT1 as a dynamic orchestrator of both acute stress (βAR/cAMP signaling) and sustained energy crisis (AMPK-mediated changes in PGC-1a phosphorylation and NAD^+ concentration) (Gerhart-Hines et al., 2007, 2011; Chao and Tontonoz, 2012).

CONCLUSIONS

Oxidative stress represents the *primum movens* of several chronic degenerative diseases, especially of the cardiovascular system (Ferrara et al., 2006; Conti et al., 2012b). In the last decades several studies have demonstrated as the β-adrenergic system represents the target of the oxidative damage and, in turn, the responsible of oxidants production. The sirtuins, a new class of histone-deacetylases, seem to be the best defense of the cell to counterbalance the oxidative stress through the action on different pathways. Most part of the research on these molecules in the last years has been focused on the sirtuins activators, showing as the caloric restriction, the resveratrol and in particular the exercise training are able to mediate their beneficial effects by induction of sirtuins activity.

More recently, the use of a SIRT1 activator SRT2104 on cardiovascular function provided positive effects on lipid profiles, but were unable to demonstrate beneficial effects on vascular, endothelial, or platelet function compared with placebo (Venkatasubramanian et al., 2013).

Therefore, as suggested by Merksamer et al. (2013), for the future it will be important to develop experimental models in which the levels of oxidative stress and the activities of sirtuins can be precisely modulated to determine if sirtuins have a causative role in lifespan extension.

Moreover, as discussed above and showed in **Figure 1**, whereas the mechanisms involved in the cellular response to oxidative stress are represented by the same actors, very few studies have been performed to link the β-adrenergic system and sirtuins activity, and most of them are only focused on the metabolic pathway.

Therefore, more studies are needed to better clarify the involvement of sirtuins in the β-adrenergic response and, overall, to better define the mechanisms by which tools such as exercise training are able to counteract the oxidative stress, by both activation of sirtuins (Ferrara et al., 2008) and inhibition of GRK2 (Rengo et al., 2010) in many cardiovascular conditions.

The activation or overexpression of sirtuins leads to measurable increases in health and resistance to different stress, making

them an appealing target for the development of interventions to promote improvements in health. However, more research is needed before we can effectively target sirtuins for therapeutic purposes. So, currently, although sirtuins represent promising therapeutic targets, their role in the regulation of mammalian lifespan remains an open question (Accili et al., 2011). Then, the future perspective could be represented by studies performed to identify the efficacy of sirtuin activators in the prevention and/or treatment of cardiovascular diseases such as heart failure.

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Adrenoreceptors and nitric oxide in the cardiovascular system

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Nitric Oxide (NO) is a small molecule that continues to attract much attention from the scientific community. Since its discovery, it has been evident that NO has a crucial role in the modulation of vascular tone. Moreover, NO is involved in multiple signal transduction pathways thus contributing to the regulation of many cellular functions. NO effects can be either dependent or independent on cGMP, and rely also upon several mechanisms such as the amount of NO, the compartmentalization of the enzymes responsible for its biosynthesis (NOS), and the local redox conditions. Several evidences highlighted the correlation among adrenoreceptors activity, vascular redox status and NO bioavailability. It was suggested a possible crosstalk between NO and oxidative stress hallmarks in the endothelium function and adaptation, and in sympathetic vasoconstriction control. Adrenergic vasoconstriction is a balance between a direct vasoconstrictive effect on smooth muscle and an indirect vasorelaxant action caused by α_2- and β-adrenergic endothelial receptor-triggered NO release. An increased oxidative stress and a reduction of NO bioavailability shifts this equilibrium causing the enhanced vascular adrenergic responsiveness observed in hypertension. The activity of NOS contributes to manage the adrenergic pathway, thus supporting the idea that the endothelium might control or facilitate β-adrenergic effects on the vessels and the polymorphic variants in β_2-receptors and NOS isoforms could influence aging, some pathological conditions and individual responses to drugs. This seems to be dependent, almost in part, on differences in the control of vascular tone exerted by NO. Given its involvement in such important mechanisms, the NO pathway is implicated in aging process and in both cardiovascular and non-cardiovascular conditions. Thus, it is essential to pinpoint NO involvement in the regulation of vascular tone for the effective clinical/therapeutic management of cardiovascular diseases (CVD).

Keywords: adrenoreceptors, endothelium, nebivolol, nitric oxide, vascular tone

INTRODUCTION

Nitric Oxide (NO) is a small gaseous molecule recognized as a ubiquitous intercellular messenger modulating crucial functions including blood flow, platelet aggregation, and neural activity (Moncada, 1994).

This molecule is synthesized from L-arginine by three isoforms of Nitric Oxide Synthases (NOSs) and all of them (nNOS, Inos, and eNOS) concur to regulate the autonomic nervous system.

NO exerts its activity essentially by stimulating soluble Guanylyl Cyclase (GC) to increase the levels of the second messenger cGMP, which in turn modulates the performance of adrenergic receptors (ARs).

Recently, many studies highlighted an important role in the regulation of the vasomotor tone of the β-adrenoreceptor subtype β_3, which, differently from the classical β_1- and β_2-ARs, induces a negative inotropism in the human heart (Balligand, 1999; Salazar et al., 2013).

NO effects depend, among others, on NO concentration, compartmentalization of NOS enzymes and local redox conditions of cells and tissues and, to date, many evidences collected by both *in vivo* and *in vitro* experiments suggest a crosstalk between NO, ARs and oxidative stress in the control of endothelium homeostasis, and in the sympathetic regulation of the vascular tone (Graves and Poston, 1993; Lembo et al., 2000; Selemidis et al., 2007).

The NO pathway is directly implicated in the development and progression of diseases such as hypertension and heart failure (HF) and, recently, this molecule has been considered a promising target to develop new clinical strategies against cardiovascular pathologies (Levy et al., 2009).

In addition, it is worth noting that some studies showed that polymorphisms in genes encoding for ARs and NOS enzymes

could influence aging, onset and progression of cardiovascular diseases (CVD), and response to therapy (Jáchymová et al., 2001; Garovic et al., 2003).

The main focus of this review is the mechanisms underlying the interconnection between β-ARs and NO in the cardiovascular system, and the therapeutic potential of new discoveries in this field.

NO MODULATES VASOMOTOR TONE BY INTERFERING WITH SYMPATHETIC AUTONOMIC NERVOUS SYSTEM

In 1980s the Endothelium-Derived Relaxing Factor (EDRF), discovered by Moncada, was identified as NO (Hutchinson et al., 1987; Palmer et al., 1987) and, from that moment, several studies shed light on a countless number of important roles played by this molecule which was proclaimed Science's "Molecule of the Year 1992" (Nathan, 1992, 1995; Bredt and Snyder, 1994).

Since its discovery, it was clear that NO acts as a key modulator of the vascular tone and that its vascular effects are generally mediated by Guanosine 3′,5′-cyclic MonoPhosphate (cGMP) through the activation of guanylate cyclase. In fact, several experiments using NO donors and/or cGMP analogs have shown that cGMP is a critical and multifunctional second messenger that mediates several functions in cardiac and vascular tissues as well as the etiology and pathophysiology of cardiovascular disorders (Tulis, 2008). Both neurotransmitters and hormones released from autonomic nervous system cooperate to preserve the balance between vasoconstriction and vasorelaxation and to control cardiac muscle cells function, and it is now generally accepted that NO exerts a critical role in this context. Balligand et al., which investigated the effects of NOS inhibitors in isolated neonatal and adult rat ventricular myocytes, exposed to either muscarinic or adrenergic agonists, concluded that the physiological response of the cells to both muscarinic cholinergic and β-adrenergic stimulation is mediated, at least in part, by NO production (Balligand et al., 1993).

Cardiovascular homeostasis is regulated by NO produced by all three NOS isoforms. Several studies demonstrated, both *in vivo* (Schwarz et al., 1995) and *in vitro* (Horackova et al., 1995), that NO produced by neuronal NOS (nNOS) controls catecholamines release in response to electrical adrenergic nerve stimulation. This is very important also in consideration that elevated levels of cathecolamines are associated to several pathologic conditions such as HF (Rengo et al., 2012a).

The inducible NO Synthase (iNOS) has been also involved in several aspects of cardiovascular biology such as the defence against intracellular microorganisms (Balligand and Cannon, 1997).

Moreover, endothelial cells express, in heart and vessels of a variety of species including humans, endothelial NO Synthase (eNOS), an isoform that is activated to produce NO in response to stimulation of both adrenergic and muscarinic cholinergic receptors in cardiac myocytes (Balligand et al., 1995).

Many studies demonstrated that vascular endothelial cells might also express β-adrenoceptors (Buxton et al., 1987; Molenaar et al., 1988), thus supporting the idea that the endothelium might control or facilitate β-adrenergic effects on the vessels. The main mechanism leading to increased eNOS activity in endothelial cells is calcium-dependent (Wu, 2002), but phosphorylation at several loci of the NOS proteins has been recognized as an additional pathway to induce both activation and inhibition of eNOS activity (Bauer et al., 2003; Fleming and Busse, 2003).

Both *in vivo* and *in vitro* studies suggested that the vascular endothelium might mediate β-adrenergic vasorelaxation, though not all the results presented are in agreement with each other. For instance, it was observed that rat mesenteric resistance arteries can be relaxed by NO release upon β_1-adrenoreceptor stimulation (Graves and Poston, 1993). Priest et al. showed an involvement of NO in β-mediated vasorelaxation in large but not in small rat arteries suggesting a role of NO strictly dependent on the vascular area (Priest et al., 1997).

Ferro et al. verified that the stimulation of β_2-adrenoreceptors led to an increase in NO, which in turn caused relaxation of Human Umbilical Vein Endothelial Cells (HUVEC). In this study, the authors provided also a comparison between β-adrenoreceptor function measured in HUVEC and the response to β-adrenergic stimuli in intact vessels, showing the importance of endothelium in maintaining vascular homeostasis (Ferro et al., 1999). In addition, Lembo et al. suggested the existence of an endothelium NO component essential for the insulin modulation of α_2- and β-adrenergic vascular responses. An impairment of the equilibrium between endothelial and vascular smooth muscle adrenergic signaling could contribute to the increase of vascular resistance, a pivotal phenotypical trait of essential hypertension (Lembo et al., 1997).

ROLE OF β_3-ADRENOCEPTOR

Emerging evidences highlighted a role played by a third β-adrenoreceptor subtype (β_3), traditionally known as a modulator of lipolysis in adipose tissue, as a regulator of the vasomotor tone in conjunction with β_1- and β_2-ARs (Trochu et al., 1999).

The involvement of a β-receptor, other than classical β_1- and β_2-ARs, has been suggested in several experiments which used a different concentration of non-selective β-blockers (Clark and Bertholet, 1983; Doggrell, 1990; Oriowo, 1995) and preferential β_3-AR agonists (Berlan et al., 1995).

It is now widely accepted that the vasoactive effects dependent on the stimulation of β-ARs are strongly associated with NO production and activation (Trochu et al., 1999).

Recently, many investigations focused on β_3-ARs, which are detected in human endothelial cells and cardiac myocytes of the human heart. These receptors subtypes are highly expressed in the atrium and, in contrast to β_1- and β_2-ARs, are responsible of a negative cardiac inotropic effect (Moniotte et al., 2001). Moreover, on the basis of differential expression of β_3-ARs in the human myocardium chambers, Brixius et al. found that eNOS is activated by β_3-AR predominantly via phosphorylation in the left ventricle and through a translocation process in the atrium (Brixius et al., 2004).

The link between β_3 AR, eNOS and vasodilation mechanism was demonstrated also in *in vivo* models; recent advances in the field were achieved by using nebivolol, a β_1-blocker. Dessy et al. verified that nebivolol dilates human

and rodent coronary resistance microarteries, and showed that this effect is sensitive to NOS inhibition and is hampered in β_3-adrenoreceptor-deficient mice. Moreover, the authors showed proangiogenic properties of nebivolol, which are dependent by both eNOS and β_3-adrenoreceptors (Dessy et al., 2005).

Interestingly, β_3-AR is upregulated during cardiomyophaties in humans and this characteristic, together with its peculiar differential expression in the human myocardium, makes it an attractive target for the development of new clinical strategies against CVD (Moniotte et al., 2001).

Many studies investigated the involvement of β_3-AR in the onset and progression of cardiovascular clinical conditions both in animal and human models, and it seems conceivable that the stimulation of β_3-AR leads to NO-mediated protective effects in vascular beds (Dessy et al., 2004, 2005). In addition, in case of neurohormonal stress, β_3-ARs expressed in the endothelium promote coronary perfusion through their vasodilator and proangiogenic effects (Balligand, 2009).

Recently, a randomized trial, named SENIORS (Study of the Effects of Nebivolol Intervention on Outcomes and Rehospitalization in Seniors with HF) was performed to investigate the effects of nebivolol on the ejection fraction in aged patients ($\geq$70 years).

Notably, nebivolol possesses vasodilator ancillary properties, probably mediated by the endothelial L-arginine NO pathway. In particular, it was demonstrated that the favorable hemodynamic profile of nebivolol, including the lowering of blood pressure, is partially due to NO release from endothelial cells (Maffei et al., 2006). In addition, thanks to its antioxidant activity, nebivolol prevents the detrimental effect on NO bioavailability associated to oxidative stress (Ignarro, 2004).

To date, preclinical and clinical data confirm that NO benefits might due to β_3-ARs overexpression. However, caution should be used, as the long term effects of β_3-ARs agonists on left ventricular function in the heart have not yet been fully evaluated.

Furthermore, many studies investigated the impact of lifestyle changes and non-pharmacological interventions on the cardiovascular homeostasis, and convincing evidences showed a favorable role exerted by diet and caloric restriction. For example, Nisoli et al. demonstrated that caloric restriction leads to enhanced mitochondrial biogenesis, at least in part, by inducing the activation of eNOS (Nisoli et al., 2005), and Cerqueira et al. observed a time-dependent increase of eNOS activation and NO bioavailability in vascular cells conditioned with serum of caloric restricted rats (Cerqueira et al., 2012).

Also exercise training (ET), currently used in cardiac rehabilitation (CR) programs, was recognized to improve some cardiovascular outcomes by inducing NO levels to increase, even if this strongly depends on both the type and intensity of exercise (Conti et al., 2012a, 2013).

Jenkins et al. suggested that a regular physical activity in combination with dietary restriction positively influences, in a NO-dependent manner, the expression of β-AR and natriuretic peptide receptors in adipose tissues of obese rats (Jenkins et al., 2013). In addition, Calvert et al. showed that an ET-dependent stimulation of β_3-ARs is useful to counteract myocardial ischemia-reperfusion injury by increasing NO signaling (Calvert et al., 2011).

These and many other studies remarked a crucial role played by NO in cardiovascular homeostasis.

ADRENORECEPTORS STIMULATION, VASCULAR REDOX STATE AND NITRIC OXIDE BIOAVAILABILITY

Experimental *in vivo* and *in vitro* evidences suggest a crosstalk between NO, adrenoreceptors and oxidative stress in the function and adaptation of endothelium, and in the sympathetic control of the vascular tone.

Excess of ROS and/or failure of antioxidant endogenous defense may result in ROS-mediated reduction of NO bioavailability in the cells. The influence of oxidative stress on the pathway of NO biosynthesis has been extensively investigated and its effects, due to either direct quenching or impaired synthesis of NO, strongly affect the vasodilation mechanism (Förstermann and Sessa, 2012).

It has been showed that β_2-ARs excitation increases cellular uptake of L-arginine, an eNOS substrate, and eNOS activity can be specifically stimulated by these AR subtypes in human endothelial cells. Moreover, β_2-ARs stimulation hyperpolarizes cellular membrane, and L-NAME, a well known NOS inhibitor, may hinder this process (Wyatt et al., 2002; Queen et al., 2006).

In vitro experiments using endothelial cells showed an increase of β_2-ARs-mediated eNOS phosphorylation at serine-1177, highlighting a β_2-AR-dependent NOS activation through a Ca^{2+} insensitive mechanism (Dimmeler et al., 1999; Queen et al., 2006; Cannavo et al., 2013a,b).

Recently, Davel et al. investigated the role of oxidative stress in sympathetic-dependent contractility of human endothelium. The authors observed an increase of contractile response in β_2-knockout (KO) mice and showed that the loss of function of these receptors in vascular tissue can induce ROS-mediated NO impairment. Administration of α1-agonist phenylephrine in the aorta of β_2-KO mice suggested a key role played by NO in the control of vasomotor tone. In fact, the reduction of basal content of NO enhanced the vasoconstriction rate in the aorta of mice deficient for β_2-adrenoreceptors. These experiments suggested that β_2-ARs in vascular tissues are necessary to maintain basal levels of NO, thus concurring to modulate vascular homeostasis (Davel et al., 2012). The lack of functional β_2-receptors led to an increase of oxidative stress in the aorta of β_2-KO mice, and a treatment with antioxidant superoxide dismutase was sufficient to limit the vasoconstrictor response to phenylephrine. These results suggest the existence of an important link between adrenergic pathway, NO bioavailability and oxidative stress.

Indeed, several studies have showed antioxidant properties of NO, confirming the role of this molecule to counteract superoxide anions production by NADPH oxidase, the major source of superoxide in blood vessels. Several stimuli, such as oscillatory shear stress, hyperglycemia and lipid peroxidation could cause impairment in the NADPH oxidase system that, in turn, produces accumulation of ROS and reduction of NO content (Vecchione et al., 2006).

Selemidis et al. showed that prolonged exposure of human endothelial cells to NO donors, such as long-acting nitrates,

induced a significant decrease of ROS via inhibition of p47phox NADPH oxidase subunit (Selemidis et al., 2007). By reducing the oxidative stress, NO donors may exert several vascular protective effects and they could be used not only for a symptomatic treatment, but also to prevent and eventually revert many aspects of CVD.

NO antioxidant properties and the ability of NO donors to counteract NADPH oxidase-dependent superoxide production are well established. However, ROS other than superoxide anions could play a role in determining redox state imbalance and the resulting detrimental effects on NO biosynthesis. Indeed, cardiovascular side effects of drugs, such as acetylsalicylic acid and other ciclooxygenase inhibitors may be due to their influence on oxidative stress hallmarks in coronary circulation, and coronary perfusion. In this context, it was suggested that those effects can be modulated by inhibition of NOS following the increase of superoxide, hydrogen peroxide and lipid peroxidation (Barudzic et al., 2013).

Further studies in the field of the antioxidant effect of adrenergic-dependent NO modulation could be essential to develop new drugs and clinical strategies to modulate oxidative stress in vascular diseases.

NITRIC OXIDE AND CARDIOVASCULAR DISEASES

Given its crucial role in the autonomic nervous system control, the NO pathway is directly implicated in diseases, such as hypertension and HF.

The generation of NO in the vascular endothelium ensures the maintenance of the vasodilator tone that is required for the regulation of blood flow and pressure.

Moreover, NO bioavailability plays an important role in the pathophysiology of CVD and its reduction in endothelial cells is strictly associated to endothelial dysfunction and hypertension (Lyons, 1997; Yetik-Anacak and Catravas, 2006).

The link between endothelial dysfunction and vascular diseases is well established (Rengo et al., 2013a). It is known, for instance, that impairment of endothelial function precedes atherosclerosis (Brush et al., 1992).

Stimulation of endothelial β-adrenoreceptors improves eNOS-derived NO production. The importance of such strong molecular interconnection has been recently demonstrated in several studies on nebivolol conducted in animals and in humans. Nebivolol is a third generation β-blocker used in the treatment of hypertension which induces vasodilation by increasing NO production.

Nebivolol has a distinctive profile among β-blockers, with the greatest selectivity for cardiac β_1-ARs and the highest β_1-/β_2-selectivity compared with other β-blockers, and no effect on α-receptors. Moreover, nebivolol could enhance NO release and promote neoangiogenesis in cardiac tissue via stimulation of β_3-ARs, thus reducing heart rate and blood pressure and improving systolic and diastolic function (Toblli et al., 2012).

Many studies underlined the importance of NO in the vasorelaxation mechanism of nebivolol in humans suggesting the occurrence of an additional vascular protection in hypertension. For instance, Cockcroft et al. investigated the effects of nebivolol on human forearm and demonstrated that the drug induced a potent vasodilation hampered by NO inhibitors, such as L-NAME (Cockcroft et al., 1995). Moreover, by comparing the effects of nebivolol and atenolol, another β_1-antagonist, on the endothelial function of hypertensive patients, Tzemos et al. showed that nebivolol, differently from atenolol, was able to lower blood pressure with a concomitant reversing action on endothelial dysfunction (Tzemos et al., 2001).

The mechanism by which nebivolol acts on NO bioactivity is still unclear, but it is conceivable that the drug increases intracellular free calcium concentration by activating phospholipase C.

Studies suggested that the NO-mediated vascular effects of nebivolol may be explained considering a pharmacological cross-reactivity between serotonin 5-HT_1 receptor and β-ARs (Fargin et al., 1998).

In addition, nebivolol exerts systemic antioxidative properties and this effect was hypothesized as an additional factor for increasing NO bioavailability. For example, nebivolol and atenolol similarly reduced blood pressure values in hypertensive patients, but oxidative stress markers, such as LDL hydroperoxides, 8-isoprostanes, ox-LDL were significantly improved only in patients treated with nebivolol (Troost et al., 2000; Fratta Pasini et al., 2005; Wojciechowski and Papademetriou, 2008).

The antioxidative property of nebivolol concurs to consider it as an optimal therapeutic presidium. In fact it was demonstrated that permanent β-ARs stimulation, typically observed during CVD, could induce an over-expression and an activation of eNOS which in turn lead to oxidative stress through superoxide anion generation and a paradoxically consequent decrease of NO bioavailability (Davel et al., 2006).

HF is another disease in which the NO pathway is recognized to have a crucial role. It is a very complex pathology characterized by cardiovascular dysfunction and also by diminished vascular NO bioavailability (Recchia et al., 1998; Sun et al., 2000; Wiemer et al., 2001; Rengo et al., 2012c).

By using down and up-regulation of all types of human NOS genes in genetically modified mice, the involvement of NO in the pathogenesis of HF has been largely investigated and many experimental studies have demonstrated that eNOS isoform plays a protective role in HF.

For example, Janssens et al. showed that overexpression of eNOS enzyme preserves cardiac function and limits cardiac remodeling in transgenic mice over expressing human eNOS enzyme (Janssens et al., 2004).

Moreover, Jones et al. demonstrated that mice over expressing eNOS displayed reduction in pulmonary edema and increase in survival without differences in ventricular morphology and function, proposing that eNOS-derived NO might exert its beneficial role by decreasing vascular resistance (Jones et al., 2003).

Vice versa, eNOS deficient mice develop severe cardiac dysfunction and remodeling after myocardial infarction (MI).

Scherrer-Crosbie et al. studied the impact of eNOS in left ventricular remodeling after MI in eNOS- KO mice, concluding that eNOS has a key role in limiting cardiac dysfunction and remodeling, in part by decreasing myocyte hypertrophy in the remote myocardium (Scherrer-Crosbie et al., 2001; Cannavo et al., 2013a).

The modulation of renin-angiotensin-aldosterone axis and adrenergic system are key elements in the therapy of several pathologies, including Alzheimer Disease (Femminella et al., 2013) and CVD, such as coronary artery diseases and HF (Marciano et al., 2012; Rengo et al., 2012b, 2013b).

It has been reported that NO is involved in beneficial effects of drugs, including statins, Angiotensin Converting Enzyme inhibitors (ACE-I), Angiotensin II Type 1 receptor blockers (ARBs) and β-blockers.

Statin therapy significantly enhances NO bioavailability in endothelial cells and exert beneficial effects in several molecular aspects of the MI, including neovascularization, LV dysfunction, interstitial fibrosis, remodeling and survival (Landmesser et al., 2004).

Both ACE-I and ARBs generate cardioprotective effects in mice with post-ischemic HF by improving left ventricular function and attenuating fibrosis and hypertrophy (Cavasin et al., 2000). It was demonstrated that in eNOS-KO mice with HF the beneficial effects of these drugs were abolished, suggesting that NO is a key regulator of the ACE-I and ARBs effects (Liu et al., 2002).

Moreover, the treatment with β-blockers improves LV systolic function and produces positive cardiac remodeling (Colucci et al., 2007).

In particular, recent studies demonstrated that the third generation β-blockers possess important additional properties besides inhibiting β-adrenoceptors. Among them, nebivolol and carvedilol enhance the bioavailability of NO by both inducing endothelial NO synthesis and preventing free radicals-mediated NO inactivation. Therefore, these drugs show advantages compared to the conventional β-antagonists (Vanhoutte and Gao, 2013).

Several studies indicated carvedilol, in addition to conventional therapy, as the preferred β-blocker in the treatment of chronic HF.

Packer et al. performed a double-blind, placebo-controlled study in 1094 patients with chronic HF, demonstrating that carvedilol considerably reduced hospitalization and mortality rates for cardiovascular causes (Packer et al., 1996).

Moreover, combined results of studies in the US Carvedilol HF Trials Program revealed that mortality was significantly lower in carvedilol than in placebo recipients (Keating and Jarvis, 2003).

Nebivolol, endowed with a significant NO-associated vasodilating effect, did not provide the same results. SENIORS trial on nebivolol effects in elderly patients with HF displayed a reduction in cardiovascular mortality, but the US Food and Drug Administration did not approve this drug for the treatment of HF because the improvement in the systolic function of patients treated with nebivolol was not as substantial as with other β-blockers (Nair et al., 2012).

There is now compelling evidence that reduced NO bioavailability due to sympathetic hyperactivity is the major contributor to endothelial dysfunction. Thus, the effects on endothelial dysfunction of the last generated vasodilating β-antagonists might have important clinical implications, particularly in patients with resistant hypertension and possibly in the treatment of HF.

EXERCISE TRAINING IMPROVES NITRIC OXIDE FUNCTION

ET influences cardiovascular function and endothelial homeostasis and it is recommended to treat age-associated disorders and CVD (Leosco et al., 2008; Rengo et al., 2010; Conti et al., 2012b).

ET improves the efficiency of the endogenous antioxidant system and reduces cellular oxidation rate through the stimulation of several molecular pathways (Rinaldi et al., 2006). Oxidants, and ROS, more particularly, play an important role in several physiological processes, but their overproduction is responsible for the generation of oxidative stress, that may in turn directly or indirectly damage cellular constituents, including DNA, proteins, and lipids (Ferrara et al., 2008; Conti et al., 2013).

It has been demonstrated that ET contributes to maintain the balance between ROS and antioxidant activity (Corbi et al., 2012), thus preventing oxidative stress, which is present in all stages of both vascular and non-vascular diseases (Carrizzo et al., 2013a; Puca et al., 2013).

One of the main beneficial effects of ET on the cardiovascular system is related to its ability to enhance NO production and release.

Yang et al. demonstrated that ET induces an increase of blood flow in collateral vessels of ischemic muscles and that NO inhibition abolished this effect. The authors suggested that one of the vascular adaptations induced by ET is an increase of the NO-mediated actions, which eventually culminate in the improvement of the endothelial function (Yang et al., 2008).

The endothelial function, strongly influenced by NO, may improve after exercise both in animal models and in humans; several studies in both healthy subjects and patients with impaired NO-related vasorelaxation remarked ET ability to improve vascular structure and function and endothelial homeostasis (Green et al., 2004).

Endothelial dysfunction play a fundamental role both in the onset and progression of CVD and it has been suggested that decreased NO bioavailability could definitely favors the proatherogenic endothelial cell phenotype.

Numerous studies have underlined a fundamental role played by endothelial dysfunction in both onset and progression of CVD and it has been suggested that decreased NO bioavailability could definitely favors the proatherogenic endothelial cell phenotype (Stary et al., 1994; Libby et al., 2002; Taimeh et al., 2013). CVD progression can be slowed, stopped, or even reversed by life-style interventions, including regular physical activity and these effects are often associated with an increase in NO bioavailability and NO metabolites (Rush et al., 2005; Carrizzo et al., 2013b).

Exercise-based CR is now considered a valid therapeutic approach against CVD since it reduces morbidity and mortality. Exercise benefit depends almost in part on the exercise-based increase of NO generation, which in turn improves the endothelial function (Linke et al., 2008).

Laurent et al. investigated the effects of water-based exercises in patients with stable chronic HF or coronary artery disease, and found that this type of CR was effective in increasing the basal level of plasma nitrates. Such modification may be related to an improvement of the endothelial function and may be of significance for patients' health (Laurent et al., 2009).

In recent years evidences about the relationship among ET, adrenergic system and NO, have been accumulating. Calvert et al. clearly demonstrated that exercise protects the heart by stimulating β_3-ARs and increasing cardiac storage of NO metabolites. The authors observed an increase of NO generation and of cardiac nitrite and nitrosothiol levels in exercised mice. In addition, they remarked a critical role played by β_3-ARs in regulating the phosphorylation (activation) of eNOS and the generation of NO in response to exercise (Calvert et al., 2011).

Due to its short half-life, it is very difficult to assess NO endothelial production in humans and all NO bioassays, albeit undoubtedly representing a practical surrogate to measure endothelial function *in vivo*, show some relevant limitations (Green et al., 2004).

As a consequence, novel strategies to unravel the molecular mechanisms influenced by NO are required. In this context, ET could be for example considered as a practical indirect approach to study NO effects in the endothelial cells (Conti et al., 2013).

NITRIC OXIDE SYNTASES AND ADRENORECEPTORS GENETIC VARIABILITY

Extensive evidence has been recently accumulated that polymorphisms in genes encoding for ARs and NO synthase enzymes might influence aging, onset and progression of CVD and therapy response.

Montesanto et al., for instance, investigated the genetic variability linked to the three enzymatic isoforms of NO synthase (nNOS, iNOS and eNOS), and observed that genetic variants of NOS genes influenced both aging phenotypes and longevity in humans. The Authors verified the presence of a correlation between nNOS and iNOS polymorphisms and longevity from one side, and between nNOS and eNOS variants with the presence, respectively, of depression symptoms and disability from the other (Montesanto et al., 2013).

In addition, increasing evidence suggests that genetic polymorphisms are responsible for different cardiovascular outcomes following the use of antihypertensive drugs. Jáchymová et al. analysed the common polymorphism

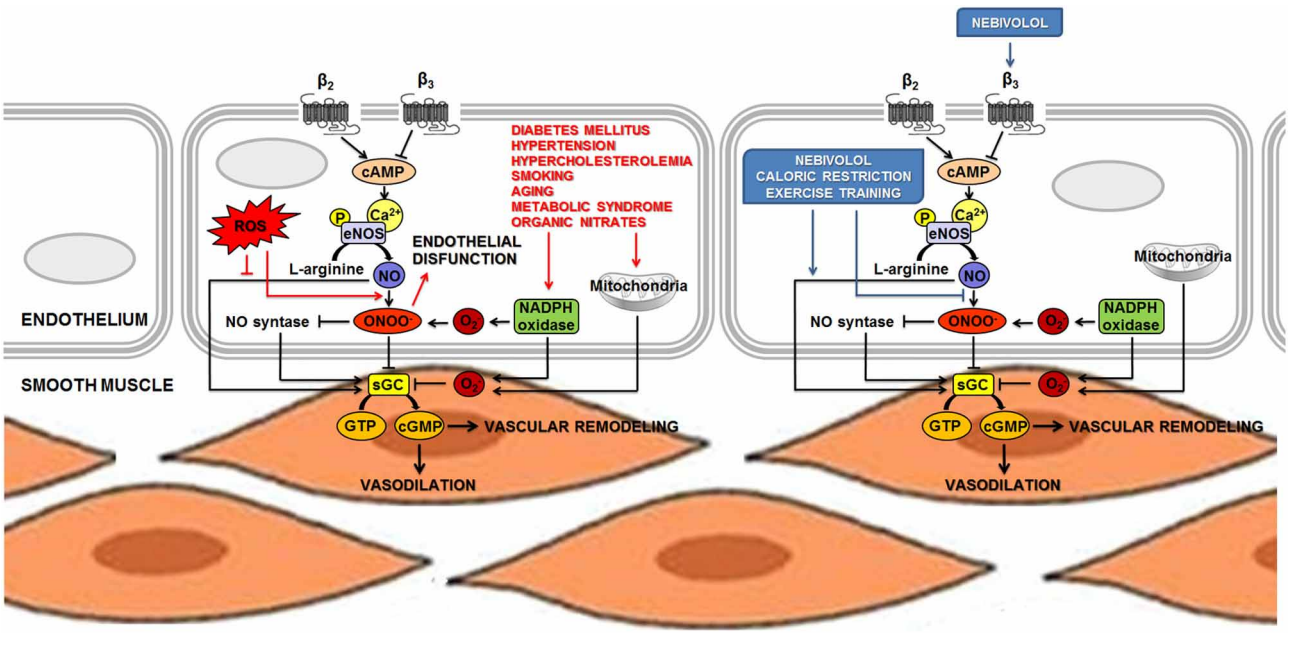

FIGURE 1 | NO is involved in the sympathetic regulation of vascular tone and in the control of endothelium homeostasis. The main mechanism leading to increased eNOS activity in endothelial cells is calcium-dependent, but phosphorylation at several loci of the NOS proteins has been recognized as an additional pathway to induce both activation and inhibition of eNOS activity. NO diffuses to vascular smooth muscle and produces relaxation by stimulating sGC to increase the levels of the second messenger cGMP. Vascular endothelial cells might also express β-adrenoceptors, thus supporting the hypothesis that the endothelium might control or facilitate β-adrenergic effects on the vessels. Acute β-adrenergic activation caused by β-adrenoceptor agonists stimulates eNOS activity and could increase release of endothelial NO. Permanently high catecholamine levels could lead to overactivation of β-adrenoceptors, increasing eNOS activity and expression. This condition may lead to the uncoupling of eNOS, which produces O_{2-} and $ONOO^-$ (ROS). An unbalanced production of NO and O_{2-} is responsible for the formation of $ONOO^-$, thus provoking vascular dysfunction. Several stimuli, such as oscillatory shear stress, hyperglycemia and lipid peroxidation could cause impairment in the NADPH oxidase system that, in turn, produces accumulation of ROS and reduction of NO content. Nebivolol, a β-blocker with a distinctive profile, combines the properties of a β_1-AR antagonist and β_3-AR agonist. Nebivolol could enhance NO release via stimulation of β_3-ARs and, thanks to its antioxidant activity, it prevents the detrimental effect on NO bioavailability associated to oxidative stress. Life-style changes and non-pharmacological interventions (such as caloric restriction and exercise training) show a positive role on the maintenance of cardiovascular homeostasis, at least in part, by inducing the activation of eNOS and increasing NO bioavailability. Abbreviations: AR, adrenoreceptor; cGMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; GTP, guanosine 5′-triphosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; O_2^-, superoxide; $ONOO^-$, peroxynitrite; P, phosphoryl; ROS, reactive oxygen species; sGC, soluble guanylate cyclase. ↓ Activation; ⊥ inhibition.

Glu298Asp, located in the eNOS gene, in a group of patients with hypertension and in age-matched healthy subjects. They found that this polymorphism was associated with an insufficient response of patients to conventional therapy, thus suggesting that this genetic variant may concur to the pathogenesis of essential hypertension (Jáchymová et al., 2001).

Zhang et al. studied several eNOS polymorphisms, highlighting a significative correlation between the presence of these variants, coronary heart diseases (CHD) and HF. In particular, this study showed that patients bearing minor allele of −690 C > T polymorphism had higher risk in CHD and minor allele carriers for −922 A > G variant had higher risk in HF.

Moreover, a genotype-dependent variability in the therapy response of patients randomized to the amlodipine or to the lisinopril and clorthalidone treatments was described (Zhang et al., 2012). Indeed, minor allele carriers treated with amlodipine showed better outcomes, when compared exclusively to those treated with lisinopril, including changes in systolic and diastolic blood pressure. These pharmacogenetic data suggested that eNOS genotyping might be useful to select the most effective and safe treatment to obtain the best individual therapy response.

Previous studies have suggested that eNOS Glu298Asp polymorphism could influence NO synthesis through the expression of a protein with different susceptibility to cleavage (Tesauro et al., 2000) and the same variant has been correlated with endothelial function (Savvidou et al., 2001; Leeson et al., 2002). These results, together with other epidemiological data, suggested that eNOS polymorphisms, other than the Glu298Asp, could play a role in influencing the onset and progression of vascular diseases, including CHD, HF and hypertension (Benjafield and Morris, 2000).

Besides the pharmacogenetic effect linked to conventional drug therapies, it was showed that Glu298Asp eNOS gene polymorphism might interact with environmental and dietary factors, such as smoking and n-3 fatty acid, influencing endothelial function (Leeson et al., 2002).

In addition, also ET-associated antihypertensive effects have been reported to vary on the basis of the individual genetic background. It is worth to note that a polymorphism (−786 T > C) in the promoter region of the eNOS gene was indicated as an influencing factor of exercise beneficial effects (Augeri et al., 2009).

Some studies suggested that changes in NO synthesis contribute to a vasodilator response variability to β_2 agonists. It was observed that forearm blood flow response to isoproterenol is impaired in men with hypercholesterolemia, a condition associated with dysfunctional NO activity (Chowienczyk et al., 1992). Moreover, the β-adrenergic vasorelaxation in the human forearm is reduced by N-monomethyl-l-arginine (L-NMMA), an inhibitor of nitric oxide synthase, confirming the crucial role played by NO in the sympathetic regulation of vascular tone (Dawes et al., 1997).

Several polymorphisms located in the gene encoding β_2-receptors have been correlated to the difference in the expression, coupling and agonist regulation of these receptors. Polymorphisms affecting amino acids 16, 27, and 164 are the most common genetic variants and, for example, the Arg16Gly is known to predispose to agonist-induced down-regulation and desensitization of the receptors, probably concurring to the pathogenesis of asthma severity (Green et al., 1993; Reihsaus et al., 1993).

To confirm the involvement of the NO pathway in the β_2-adrenoreceptor-dependent vasodilation control, Garovic et al. showed that the forearm blood flow response to isoproterenol was greater in Gly 16 then in Arg 16 homozygotes and that response was inhibited by L-NMMA (Garovic et al., 2003).

To date, many authors reported that adrenoreceptor (in particular, β_2-receptors) polymorphisms are strongly associated to cardiovascular outcomes, including blood pressure, and to predisposition (Timmermann et al., 1998; Bray et al., 2000) and treatment (Johnson and Terra, 2002; McNamara et al., 2002; Taylor and Bristow, 2004) of CVD.

The studies described above confirm that analysis of patients DNA may be useful to understand sympathetic vasodilation mechanism and implication of NO pathway and to create new therapeutical strategies against CVD.

CONCLUSIONS

The maintenance of myocardial and vascular homeostasis is one of the many diverse physiological functions mediated by NO, a versatile and nearly ubiquitous molecule that plays a key function as a signaling molecule throughout the body. An imbalance in either the production or release of this molecule is correlated to CVD such as hypertension and HF.

It is now evident that the control of endothelium homeostasis and the sympathetic regulation of the vascular tone are the result of a complex crosstalk between NO, β-adrenoreceptors (in particular the β_3 subtype) and oxidative stress. As an example, recent advances on the β_1-selective antagonist nebivolol remarked the importance of NO bioavailability in the maintenance of myocardial and vascular homeostasis. A scheme representing some of the functions in which the pathway of NO is strongly involved was presented in **Figure 1**.

It is our opinion that the effective clinical/therapeutic management of CVD requires the understanding of the molecular determinants responsible for this crosstalk to identify new targets and develop new clinical strategies.

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New advances in beta-blocker therapy in heart failure

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The use of **β**-blockers (BB) in heart failure (HF) has been considered a contradiction for many years. Considering HF simply as a state of inadequate systolic function, BB were contraindicated because of their negative effects on myocardial contractility. Nevertheless, evidence collected in the past years have suggested that additional mechanisms, such as compensatory neuro-humoral hyperactivation or inflammation, could participate in the pathogenesis of this complex disease. Indeed, chronic activation of the sympathetic nervous system, although initially compensating the reduced cardiac output from the failing heart, increases myocardial oxygen demand, ischemia and oxidative stress; moreover, high catecholamine levels induce peripheral vasoconstriction and increase both cardiac pre- and after-load, thus determining additional stress to the cardiac muscle (1). As a consequence of such a different view of the pathogenic mechanisms of HF, the efficacy of BB in the treatment of HF has been investigated in numerous clinical trials. Results from these trials highlighted BB as valid therapeutic tools in HF, providing rational basis for their inclusion in many HF treatment guidelines. However, controversy still exists about their use, in particular with regards to the selection of specific molecules, since BB differ in terms of adrenergic β-receptors selectivity, adjunctive effects on α-receptors, and effects on reactive oxygen species and inflammatory cytokines production. Further concerns about the heterogeneity in the response to BB, as well as the use in specific patients, are matter of debate among clinicians. In this review, we will recapitulate the pharmacological properties and the classification of BB, and the alteration of the adrenergic system occurring during HF that provide a rationale for their use; we will also focus on the possible molecular mechanisms, such as genetic polymorphisms, underlying the different efficacy of molecules belonging to this class.

Keywords: beta blockers, heart failure, clinical trials as topic, elderly patients, pharmacogenomics

INTRODUCTION

The use of β-blockers (BB) in heart failure (HF) has been considered a contradiction for many years. Considering HF simply as a state of inadequate systolic function, BB were contraindicated because of their negative effects on myocardial contractility. Nevertheless, evidence collected in the past years have suggested that additional mechanisms, such as compensatory neuro-humoral hyperactivation or inflammation, could participate in the pathogenesis of this complex disease. Indeed, chronic activation of the sympathetic nervous system, although initially compensating the reduced cardiac output from the failing heart, increases myocardial oxygen demand, ischemia and oxidative stress; moreover, high catecholamine levels induce peripheral vasoconstriction and increase both cardiac pre- and after-load, thus determining additional stress to the cardiac muscle (Kubon et al., 2011). As a consequence of such a different view of the pathogenic mechanisms of HF, the efficacy of BB in the treatment of HF has been investigated in numerous clinical trials. Results from these trials highlighted BB as valid therapeutic tools in HF, providing rational basis for their inclusion in many HF treatment guidelines. However, controversy still exists about their use, in particular with regards to the selection of specific molecules, since BB differ in terms of adrenergic β-receptors (β-ARs) selectivity, adjunctive effects on α-receptors, and effects on reactive oxygen species and inflammatory cytokines production. Further concerns about the heterogeneity in the response to BB, as well as the use in specific patients, are matter of debate among clinicians. In this review, we will recapitulate the pharmacological properties and the classification of BB, and the alteration of the adrenergic system occurring during HF that provide a rationale for their use; we will also focus on the possible molecular mechanisms, such as genetic polymorphisms, underlying the different efficacy of molecules belonging to this class.

HISTORY, STRUCTURE AND CLASSIFICATION OF ADRENERGIC BETA RECEPTORS

To date, three subtypes of β-receptors (β1-AR, β2-AR and β3-AR) have been identified; the existence of a fourth subtype, called β4, has been proposed to explain the sympathomimetic effects of some atypical β-agonists (such as CGP-12177) known to be inactive on the "classical" β-receptors; however recent evidence suggest that the putative β4 receptor is more likely a novel functional state of β1 receptor (Granneman, 2001).

β-ARs display peculiar tissue distribution and pharmacological properties: β1 is the "cardiac" receptor, while β2 is expressed predominantly in smooth muscle cells, and β3 in

the adipose tissue. Structurally, β-ARs belong to the family of G protein-coupled receptors, with seven transmembrane domains, an extracellular N-terminal region, and an intracellular C-terminus. Although, in the heart, β1 is the predominant AR subtype, cardiac cells express also β2 and, to a less extent, β3, with quantitative differences depending on age and pathological conditions. In particular, β-AR population of the non-failing heart is composed of β1 and β2, in a ratio of 8:2; however, in both ageing and HF, the proportion of β1 subtypes decreases due to mRNA down-regulation, while levels of β2-AR remain stable, thus achieving a 1:1 ratio of β1- and β2-ARs (Lohse et al., 2003). In the heart, stimulation of β-ARs pathway leads to the activation of the Gs protein, with consequent stimulation of the adenylate cyclase, increase in intracellular cAMP, and protein kinase A-dependent phosphorylation and modulation of the activity of important proteins involved in myocardial contractility, such as L-type Ca^{2+} channels, troponin I and sarcoplasmic reticular Ca^{2+}/ATPase inhibitory protein (Wachter and Gilbert, 2012). Moreover, through the βγ-subunits of the heterotrimeric G-protein, β-AR stimulation activates members of the G-protein receptors kinase family, named βARK (βγ-AR kinase) or GRK2 (G-protein receptor kinase 2), which are a primary mechanism of self-regulation of adrenergic stimulation. Indeed, by phosphorylating residues in the C-terminal region of the receptor, GRK2 induce the binding of protein such as β-arrestin to the receptor, thus causing its uncoupling from the Gs protein and its transductional pathways; moreover, GRK2-induced phosphorylation also cause an increase in the affinity of β-AR for the inhibitory G protein Gi, thus accelerating receptor desensitization (Rengo et al., 2012b).

Although the main intracellular pathway activated by β-ARs is Gs signaling, it has been demonstrated that stimulation of β-ARs can also activate Gi proteins, MAP kinases and other proteins involved in the control of cell cycle and apoptosis (Bogoyevitch et al., 1996), in a subtype-specific manner, thus differentially influencing cardiomyocytes fate. These evidence suggest that modulation of β-ARs can impact cardiac pathophysiology in different and multiple ways, well beyond than simply controlling heart mechanics.

MOLECULAR MECHANISMS UNDERLYING β ADRENERGIC SYSTEM DYSREGULATION IN HF AND THE EFFICACY OF β-BLOCKERS

HF is associated to dramatic changes in neurohormonal balance. To compensate the reduced cardiac output, an increased activity of the adrenergic nervous system (ANS), as well as an activation of the Renin-Angiotensin-Aldosterone System (RAAS) occur. As a consequence of ANS hyperactivity, norepinephrine and epinephrine plasma levels increase, due to adrenal gland secretion and adrenergic terminals spillover, thus leading to chronic sympathetic stimulation of the heart. Such stimulation of cardiac β-ARs increases oxygen demand and myocardial work, thus contributing to cardiac muscle stress. In adult rat myocytes, β1-AR mediates apoptotic signaling, whereas the β2 subtype seems to stimulate antiapoptotic pathways coupling to the inhibitory G protein (G_i) (Bristow, 2000). Stimulation of renal iuxtaglomerular β1-AR can also activate RAAS, thus causing an increase in angiotensin-related cardiac remodeling and apoptosis (Lymperopoulos et al., 2013). Negative effects of ARs hyperactivity are also recapitulated by transgenic mouse models; indeed, transgenic mice overexpressing β1-AR (Engelhardt et al., 1999) or cardiac Gαs (Iwase et al., 1996) show a cardiomyopathy with ventricular dilatation and systolic dysfunction; by contrast, cardiac overexpression of β2-AR improves contractility in the healthy heart (Akhter et al., 1997). Chronic ANS activation is also responsible for the selective down-regulation of β1-AR and for the functional uncoupling of both β1- and β2-ARs from their intracellular coupling mechanisms; these events are determined by activation of GRKs, particularly GRK2. Indeed, it has been demonstrated that, in chronic HF, GRK2 is up-regulated in cardiomyocytes, thus leading to a reduced responsiveness of the cardiac muscle to catecholamines stimulation (Rengo et al., 2011). GRK2-induced ARs uncoupling and down-regulation also occur in the adrenal gland, where the inhibitory feedback on catecholamine release mediated by inhibitory α2-ARs is reduced, thus determining an increase of circulating epinephrine (Lymperopoulos et al., 2007). GRK2 inhibition by a small peptide (βARK ct) increases cardiac contractility, normalizes neurohormonal axis activity, and improves survival in several animal models of HF (Rockman et al., 1998).

The molecular evidence here briefly reviewed have reinforced the hypothesis that the changes in the ANS during HF are not merely adaptive, but rather play a direct pathogenetic role, opening novel avenues to interpret the efficacy of drugs acting by blocking β-ARs in HF. In fact, BB might exert beneficial effects well beyond those exerted in cardiac muscle (limiting AR decrease in number and functional desensitization) (Iaccarino et al., 1998), such as counteracting some aberrant maladaptive responses of ANS and RAAS neurohormonal systems occurring in HF. Indeed, it has been recently demonstrated that bisoprolol normalized the adrenal catecholamine production by reducing GRK2 levels, thus restoring the negative feedback on epinephrine release exerted by α2-AR (Rengo et al., 2012a). In addition, atenolol and bisoprolol treatment have been also shown to improve myocardial perfusion by enhancing neoangiogenesis in the failing heart, via activation of VEGF signaling pathway (Dedkov et al., 2005; Rengo et al., 2013).

BETA-BLOCKERS: CLASSIFICATION AND PHARMACOLOGY

BB are a wide and heterogeneous group of molecules acting as competitive and reversible antagonists of β-ARs. In addition to their ability to block β-ARs-signaling, BB show a variety of adjunctive actions that are often used as criteria for their classification (see **Table 1**).

BB can be classified according to:

β1-AR-SELECTIVITY

Propranolol and timolol are the prototypical non-selective, "first generation" BB, showing the same affinity for both β1 and β2 subtypes. Subsequent research, aiming to find cardioselective drugs, led to the synthesis of molecules such as atenolol, bisoprolol and metoprolol, preferentially blocking "cardiac" β1-AR.

Table 1 | Main pharmacological properties of BB.

Drug	Selectivity	ISA	α–AR blockade	Membrane-stabilizing activity	Bioavailability (%)	Half-life (hrs)
β non-selective						
Propranolol	0	0	0	++	25	3–5
Nadolol	0	0	0	0	35	10–20
Timolol	0	0	0	0	50	3–5
Pindolol	0	++	0	±	75	3–4
Labetalol	0	+	+	±	20	4–6
Carvedilol	0	0	+	0	30	7–10
β1-selective						
Metoprolol	++	0	0	±	40	3–4
Atenolol	++	0	0	0	50	5–8
Esmolol	++	0	0	0	–	0.13
Acebutolol	+	+	0	+	40	8–12 (diacetolol)
Bisoprolol	++	0	0	0	90	9–12
Nebivolol	++	0	0	0	12–96*	10–30*

Abbreviations: ISA: intrinsic sympathomimetic activity; α-AR: alpha adrenergic receptor.

**Depending on CYP polymorphisms.*

Data are mainly from refs. (Rockman et al., 1998) and (Goodman and Gilman's, 2011).

α-AR-ANTAGONISM AND VASODILATOR ACTIVITY

Labetalol, bucindolol and carvedilol also act as α1-AR-antagonists. This pharmacological effect is particularly important in HF, since the peripheral vasodilatation induced by α1-AR blockade decreases both pre- and after-loads, thus reducing myocardial oxygen consumption and work. Other BB such as nebivolol, although not provided of α1-AR antagonism, show marked vasodilator properties; possible mechanisms explaining this effect are: stimulation of nitric oxide production, blockade of Ca^{2+} entry, opening of K^{+} channels, and antioxidant activity. In particular, nebivolol has been demonstrated to activate endothelial β3-AR, thus stimulating NO production and dilation of coronary arteries (Rozec et al., 2006).

INTRINSIC SYMPATHOMIMETIC ACTIVITY (ISA)

Although classified as blockers, some molecules belonging to this class can act as partial agonists, thus activating β1-AR. The intrinsic sympathomimetic activity of pindolol, acebutolol and celiprolol can improve BB tolerability, since they do not cause severe bradycardia or excessive negative inotropic effects at rest. On the other hand, some BB also act as inverse agonists, thus inducing a negative response of receptor signaling in absence of the natural agonist. This characteristic has clinical consequences, since drugs as bucindol (with low inverse agonist activity) decrease mean and peak heart rate, while they do not reduce minimum heart rate (Wachter and Gilbert, 2012).

PHARMACOKINETICS

BB widely differ in their physico-chemical properties. In particular, lipophilic compounds such as metoprolol, bucindolol, carvedilol and nebivolol, when given orally, are rapidly adsorbed in the gastrintestical tract and are extensively metabolized by the liver, therefore often presenting with a shorter half-life when compared to other BB. Moreover, the high lipophilicity and the resulting higher penetration across the blood-brain barrier could also explain the increased number of brain-related adverse events, as well as the membrane-stabilizing (quinidine-like) properties of antiarrhythmic molecules which appear independent of their BB activities (Murray et al., 1990).

OTHER PROPERTIES

Carvedilol and its metabolites are also endowed with antioxidant activity, a property that can be useful in the treatment of HF; propanolol and carvedilol seem to decrease vascular smooth muscle cells proliferation. Moreover, carvedilol and bucindolol can modulate guanine-nucleotide binding to its receptor (Bristow et al., 1992). It has been also demonstrated that carvedilol ameliorates insulin sensitivity (Jacob et al., 1996).

PHARMACOKINETICS

As stated before, lipophilicity is one of the chemical characteristics influencing bioavailability and, consequently, administration schedule. High lipophilic molecules such as propanolol are rapidly adsorbed but, at the same time, they become extensively metabolized by the liver (first-pass metabolism); by contrast, drugs with intermediate lipophilic properties (bisoprolol) are efficiently adsorbed in the gut but poorly removed by the liver first-pass metabolism, so that they display high bioavailability (90%). Many BB are metabolized by the liver through the CYP pathway, in particular via the CYP2D6 isoform; in humans, the CYP2D6 enzyme is highly polymorphic and this characteristic has been often referred to explain the inter-individual variability observed in the plasma levels of drugs such as carvedilol and in the responses to treatment with BB in HF. Other CYP isoforms (CYP1A2, CYP2C9, CYP2C19 and CYP3A4) can contribute, though to a lesser extent, to BB hepatic metabolism, while other molecules, such as bisoprolol, are excreted unmodified via the kidney. As a consequence, dose modification of BB should

be considered in patients with pathological conditions impairing both liver and kidney functions, or for patients treated also with drugs metabolized by the same CYP isoforms (antidepressants, antipsychotics). Moreover, it should be mentioned that peak plasma concentrations, as well as half-lives, are strongly influenced by the formulation of the molecule; this has important consequences on clinical outcomes. A paradigmatic example is represented by the two different formulations of metoprolol used in controlled clinical trials; indeed, in the COMET study (Carvedilol or Metoprolol European Trial), the reduced efficacy demonstrated in the metoprolol-tartrate arm with respect to the carvedilol arm has been largely explained by the shorter half-life of metoprolol-tartrate when compared to carvedilol. It has been suggested that this pharmacokinetic difference led to a different degree of beta blockade in patients enrolled in the two study arms, and, therefore, that the dose of metoprolol tartate administered in this study was inadequate (Poole-Wilson et al., 2003). In a subsequent trial, MERIT-HF (Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart failure), a different and longer-lasting formulation, metoprolol-succinate, was demonstrated to reduce mortality and hospitalization in HF patients (Hjalmarson et al., 2000); the degree of these protective effects were now similar to those demonstrated for other BB with longer plasma half-lives. Based on these evidence, FDA has approved metoprolol-succinate, but not metoprolol-tartrate, for the treatment of patients with HF.

TOLERABILITY OF β-BLOCKERS

The most common adverse events of BB result from their mechanism of action. The blockade of sympathetic stimulation may have both acute or chronic consequences, mainly giving rise to symptoms of cardiovascular, respiratory, central nervous system and metabolic origins.

In particular, acute blockade of catecholamines effects can induce bradycardia that can be potentially life-threatening in patients with defects in atrio-ventricular conduction; moreover, acute blockade of β-ARs can worsen myocardial contractility and, consequently, induce or deteriorate HF in patients with myocardial infarction, cardiomegaly or compensated HF. Starting with low doses and slowly titrating up (in order to reach the optimal dose in several weeks) is a widely used and well known strategy to reduce these risks. In the same vein, given that a prolonged BB treatment may induce β-ARs up-regulation and, consequently, an enhanced sensitivity to catecholamines, abrupt withdrawal of BB should be avoided in order to prevent angina and risk of sudden death. BB treatment can also induce Raynaud's phenomenon and worsen peripheral vascular disease.

As β2-ARs-blockade cause bronchoconstriction, BB have been considered as contraindicated in patients suffering from asthma or Chronic Obstructive Pulmonary Disease (COPD). Thus, concerns exist regarding the use of BB in patients with HF and COPD, in particular for non-cardioselective molecules; however, the risk to increase bronchoconstriction, thus worsening COPD symptoms, is often overcome by the beneficial effects on HF. For these reasons, COPD is currently considered as a relative contraindication for BB treatment, and a careful risks-benefits assessment should be made to avoid undertreatment of HF patients with respiratory comorbidities (Ellison and Gandhi, 2005).

BB also impact glucose homeostasis, since catecholamines, mainly through β2-AR, promote glycogenolysis and glucose-mobilization in response to hypoglycemia, thus increasing blood glucose levels. Therefore, BB can conceal symptoms of hypoglycemia or cause an excessive reduction of blood glucose in susceptible diabetic patients treated with insulin or oral hypoglycemic agents. β1-AR selective antagonists, as well as carvedilol, the latter shown to improve insulin-sensitivity, could possibly represent a valid alternative for HF patients with concomitant diabetes.

EFFICACY OF BB IN HF

The efficacy of BB in the treatment of HF has been evaluated in several randomized, controlled clinical trials (**Table 2**). Patients enrolled in these studies suffered from HF with different etiology and showed an impaired systolic function. Taken together, the aforementioned clinical trials have demonstrated that three BB (metoprolol succinate, bisoprolol and carvedilol) are able to improve ventricular ejection fraction and HF symptoms, and to reduce mortality and hospitalizations. Of note, numerous trials were prematurely discontinued for mostly ethical reasons, since interim analysis showed a reduced mortality in the BB arm compared to placebo-arm. Bucindolol and nebivolol have also been tested in clinical trials, with different results.

METOPROLOL

Metoprolol has been one of the first BB to be studied in HF treatment. 383 patients with systolic disfunction secondary to dilated cardiomyopathy were randomized to placebo or metoprolol at a target dose of 100–150 mg/daily. Metoprolol was shown to reduce mortality and need of transplantations by 34% with respect to placebo arm (Waagstein et al., 1993). A subsequent wider trial (3991 patients in 14 countries), MERIT-HF, investigated the efficacy of metoprolol succinate in mild-moderate HF patients with impaired systolic function (left ventricular ejection fraction-LVEF <40%) and New York Heart Association (NYHA) functional class II–IV; in this study, a long-lasting release metoprolol formulation was used, with a mean daily dose of 159 mg (Hjalmarson et al., 2000). The authors found a 34% statistically-significant decrease in mortality and a reduction in combined endpoint "all-cause mortality and hospitalization" (risk reduction by 19%); the beneficial effects of metoprolol were even higher on cardiac death and non-fatal acute myocardial infarction, with a 39% risk reduction.

BISOPROLOL

Following a previous Cardiac Insufficiency Bisoprolol Study (CIBIS), in which bisoprolol administration showed a non-statistically significant trend toward improved survival in HF patients (1994), a subsequent CIBIS-II trial enrolled a higher number of subjects ($n = 2647$) with LVEF <35% and NYHA class III–IV. As for metoprolol, a reduction in all-cause mortality was demonstrated for bisoprolol-treated patients when compared to placebo-arm (11.8 vs. 17.3%, respectively), with a follow-up of about 1 year (1999). More recently, a third study with bisoprolol (CIBIS III) has addressed the relevant issue of whether

Table 2 | Summary of main clinical trials reported in the text investigating the efficacy of BB in HF.

Study name (reference)	No patients	BB used	Description	Main findings	Additional findings and comments
MDC (Waagstein et al., 1993)	383	Metoprolol	HF secondary to Dilated Cardiomyopathy (EF <40%)	34% decrease in mortality or need for transplantation	No significant difference in mortality alone
MERIT-HF (Hjalmarson et al., 2000)	3991	Metoprolol succinate	Mild-moderate HF (EF <40%) NYHA II-IV	<34% decrease in all cause mortality	<39% decrease in cardiac death and non-fatal MI
CIBIS (CIBIS Investigators and Committees, 1994)	641	Bisoprolol	Moderate HF (EF <40%) NYHA III-IV	No significant difference in mortality	Significant improvement of functional status of the patients
CIBIS II (CIBIS-II Investigators, 1999)	2647	Bisoprolol	Moderate HF (EF <35%) NYHA III-IV	32% decrease risk of mortality and hospitalization for HF	Greatest effects in patients with ischaemic HF and NYHA III at baseline
CIBIS III (Willenheimer et al., 2005)	1010	Bisoprolol (vs. enalapril)	Mild moderate HF (EF <35%) NYHA II-III	Non-inferiority of bisoprolol vs enalapril in reducing mortality as first treatment in ITT	Non-inferiority of bisoprolol was not proven in *per-protocol* analysis
US Carvedilol study (Packer et al., 1996)	1094	Carvedilol	Mild moderate HF NYHA II-IV	65% mortality reduction	38% reduction in death or hospitalization for cardiovascular reasons
COPERNICUS (Packer et al., 2002)	2289	Carvedilol	Severe HF (EF <25%) NYHA III-IV	35% in risk of death	27% decrease death or hospitalization for a cardiovascular reason
CAPRICORN (The CAPRICORN Investigators, 2001)	1959	Carvedilol	Patients with recent MI and left ventricular dysfunction (EF <40%)	23% reduction in mortality	No significant difference in primary endpoint (all-cause mortality or hospitalization for cardiovascular problems)
COMET (Poole-Wilson et al., 2003)	3029	Carvedilol vs. Metoprolol tartrate	Mild moderate HF (EF <35%) NYHA II-IV	17% decrease in carvedilol- vs. metoprolol- arm	Concerns about metoprolol formulation
BEST (BEST Investigators, 2001)	2708	Bucindolol	Mild moderate HF (EF <35%) NYHA III-IV	No significant overall survival benefit	Reduction in mortality in patients homozygous for Arg389 (subsequent pharmacogenetic analysis)
SENIORS (Flather et al., 2005)	2128	Nebivolol	Mild moderate HF (EF <35% in last 6-months) Age >70yrs	14% reduction mortality and hospitalizations	Significant increase of LVEF and decrease in end-systolic volume

The Table re-elaborates and integrates the data reported in Table I of Kubon et al., 2011.

BB could be as effective as renin-angiotensin-aldosterone system (RAAS) inhibitors as first-line drugs in HF. In general, therapy in CHF patients is initiated with an ACE inhibitor or an AngII-receptor blocker (ARB), and thereafter a β-blocker is introduced. This drug sequence seems to result largely from the fact that, historically, the beneficial effects of ACE inhibitors were documented first. The order of initiation of these agents is of great relevance, because the first agent initiated is more likely to be titrated up to its target dose, whereas the second agent is often given at a suboptimal dose or not initiated at all. Theoretically, sympathetic system alterations occur before RAAS dysfunction during chronic HF; moreover, it is well known that stimulation of β1-ARs in iuxtaglomerular cells can increase renin production, thus contributing to RAAS over activation. The CIBIS III

trial compared bisoprolol to enalapril, one of the most used first-line drug for HF. Patients with LVEF <35% were randomized to bisoprolol- or enalapril; after a 6-months mono-therapy period, patients received both drugs. The results obtained showed the non-inferiority of bisoprolol-first versus enalapril-first strategy, in the Intention to treat population but not in per-protocol analysis, thus requiring more data to better clarify this issue (Willenheimer et al., 2005).

CARVEDILOL

This non-selective, third generation BB has been tested in different trials. The first randomized study evaluated the efficacy of carvedilol on 1094 US patients with LVEF <35%, stratified according to their performance on exercise tests. Carvedilol was shown to reduce mortality risk by 65% when compared to placebo; these data induced the Monitoring Board to terminate the study before its scheduled completion (Packer et al., 1996). The efficacy of carvedilol has been evaluated also in patients with severe HF, a group often not included in randomized trials. The COPERNICUS study enrolled 2289 patients with symptoms of HF at rest or on minimal exertion and with LVEF <25%, randomized to carvedilol or placebo; in the carvedilol arm, a reduction by 35% in the risk of death and an improvement in HF symptoms were observed (Packer et al., 2002). Carvedilol has been evaluated also in patients with left ventricular dysfunction, with or without HF, secondary to myocardial infarction. The aim of the CAPRICORN study was to evaluate whether addition of carvedilol to standard modern management of acute myocardial infarction would improve outcome in terms of mortality and morbidity. The results obtained confirmed that carvedilol decreased the risk of mortality by 23% when compared to placebo (2001).

It should be mentioned that clinical trials have investigated efficacy of BB in patients with impaired LVEF (<40%); however, since BB lower myocardial work by reducing heart rate and oxygen demand, it seems desirable to extend these studies also to HF patients with preserved systolic ejection fraction such as the elderly population.

COMPARISON BETWEEN BB

Collectively, results emerged from clinical trials demonstrated that blockade of β-AR signaling has beneficial effects in HF, both reducing mortality and improving symptoms of impaired cardiac function. Such protection has been demonstrated for different molecules belonging to BB class, and the degree of the beneficial effects was quite similar for any of the chosen drug. Thus, the possibility existed that BB efficacy in HF was due to a "class effect." On the other hand, many researchers have pointed out that peculiar pharmacodynamic and/or pharmacokinetic properties (β1-AR-selectivity, vasodilating actions, anti-oxidant activity, "pleiotropic effects", good bioavailability, low drug-drug interaction risk) could represent valuable characteristics for preferring a specific molecule over other BBs in the treatment of HF. To verify this hypothesis, a specific clinical trial was conducted in 2003. The COMET study aimed to compare the mortality risk-reducing effects in 3029 patients with HF randomized to receive either carvedilol or metoprolol, after a mean follow-up of 58 months. The results demonstrated that carvedilol decreased mortality by 17% with respect to metoprolol (Poole-Wilson et al., 2003); however, as mentioned before, subsequent analyses showed that the degree of β-blockade reached with the formulation of metoprolol used in the study (metoprolol tartrate) was not adequate, and probably not similar to that of the carvedilol arm. Basically, metoprolol tartrate should have been titrated up in the COMET study (Talber, 2004).

A solution to this issue has been also pursued by several meta-analyses; most of these have been focused on carvedilol, possibly because of its unique pharmacodynamic profile. Nevertheless, results from these studies seem to be conflicting; in a systematic review of 11 randomized controlled trials in 5,207 patients, it was found that carvedilol reduced all-cause mortality in patients with HF significantly more when compared to other BB such as atenolol, bisoprolol and metoprolol. According to the Authors, the superiority of carvedilol over other BB could be explained by the peculiar actions exerted by carvedilol, such as: antiarrhythmic effect (and consequent reduction in the risk of sudden death), a more sustained increase in LVEF when compared to other BB, blockade of up-regulated β2-AR, vasodilation. (DiNicolantonio et al., 2013). By contrast, in their meta-analysis of 21 trials including 23122 HF patients, Chatterjee et al. found no significant differences in mortality outcome among the BB considered (atenolol, bisoprolol, bucindolol, carvedilol, metoprolol, and nebivolol), thus concluding that the beneficial of BB were due to a class effect. Nonetheless, carvedilol showed the lowest cardiac mortality among all β blockers tested, although this was not statistically significant; in consideration also of the its beneficial effects on lipid and glucose profile, the Authors suggest the use of carvedilol as an empiric initial treatment in patients with cardiovascular comorbidities (Chatterjee et al., 2013).

Lack of clear data from clinical trials regarding the superiority of a given BB are reflected in current guidelines for the management of HF, in which the use of a particular BB over the others is not specified.

PHARMACOGENOMICS OF BB

As for many other pharmacological treatments, variability in clinical and functional outcomes exists also for BB administration in HF patients. Actually, BB treatment fails to improve LVEF in a variable proportion of subjects, while a minority of patients experience worsening of HF symptoms during BB titration (Talameh et al., 2012); such differences could be explained, at least in part, by genetic variation influencing BB pharmacodynamics and/or pharmacokinetics. To date, polymorphisms in β1-AR, β2-AR, GRK-5, CYP2C6, NET and UGT1A1 have been associated to variability in BB response.

β1-AR

The most studied polymorphism in the β1-AR gene (ADRB1) is the Arg389Gly. β1-ARs possessing arginine residue show greater activity, both in basal condition and after agonist-stimulation. Therefore, in these patients, β-blockade might potentially have a greater effect by reducing the high sympathetic stimulation determined by the β1-Arg receptor (Talameh and Lanfear, 2012). On the other hand, the high sympathetic activity could require higher

dose of BB. The most convincing result from the literature concerning the possible role of Arg389Gly variant in BB treatment comes from BEST study, a trial investigating the effects of bucindolol in HF. Despite the main study failed to demonstrate the efficacy of bucindolol in all the population studied, subsequent pharmacogenetic analysis clearly demonstrated that bucindolol significantly reduced mortality when compared to placebo in patients homozygous for Arg389 (2001). Nevertheless, different studies, such as the pharmacogenetic sub-study of MERIT-HF, failed to find association between Arg389 allele and mortality outcome, suggesting that results from BEST depended on bucindolol peculiar properties (in particular to its marked ability to suppress β1-ARs), and could not be extended to other BB.

Another pharmacogenetic sub-study, the HF-ACTION DNA, demonstrated that patients with the Arg/Arg genotype required a higher dose of BB to achieve a mortality risk reduction similar to that of Gly carriers (Fiuzat et al., 2013).

Another variant of ADRB1, Ser49Gly, has been associated to differential outcome in HF and response to β-AR antagonists. The presence of the glycine residue is thought to enhance agonist-promoted down-regulation of β1-AR with respect to Ser49 (Levin et al., 2002), thus preserving failing myocardium from toxic effects exerted by catecholamines. However, data about its possible role in determining BB response are poor and inconsistent.

β2-AR

As stated before, β2-AR signaling plays a more relevant role in the failing heart, since β1-AR expression is down-regulated. Thus, variants in ADRB2 gene could modulate response to BB treatment. Among the three polymorphisms identified in β2-AR gene, Gln27Glu variation has been investigated; Glu27 β2-AR shows an enhanced resistance to desensitization. Results from clinical trials are conflicting: some studies found a lower proportion of responders to BB treatment for patients homozygous for Gln27, other trials failed to find associations. However, these negative data could be explained by the reduced number of participants and by the inclusion of β1-AR-selective blocker (Talameh et al., 2012).

$\alpha 2_C$-AR AND GRK-5

Genetic variants of the presynaptic α2 receptor and of the G-protein coupled receptor kinase-5 seem to contribute to the variability in BB response. In particular, association of polymorphisms of $\alpha 2_C$-AR and GRK-5 with the Arg389 variant in β1-AR might impact response to BB. Indeed, a variant of the $\alpha 2_C$-AR gene leading to a deletion of amino acids 322–325, which resulted in an increase of catecholamine stimulation, when associated to the Arg389 variant in the ADRB1, caused a more pronounced increase in LVEF with respect to other genotypes. In the same way, patients with the Gln41variant in GRK5 and Arg389 in β1-AR showed advantages in term of reduced mortality after BB treatment (Talameh et al., 2012).

CYP2D6 AND UGT1A

As mentioned before, CYP2D6 is the main CYP isoform involved in BB metabolism. Thus, genetic variants in this gene have been suggested to modulate BB response. In the same vein, polymorphisms in UGT1A1 have been also proposed to modify BB metabolism and, consequently, response to pharmacological treatment. The study of (Baudhuin et al., 2010) retrospectively analyzed 93 patients characterized as responders or non-responders to metoprolol or carvedilol therapy. These patients have been also classified according to their genotype in different classes, ranging from poor to extensive metabolizer, for both CYP2D6 and UGT1A1 variants. The Authors did not find any association between CYP2D6 and UGT1A1 polymorphisms and response to therapy with carvedilol or metoprolol; nevertheless, patients who were poor metabolizer for CYP2D6 required a higher dose of carvedilol. These data might be relevant in patients also carrying ADRB1 Gly/Gly variant, since higher dose of carvedilol should be used to reach beneficial effects on HF (Baudhuin et al., 2010).

Taken together, these results, although suggestive of the possibility to select BB according to a particular genotype, are not conclusive, thus requiring large prospective clinical trials.

BB IN SPECIFIC GROUP OF PATIENTS

Although their efficacy in HF has been demonstrated, BB are not given or are inadequately administered to some categories of patients. Among them, elderly patients are frequently undertreated, because of comorbidities, reduced tolerability to BB, and risk to worsen symptoms of HF. Moreover, elderly patients frequently show preserved LVEF, thus making even harder the decision to start BB therapy. Nevertheless, analysis of randomized trials have shown that BB reduce mortality and improve quality of life also in patients >70 years, as well as in younger ones. Among BB, nebivolol has been considered as the most interesting for HF in elderly because of its unique pharmacodynamic profile. Indeed, the β1-AR-selective antagonist nebivolol is not provided with vasoconstrictor activity and should not interfere with respiratory function; moreover, stimulation of NO release might improve diastolic function. These characteristics might be relevant in elderly patients, frequently showing comorbidities and commonly less tolerant to peripheral vasoconstriction (Del Sindaco et al., 2010). Based on these evidence, two clinical trials have investigated efficacy of nebivolol in aged HF patients. While the ENECA study demonstrated an improvement in LVEF in patients >65 years (Edes et al., 2005), the SENIORS trial showed that nebivolol reduce mortality and hospitalizations by 14% when compared to placebo. Nebivolol was also well tolerated, including in patients with impaired renal function, and the proportion of patients discontinuing treatment due to adverse events was similar in nebivolol and placebo arms (Flather et al., 2005).

As stated before, COPD is considered a contraindication to BB treatment because of the risk to induce bronchoconstriction, thus worsening symptoms. To this aim, cardioselective BB have been proposed to overcome the possible lack of tolerability by HF patients with concomitant COPD. A recent analysis by Mentz et al. has compared β1-selective versus non-selective drugs in determining worse outcomes in HF patients enrolled in the OPTIMIZE-HF trial. The Authors found no evidence that cardioselectivity was associated with better outcomes, in terms of both mortality and tolerability. The Authors also suggest that use of non-selective BB might be helpful for HF (antagonism on β2-AR, whose signaling has a relevant role in the failing heart) and

COPD (reduction of pulmonary desensitization caused by β2-agonists). These data support the hypothesis that BB might be safe and well tolerated also in patients with HF and COPD (Mentz et al., 2013).

CONCLUSIONS

BB use is strongly recommended in all current guidelines for patient with symptomatic HF and impaired systolic function, unless there is a contraindication. Although wide, randomized, controlled clinical trials have investigated efficacy of three molecules, thus driving to the registration of carvedilol, metoprolol succinate and bisoprolol for the treatment of HF, it appears reasonable to suppose that BB efficacy lies in their ability to counteract adrenergic overactivation, more than in additional, molecule-related properties. Such consideration is supported by different meta-analyses suggesting that BB efficacy should be considered as a class effect. However, peculiar mechanisms of action of distinct BB (i.e., vasodilation, NO release, anti-oxidant activity, anti-proliferant actions on vascular smooth muscle cells) might represent additional and useful tools for HF therapy and to increase treatment tolerability, mainly in selected groups of patients such as elderly; a careful evaluation of the patient and his clinical condition should be made. For instance, molecules such as carvedilol, which have been demonstrated to ameliorate insulin sensitivity, could be useful in patients with concomitant diabetes. Moreover, analysis of genetic polymorphisms in β-ARs and metabolic enzymes, might also contribute to find "the best drug to the best patient."

In conclusion, BB are currently a cornerstone in HF therapy, and their use should be extended also to groups of patients commonly undertreated, such as elderly or comorbid patients.

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Targeting cardiac β-adrenergic signaling via GRK2 inhibition for heart failure therapy

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Cardiac cells, like those of the other tissues, undergo regulation through membrane-bound proteins known as G protein-coupled receptors (GPCRs). β-adrenergic receptors (βARs) are key GPCRs expressed on cardiomyocytes and their role is crucial in cardiac physiology since they regulate inotropic and chronotropic responses of the sympathetic nervous system (SNS). In compromised conditions such as heart failure (HF), chronic βAR hyperstimulation occurs via SNS activation resulting in receptor dysregulation and down-regulation and consequently there is a marked reduction of myocardial inotropic reserve and continued loss of pump function. Data accumulated over the last two decades indicates that a primary culprit in initiating and maintain βAR dysfunction in the injured and stressed heart is GPCR kinase 2 (GRK2), which was originally known as βARK1 (for βAR kinase). GRK2 is up-regulated in the failing heart due to chronic SNS activity and targeting this kinase has emerged as a novel therapeutic strategy in HF. Indeed, its inhibition or genetic deletion in several disparate animal models of HF including a pre-clinical pig model has shown that GRK2 targeting improves functional and morphological parameters of the failing heart. Moreover, non-βAR properties of GRK2 appear to also contribute to its pathological effects and thus, its inhibition will likely complement existing therapies such as βAR blockade. This review will explore recent research regarding GRK2 inhibition; in particular it will focus on the GRK2 inhibitor peptide known as βARKct, which represents new hope in the treatment against HF progression.

Keywords: heart failure, β adrenergic system, β blockers, G-protein coupled receptors, G-protein-coupled receptor kinase 2

INTRODUCTION

Heart failure (HF) is a major and growing public health problem and despite effective therapy outcomes remain poor with a 5-year mortality at 50% (Braunwald, 2001; Hunt, 2005). Clinically, HF is a chronic and severely debilitating syndrome that generally ends up in a vicious cycle of progressive functional decline and it is characterized by the insufficient pumping of blood to meet the needs of the body. During HF, sympathetic nervous system (SNS) activity and levels of catecholamines are increased in an attempt to drive pump function higher. Initially, SNS hyperactivity serves to compensate for the reduced cardiac output, but long-term exposure to high levels of circulating catecholamines causes maladaptive changes to the heart through dysregulation of their β-adrenergic receptor (βAR) targets and also causes myocyte death that leads to maladaptive remodeling of the stressed and failing heart (Woo and Xiao, 2012). Currently, HF therapy is palliative and protecting the heart against SNS bombardment through βAR blockers has shown to offer benefit (Barki-Harrington et al., 2003). However, β-blockers and other neurohormonal blocking strategies are still not ideal therapies as not all patients respond favorably to these agents and thus, new therapeutic strategies are urgently needed. In this regard, a deeper understanding of the underlying molecular mechanisms contributing to the development and progression of the disease represents the best case for future therapeutic advances. In the last two decades, the study of G protein-coupled receptor (GPCR) signaling in failing myocardium has led to the identification of GPCR kinase 2 (GRK2) playing a central role in HF pathology. Accordingly, the inhibition of GRK2 appears to be a powerful therapeutic approach and appears to provide complementation to β-blockade.

βAR IN CARDIAC PHYSIOLOGY AND PATHOLOGY

βARs are the most important GPCR class expressed in the human heart and represent the most powerful means to increase the pumping function of the heart. In particular, βARs are the prime modulators of heart rate and myocardial contractility in response to catecholamines originating from the SNS (Huang et al., 2011). Three βAR subtypes (β_1, β_2, and β_3) have been identified in human cardiac tissue with the β_1- and β_2ARs representing the majority of βARs in the myocyte driving functional responses and these receptors are in a 3–4:1 ratio (β_1:β_2) in the normal heart (Frielle et al., 1987; Kobilka et al., 1987; Emorine et al., 1989). The β_3AR is relatively minor although it is present and may contribute to normal and diseased myocardial regulation (Aragón et al., 2011). Following catecholamine stimulation, both β_1- and β_2ARs couple to adenylyl cyclase (AC) stimulatory G protein, Gs, leading to cAMP accumulation within the myocyte and

activation of protein kinase A (PKA). This kinase phosphorylates many Ca^{2+} handling protein and some myofilament components leading to positive inotropic, lusitropic and chronotropic effects (Bristow et al., 1990). Importantly, stimulation of cardiac β_2ARs also causes stimulation of pertussis toxin (PTX)-sensitive Gi signaling pathways (Xiao, 2001). Gi activation can lead to AC inhibition and also other signaling pathways independent of β_1AR pathways including activation of mitogen-activated protein kinase (MAPK) pathways and also PI3-kinase and Akt pathways (Xiao, 2001). Therefore, these distinct G protein-coupling characteristics of β_1ARs and β_2ARs can result in differential regulation and fate of cardiac myocytes (**Figure 1**). Known differences include myocyte cell death and survival as β_1AR stimulation leads to apoptosis while β_2AR signaling favors cell survival pathways (Communal et al., 1999; Zaugg et al., 2000). The later has been shown to be through a β_2AR-Gi-G$\beta\gamma$-PI3K-Akt cell survival signaling pathway and the inhibition of this pathway converts β_2AR signaling from survival to apoptotic (Zhu et al., 2001). β_1- and β_2ARs also manifest opposing effects on cardiac cell growth as stimulation of β_1ARs, but not β_2ARs, can cause hypertrophy in cultured neonatal and adult rat cardiac myocytes (Schafer et al., 2000; Morisco et al., 2001).

GRKs

βARs and other GPCRs undergo regulation for signal termination immediately following activation through phosphorylation by a family of kinases known as GPCR kinases (GRKs). GRK2 is a principal GRK involved in intracellular βAR signaling within the cardiac myocytes (**Figure 1**) and as discussed below, plays a crucial role in HF (Keys and Koch, 2004). Of the seven mammalian GRKs that have been identified to date, GRK2 and GRK5 represent the most abundantly expressed types in the heart and both can regulate βAR signaling (Huang et al., 2011). In addition to βARs, GRK2 and other GRKs phosphorylate many receptors and appear to have some substrate specificity in the heart (Eckhart et al., 2000). All GRKs are serine/threonine kinases with similar structural architecture as they all have a highly-conserved, central catalytic domain (∼270 aa), flanked by a variable amino-terminal (NT) domain (∼185 aa) and carboxyl-terminal (CT) domain (∼105–230 aa) that contains specific regulatory sites (Rockman et al., 1998b; Vinge et al., 2008). The NT-domain harbors several regulatory motifs including a RH domain (regulator of G protein signaling homology domain) and the CT-domain mediates the interactions with lipids and membrane proteins that control the subcellular localization of GRKs (Pitcher et al., 1998; Penela et al., 2010). Importantly, the CT-domain of GRK2 (and the related GRK3) contains a pleckstrin homology domain (PH), which interacts with phosphatidylinositol 4,5-biphosphate (PIP2) and free $G_{\beta\gamma}$ subunits. Following these interactions, GRK2 translocates to the plasma membrane enhancing activated GPCRs phosphorylation. GRK5 does not have this domain and does not use $G_{\beta\gamma}$ to target the plasma membrane and thus, GRK2 can be selectively targeted through CT-derived peptides that keep GRK2 off the membrane and can limit the desensitization of GPCRs including cardiac βARs (Koch et al., 1993, 1995).

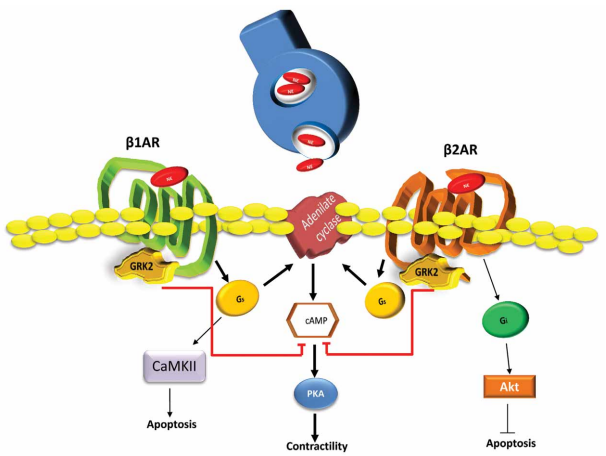

FIGURE 1 | Differential signaling pathways activated by β_1ARs and β_2ARs in cardiomyocytes. Following SNS catecholamines (Norepinephrine-NE) stimulation, activated β_1ARs and β_2ARs induce the production of cAMP through Gs activation of adenylyl cyclase (AC). Next, cAMP activates PKA resulting in positive chronotropic and inotropic effects. Secondarily, β_1AR through Gs and Calmodulin-dependent kinase (CAMKII) dependent pathway can induce myocyte apoptotic responses after increased catecholamine stimulation. In contrast, β_2ARs can induce the activation of Gi dependent cell survival and anti-apoptotic signaling through Akt. Both β ARs are regulated by GRK2 phosphorylation causing the dampening of G protein activation and chronically this leads to receptor down-regulation and the loss of inotropic reserve. Heightened GRK2 activity, as seen in human HF, appears to have a net pro-death effect due, at least in part to desensitization of β_2ARs (Brinks et al., 2010) and also due to novel GPCR-independent mitochondrial targeting (Chen et al., 2013).

GRK2 AND βAR SIGNALING IN HF

As mentioned above, GRKs phosphorylate activated receptors leading to desensitization to control their over-stimulation (Freedman et al., 1995; Rockman et al., 2002) and since βARs are an important part of the heart's response toward stress and injury they are GRK targets. Following GRK-mediated phosphorylation of βARs in the heart, β-arrestins are recruited to these receptors and these adaptor molecules block further G protein activation (Ferguson, 2001). β-arrestins then internalize receptors leading to their degradation, resensitization and return to the membrane or induce G protein-independent signaling (Pitcher et al., 1998). Overall, βAR signaling is critical for both normal and diseased heart function and as introduced above their dysregulation in injured/stressed myocardium is a cardinal characteristic of HF. Over the last two decades GRK2 has been shown to be the central culprit in cardiac βAR dysregulation as its up-regulation causes a loss of βAR responsiveness that is marked by both chronic receptor desensitization and down-regulation, which originally occurs due to heightened SNS activity (Bristow et al., 1982; Iaccarino et al., 1998; Rengo et al., 2009; Raake et al., 2013). Importantly, the major βAR down-regulation that occurs is selective for the β_1AR and this causes a change in β_1:β_2 ratio to closer to equal (Bristow et al., 1990; Rockman et al., 1998a; White et al., 2000). Certainly, the up-regulation of GRK2 (and also probably GRK5) caused by SNS activation acts as a "brake" for cardiac βAR signaling and this is a partial reason why β-blockers have some beneficial effects in HF as they can block the noxious effects of catecholamines on the

cardiac myocyte. However, increased GRK2 activity has proven to be maladaptive in HF and its inhibition has emerged as a therapeutic target (Lymperopoulos et al., 2012). Interestingly, chronic β-blocker use has shown including in HF models, to decrease GRK2 expression levels, which from data with GRK2 lowering alone, could contribute to their therapeutic effects (Iaccarino et al., 1998; Rengo et al., 2009).

YIN AND YANG OF HF THERAPY-BETA ADRENERGIC RECEPTORS BLOCKERS

β-blockers are currently considered as the mainstay of HF therapy. Behind pharmacologic β-blockade action is the ability of these molecules to inhibit the excessive catecholamine stimulation of inotropic βARs that induce myocyte cell death so resulting in cardiac deterioration, and changes in ventricular mass and that promote ventricular dilation through HF (Mann, 1999; Bristow, 2011). Importantly, although β-blockers only indirectly address key molecular signaling alterations within the cardiac myocyte, their clinical use substantially improves HF prognosis increasing survival rate of HF patients and reducing re-hospitalization (Bristow, 1997, 2000). Interestingly, it has been observed that at molecular level, sustained therapy with β-blockers in HF is associated with resensitization of βARs, normalization of GRK2 levels and activity and as a consequence, β-blockers cause the upregulation of cardiac βARs (down-regulated in HF) increasing βAR signaling when they are activated (Leineweber et al., 2005). However, the full extent of whether these molecular changes are the true therapeutic mechanisms of action of βAR antagonists in HF is not fully understood as there are some specific differences in β-blockers classes used. In fact, β-blockers are a heterogeneous group of pharmacologic agents with a number of different actions that include β_1AR and β_2AR antagonism, intrinsic sympathomimetic activity (ISA) (Jaillon, 1990), inverse agonism (Chidiac et al., 1994) and guanine nucleotide binding regulation (Bristow et al., 1992). Further, β-blockers may possess additional properties such as vasodilatation via α_1AR antagonism and non-adrenergic pharmacological properties such as quinidine-like (or "membrane stabilizing") effects in model systems (Tritthart et al., 1969) and may have also significant antioxidant properties (Dandona et al., 2000).

Of note, β-blocker therapy has limitations in the HF patient population and it is not tolerated by all patients and is certainly not an ideal therapeutic. For example, long-term treatment with β-blockers in HF patients is associated with risk of unwanted systemic side effects. In this context it has been described that increased bradycardia (due to depression of the cardiac conduction system), hypotension, dizziness, weakness, and worsening of depression represent some of the major deleterious effects of β-blocker non-selectivity (Packer, 2001). Furthermore, dose, a critical factor for therapeutic success, has to be titrated individually for each HF patient. Finally, not all HF patients are suited for β-blocker treatment, opening a demand for new therapeutic strategies. For this reason the development of new potential molecules that minimize the unfavorable effects and potentially allow dose reduction is absolutely needed and we believe inhibiting GRK2 is an attractive target not only because it can also normalize βAR signaling but also it appears to have exciting and novel non-GPCR effects that has appeal for targeting its inhibition that can synergize with β-blocker use in HF patients (see below).

GRK2 NON-RECEPTOR FUNCTIONS IN HF

Although as discussed above it is clear that enhanced activity of GRK2 in HF negatively affect βAR signaling, we have recently demonstrated that GRK2 can induce myocardial pathology through other systems including non-GPCR activity that can negatively affect myocyte metabolism and cell survival (Brinks et al., 2010; Ciccarelli et al., 2011; Chen et al., 2013; Fan et al., 2013). For example, data now supports GRK2 being a molecular link between the excessive neurohormonal activation that follows cardiac stress and initiation of defects in myocyte energy substrate use by negatively affecting glucose uptake in the myocyte through insulin-dependent phosphorylation of insulin receptor substrate-1 (IRS1) that causes a loss of signaling (Ciccarelli et al., 2011). Thus, GRK2 appears to directly modulate signaling through this non-GPCR and participates in the physiological regulation of myocardium insulin signaling. Moreover, our group have also showed that elevated levels of GRK2 in cardiomyocytes cause excessive cell death after acute ischemic injury and targeted inhibition or genetic deletion significantly protects the heart (Brinks et al., 2010; Fan et al., 2013), which importantly argues against GRK2-up-regulation being initially adaptive as elevated GRK2 activity appears to be maladaptive at all times prior to and after cardiac stress. The pro-death effects of GRK2 may be β_2AR dependent (Brinks et al., 2010) or non-GPCR dependent as we have found that myocyte death during ischemic stress can be regulated by GRK2 levels found within mitochondria as GRK2 is associated with increased mitochondrial-dependent pro-death signaling and also caused increased Ca^{2+}-induced opening of the mitochondrial permeability transition pore, a key step in cellular injury (Chen et al., 2013). Accordingly, GRK2 inhibition reduces IRS1-phosphorylation improving insulin signaling and as a result increase glucose uptake in the ischemic cardiomyocytes and its inhibition but also reduces the pro-apoptotic pathway activated by this kinase in response to ischemia. For these reasons it is important to underline that these non-classical roles of GRK2 strongly support the idea that developing a therapy that selectively inhibits GRK2 could synergize and increase the efficacy of β-blockers as GRK2 inhibition would result in additional benefits independent of βARs. In fact, several animal studies have shown significant HF benefit when GRK2 is lowered or inhibited concurrent with βAR antagonists (Harding et al., 2001; Raake et al., 2008; Rengo et al., 2009).

GRK2 INHIBITION AS A POTENTIAL APPROACH FOR HF TREATMENT

Over the last two decades, our lab and others have shown that lowering GRK2 expression or activity in the injured, stressed or failing heart can prevent or reverse ventricular dysfunction at the functional and morphological level (Huang et al., 2011). This has been shown using a CT-derived peptide inhibitor known as the βARKct and also in GRK2 knockout (KO) mice. First, cardiac-specific βARKct transgenic mice were created showing increased inotropic reserve (Koch et al., 1993) and these mice have been

used to prevent HF in several genetic mouse models (Rockman et al., 1998a; Freeman et al., 2001; Harding et al., 2001). In addition, viral-mediated βARKct delivery to rats, rabbits, and pigs have shown significant beneficial effects including improved cardiac function and reverse ventricular remodeling (White et al., 2000; Shah et al., 2001; Tevaearai et al., 2001; Rengo et al., 2009; Raake et al., 2013). Similar HF rescue by induced KO of GRK2 in mice after HF was evident is consistent with βARKct-mediated activity being GRK2 inhibition (Raake et al., 2008). Although there are some minor differences between GRK2 inhibition with βARKct and GRK2 KO (Matkovich et al., 2006; Raake et al., 2008; Völkers et al., 2011) the data is overwhelming in supporting GRK2 targeting as beneficial in the failing heart.

Importantly, a recent pre-clinical, large animal HF study has been done that demonstrates the clinical potential of βARKct-mediated gene therapy (Raake et al., 2013). This study used adeno-associated virus serotype-6 (AAV6) to deliver βARKct to a post-ischemic HF model in the pig (Pleger et al., 2011; Raake et al., 2013). We found that AAV6-βARKct delivery via retrograde coronary venous perfusion ameliorated LV function, and suppressed adverse cardiac remodeling and fetal gene expression in this model (Raake et al., 2013). Of note, this pig study recapitulated similar results found with AAV6-βARKct delivery to a rat model of HF where it was also shown that βARKct worked significantly better that β-blockade alone and the two were complementary together (Rengo et al., 2009). Of importance, both of these studies showed that chronic GRK2 inhibition results in significant lowering of catecholamines and aldosterone demonstrating feedback to decrease the neurohormonal outflow associated with negative prognosis in HF (Rengo et al., 2009; Raake et al., 2013). These two studies in particular are important as they show reversal of the disease process and the pig study closely reflect human pathophysiology and is a pre-requisite for future clinical trials.

EMERGING SMALL MOLECULE INHIBITORS OF GRK2

Gene therapy for HF is now becoming a reality (Jaski et al., 2009; Jessup et al., 2011) and AAV6-βARKct trials are in the planning stages, GRK2 inhibition by small pharmacological agents would offer many advantages to the HF patient. Interestingly, several recent molecules have been developed and described that have GRK2 inhibitory properties. Two decades ago, heparin and related compounds were shown to block GRK2 activity however, the direct access to GRK2, the high concentration and the intrinsic cytotoxicity made them not useful for either in cell-based assays or in *in vivo* scenarios (Lohse et al., 1989; Kim et al., 1993; Hasbi et al., 2000; Kassack et al., 2000; Winstel et al., 2005). RNA molecules such as aptamers have been also investigated as a new approach to efficiently block GRK2 activity and the RNA-aptamer C13, was shown to be able to bind to GRK2 with a high affinity and inhibit GRK2-catalyzed rhodopsin phosphorylation (Mayer et al., 2008). All the studies that have analyzed the efficacy of C13, suggest that this RNA-aptamer might represent a starting point for the development of small molecules that specifically target GRK2. Unfortunately, this molecule has been tested only in *in vitro* models and no *in vivo* study at this time is present.

Interestingly, molecules have recently emerged that target the GRK2-$G_{\beta\gamma}$ protein-protein interaction and thus, have mechanisms identical to the βARKct. M119 is such a molecule (Bonacci et al., 2006; Casey et al., 2010) and it has been shown to work *in vitro* and *in vivo* on cardiac cells and in the heart preventing ventricular dysfunction after chronic catecholamine exposure and also showing positive results similar to the βARKct in a genetic model of cardiomyopathy (Casey et al., 2010). Gallein is a related molecule that blocks GRK2-Gβγ and it has also shown positive results *in vivo* (Piao et al., 2012). These are promising results, however, these compounds are not true pharmacological agents in a "drugable" sense and have severe limitations that preclude human use (Casey et al., 2010).

Recently, we have found that an existing FDA-approved drug has significant GRK2 inhibitory properties and potentially this off-target effect may be seen in humans. The serotonin reuptake inhibitor (SSRI), paroxetine has affinity for GRK2 and has significant GRK2 inhibitory properties *in vitro* and *in vivo* (Thal et al., 2012). Paroxetine binds in the active site of GRK2 and stabilizes the kinase domain in a novel conformation in which a unique regulatory loop forms part of the ligand binding site (Thal et al., 2012). Further, this drug causes increased isoproterenol-induced shortening and contraction amplitude in cardiomyocytes *in vitro*, and pretreatment *in vivo* of mice with paroxetine before isoproterenol significantly increases left ventricular inotropic reserve with no significant effect on heart rate (Thal et al., 2012). This agent used for clinical depression probably is not viable for use as a specific GRK2 inhibitor but is a great starting point for chemistry to develop novel GRK2 inhibitors that can be used eventually for cardiovascular disorders.

CONCLUSIONS AND FUTURE PERSPECTIVES

As demonstrated by us and others, targeted GRK2 inhibition primarily by βARKct expression and some emerging small molecules have shown sustained improvement of global cardiac function and reversal of LV remodeling at least in part due to the normalization of the neurohormonal signaling axis and βAR signaling. In addition, non-GPCR effects of lowering GRK2 activity, has positive effects on cardiac metabolism and on cell survival/death pathways (**Figure 2**). Importantly, the specific targeting of GRK2 appears similar whether there is inhibition by βARKct or deleting gene expression. Therefore, taken together, these results strongly suggest that the inhibition/lowering of GRK2 activity is a valid and promising novel molecular approach for treating HF. Most studies, including studies with βARKct expression in HF pigs, have shown a reversal of βAR dysfunction including receptor upregulation and a normalization of signaling; however, no doubt, there are effects of the βARKct that go beyond resensitization of cardiac βARs and these effects are currently being explored by us and others. Therefore, these results have launched a clinical gene therapy approach using the βARKct with a Phase I clinical trial being actively planned in order to obtain the best cardio-selective HF treatment that, as widely proven by all gathered data collected by us, could be used alone or in conjunction with the actual β-blockers therapy.

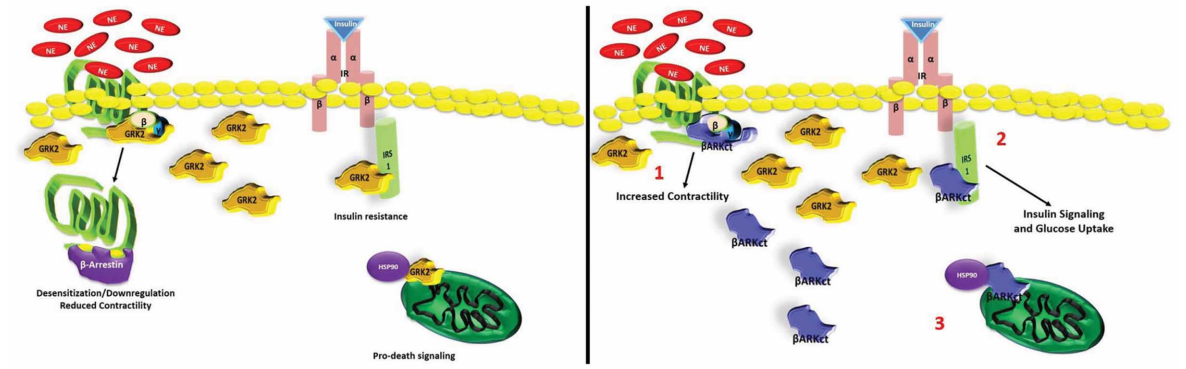

FIGURE 2 | Multiple protective role of βARKct in failing myocardium against high GRK2 levels. Schematic representation of GRK2-inhibition mediated by βARKct: (1) βARKct, like GRK2, binds to $G_{\beta\gamma}$ subunits after GPCR (e.g., βARs) activation and reduces the capability of GRK2 to induce dysregulation/downregulation of these receptors; (2) βARKct antagonizes GRK2 dependent phosphorylation of IRS1 increasing glucose uptake in myocytes; (3) βARKct blocks the ischemia-induced mitochondria localization of GRK2 through inhibition of MAPK-dependent Hsp90 binding, inhibiting pro-apoptotic signaling.

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Adrenal adrenoceptors in heart failure

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Heart failure (HF) is a chronic clinical syndrome characterized by the reduction in left ventricular (LV) function and it represents one of the most important causes of morbidity and mortality worldwide. Despite considerable advances in pharmacological treatment, HF represents a severe clinical and social burden. Sympathetic outflow, characterized by increased circulating catecholamines (CA) biosynthesis and secretion, is peculiar in HF and sympatholytic treatments (as β-blockers) are presently being used for the treatment of this disease. Adrenal gland secretes Epinephrine (80%) and Norepinephrine (20%) in response to acetylcholine stimulation of nicotinic cholinergic receptors on the chromaffin cell membranes. This process is regulated by adrenergic receptors (ARs): α2ARs inhibit CA release through coupling to inhibitory Gi-proteins, and βARs (mainly β2ARs) stimulate CA release through coupling to stimulatory Gs-proteins. All ARs are G-protein-coupled receptors (GPCRs) and GPCR kinases (GRKs) regulate their signaling and function. Adrenal GRK2-mediated α2AR desensitization and downregulation are increased in HF and seem to be a fundamental regulator of CA secretion from the adrenal gland. Consequently, restoration of adrenal α2AR signaling through the inhibition of GRK2 is a fascinating sympatholytic therapeutic strategy for chronic HF. This strategy could have several significant advantages over existing HF pharmacotherapies minimizing side-effects on extra-cardiac tissues and reducing the chronic activation of the renin–angiotensin–aldosterone and endothelin systems. The role of adrenal ARs in regulation of sympathetic hyperactivity opens interesting perspectives in understanding HF pathophysiology and in the identification of new therapeutic targets.

Keywords: heart failure, adrenergic system, GRK2, adrenal gland, catecholamine, β-adrenergic receptor, functional recovery

BACKGROUND

The sympathetic nervous system (SNS) is part of the autonomic nervous system and its activation was described by Cannon as "the fight or flight response" (Cannon, 1963). In the clinical setting, sympathetic nervous activity (SNA) can be evaluated by the analysis of plasmatic or urinary catecholamine concentrations but this estimation is variable depending on hormonal and other intra-individual adjustments, as well as on several factors such as glycemia, physical or psychological stress, and drugs. In recent years, many methods have been proposed for the assessment of SNA and, among these, microneurography and heart rate variability (HRV) are the most commonly used. Microneurography allows for a direct evaluation on electrical transmission in subcutaneous sympathetic nerves but it is not applicable for studies of large number of patients.

HRV analysis is spreading as a non-invasive technique for the evaluation of the autonomic nervous system influence on heart rate in various diseases and alterations in HRV have been shown to represent an independent predictor of mortality after myocardial infarction (Amadi et al., 1995). HRV gives an estimation on how the cardiac equilibrium between parasympathetic and sympathetic systems influences heart rate studying variations in cardiac frequencies.

Sympathetic hyperactivity has been recognized as a peculiar feature of several cardiovascular diseases as atherosclerosis, heart failure (Leimbach et al., 1986; Lymperopoulos et al., 2013), hypertension (Grassi, 1998), and syncope (Zysko et al., 2007).

Furthermore, sympathetic overdrive is associated also with non-cardiovascular pathologies: hyperglycemia and diabetes mellitus (Huggett et al., 2003), obesity and metabolic syndrome (MS) (Grassi et al., 2007), obstructive sleep apnea (Narkiewicz and Somers, 1997), and renal disease (Masuo et al., 2010).

Therefore some authors hypothesized a close connection between the components of the metabolic syndrome and augmented sympathetic activity suggesting a role of this latter in syndrome's establishment or progression (Esler et al., 2006). It is important to emphasize that most of SNA-related diseases, including HF, MS, and hypertension are major causes of morbidity and mortality worldwide. Sympathetic hyperactivity leads to an increase in arterial blood pressure and it is known to cooperate to the establishment and development of essential hypertension (Smith et al., 2004) through alterations in structural components of vessels and cardiac tissue with dysfunctional consequences.

HF is a chronic clinical syndrome characterized by the reduction in left ventricular (LV) function with the inability to adequately pump blood, maintain tissue perfusion, and support physiological functions. This leading disease represents one of the most important causes of morbidity and mortality worldwide (Go et al., 2014). Despite considerable advances in pharmacological treatment, HF represents a severe clinical and social burden.

During HF, several neurohormonal mechanisms get triggered in order to maintain cardiac output. The most important among these neurohormonal mechanisms are the SNS overdrive characterized by elevated circulating Catecholamines (CAs) and the Renin-Angiotensin-Aldosterone System hyperactivity. Consequently, sympatholytic drugs such as beta-blockers, Angiotensin-converting enzyme inhibitors, Ang-II receptor blockers and mineralcorticoid receptor antagonists are a cornerstones for the treatment of HF disease by ameliorating cardiac function (CIBIS-II Investigators and Committees, 1999; Von Lueder and Krum, 2013). The increase in circulating levels of Epinephrine (Epi) and Norepinephrine (NEpi) is initially needed to compensate heart dysfunction, according to the fundamental Frank-Starling law of cardiac function. However, if the cardiac insult persists, this law can no longer work and the process progressively becomes maladaptive and conducts to decompensated phase of HF, adversely impacting the clinical outcomes (Cohn et al., 1984; Lymperopoulos, 2013).

Body's major source of CAs is the adrenal medulla, the central part of the adrenal gland, where the chromaffin cells secrete approximately 20% NEpi and 80% Epi (Lymperopoulos et al., 2007b). The adrenal gland obtains input from the SNS through pre-ganglionic fibers and can be compared to a specialized sympathetic ganglion but it has the peculiar characteristic to secrete neurohormones directly into the blood. Chromaffin cells are post-ganglionic sympathetic neurons that have lost part of their peculiar characteristics as axons and dendrites and secrete their hormones into the bloodstream by exocytosis (Haase et al., 2011). The existing link between SNA and heart pathophysiology is very inescapable and suggestive.

In particular, since 1984 it was clear that plasma concentration of NEpi was negatively associated with survival in heart failure patients and the augmented plasma concentrations led to higher mortality (Cohn et al., 1984).

Furthermore, sympathetic overdrive in HF determines higher risk of arrhythmias and left ventricular dysfunction contributing to worsen the prognosis of this disease (Kaye et al., 1995). In addition, this linkage is more evident when evaluating cardiac consequences in Pheochromocytoma (PCC). PCC is rare neuroendocrine tumor of the adrenal glands medulla arising from the chromaffin cells (in 20% from extra-adrenal abdominal paraganglion tissue) and secreting high levels of catecholamines. Pheochromocytoma is present in 0.1–1% of patients with hypertension (Anderson et al., 1994) and it is present in phosphorylates of these tumors are mainly due to augmented CAs, particularly NEpi: tachycardia and palpitations, hypertension, acute myocardial infarction, angina, arrhythmias, left ventricular dysfunction, heart failure, and pulmonary edema. However, some Epi- and Dopamine-secreting tumors can determinate hypotension or cardiogenic shock (Bergland, 1989). Uncommon cardiac manifestations are rhythm disturbances as ventricular tachycardia, ST-segment elevation, prolongation of the QT interval and T-wave modifications.

CAs bind to adrenergic receptors (ARs) that are the principal mediators of SNS effects. So far, nine mammalian AR subtypes are known: three α1-AR, three α2-AR, and three β-AR (Bylund et al., 1994). ARs are part of the G-protein-coupled receptors (GPCRs) superfamily, membrane receptors that activate heterotrimeric G-proteins after their ligand binding. G-proteins typically stimulate (Gs-proteins) or inhibit (Gi-proteins) the enzyme adenylyl cyclase (AC) or activate (Gq-proteins) phospholipase C (PLC) (Rengo et al., 2009a). These receptors are phosphorylated by the family of GPCR kinases (GRKs) that regulates their pathway and function (Davis and Johnson, 2011). Cardiac role of βARs is the regulation of heart rate and contractility in response to CAs.

Stimulation of β1ARs (the primary subtype present on cardiomyocytes) and partially of β2ARs has inotropic, dromotropic, cronotropic, and lusitropic effects (Grossini et al., 2013).

β1ARs and β2ARs activates both Gs proteins (stimulatory G proteins); however, β2AR can switch its signaling from Gs to Gi proteins when is phosphorylated by PKA. In addition, β1AR stimulation determinates cardiomyocyte apoptosis while β2AR has antiapoptotic cardiac effects in the heart (Rengo et al., 2012c; Lymperopoulos, 2013; Lymperopoulos et al., 2013; Salazar et al., 2013).

High CAs levels determinate structural alterations in the heart: focal myocardium necrosis and monocytic inflammation, increased collagen deposition and consequent interstitial fibrosis in the arterial wall and in the myocardium (Roghi et al., 2011). Norepinephrine can increase cardiac oxygen consumption and myocytes apoptosis with consequent left ventricular alteration and dilated cardiomyopathy (Prejbisz et al., 2011). CAs conduct to cardiomyopathy by GRK2-mediated downregulation of β-adrenergic receptors in the heart (β-AR) and augmented intracellular calcium concentrations resulting in decreased cardiac contraction (Kassim et al., 2008).

Accordingly, it was shown that cardiac GRK2 levels and activity were increased in end-stage human HF and heterozygous GRK2 knockout mice have augmented cardiac contractility and function (Iaccarino et al., 1998, 1999; Iaccarino and Koch, 1999; Rengo et al., 2012b). Furthermore, transgenic mice overexpressing cardiac GRK2, have decreased cardiac function due to an excessive βAR dysfunction and oppositely mice with cardioselective expression of βARKct showed improved cardiac contractility at baseline and isoprotenerol-induced (Koch et al., 1995). GRK2 enhancement determinates cell death in ischemic cardiomyocytes, and its inhibition by an inhibitory peptide (βARKct) is cardioprotective. Recently, it has been demonstrated that GRK2 is able to localize in mitochondria but his role is controversial. Koch et al. recently showed that GRK2 has a cardiac pro-death function by mitochondrial localization in myocytes after ischemic stress while Fusco et al. demonstrated that mitochondrial GRK2 plays a protective role regulating ATP production (Fusco et al., 2012; Chen et al., 2013).

Elevated circulating CAs can determinate myocardial damage by enhancing the cardiac oxygen request and by increasing

peroxidative and lipoperoxidative metabolism and consequent free radicals production (Radtke et al., 1975). Severe LV dysfunction occurs in few patients and it seems to be secondary to genetic polymorphisms of the β-adrenergic receptors that increase the propensity to develop cardiomyopathy (higher sensitivity to catecholamines) (Small et al., 2002).

α_2-ADRENOCEPTORS

The α2-ARs are inhibitory autoreceptors that inhibit further release of CAs in adrenergic nerves in the central and in the SNS, including the adrenal gland.

The predominant inhibitory role of α2ARs in the adrenal gland results clear when considering that PC12, a rat pheochromocytoma cell line typically used as neuronal cell model, does not express these receptors and secretes abnormal CAs quantity.

However, it has been discovered that different α-AR subtypes explicate their main action in diverse organs (Brede et al., 2003). Due to the absence of selective drugs for the three α2-ARs subtypes, gene deletion in animals or cells lacking α2-AR subtypes have been necessary to understand the real function of these different subtypes. α2A-AR and α2C-AR perform their role as autoreceptors on neurons of peripheral nerve terminals and in the heart, inhibiting NEpi release. In particular, α2A-AR inhibits hormones release at high stimulation frequencies whereas the α2C-subtype plays his role at lower levels of nerve activity. Anyway low- and high-frequency stimulations are both important for synapse regulation (Hein et al., 1999). α2C-ARs is also implicated in some brain functions as vigilance, attention, stress reaction, gait, and locomotion (Sallinen et al., 1999) and some renal functions as well as tonic renal vasoconstriction and inhibition of renin release (Michel and Rump, 1996). α2B-AR subtype is mainly expressed in the central SNS and in vascular smooth (role of vasoconstriction) cells (Link et al., 1996) and it is involved in embryonic growth probably because of his function in placental angiogenesis (Macdonald et al., 1997). Moreover, the discrepancy in secretion (noradrenergic and adrenergic) in different groups of chromaffin cells should be (Hein et al., 1999) connected to different α2-AR subtypes expression.

In addition, α2-AR subtypes seem to play a part in neuronal differentiation. For this purpose Taraviras et al. studied the cellular modifications after Epi stimulation in PC12 cells expressing only one of the different α2-AR subtypes. They found that Epi can induce a diverse neuronal differentiation in a subtype-dependent way. Particularly, PC12α2B- and PC12α2C-transfected cells presented evident Epi-induced differentiation showing neurofilaments typical of differentiated neurons while PC12α2A-transfected cells didn't need Epi for their differentiation. Furthermore, they have shown that mitogen-activated protein kinase (MAPK) and Akt activation are needed for α2-AR–dependent neuronal differentiation (Taraviras et al., 2002). All these findings suggest that α2-AR subtypes differential expression in neuronal or neuron-like cells can influence not only organ tissue-specificity but also embryonic evolution and cellular differentiation. Moreover, α2-ARs could exert their neurogenic effects via the NF-kB pathway. NF-kB phosphorylation and consequent degradation of IkBα is under β-arrestins (β-arrs) control opening new interesting scenarios (Luttrell and Lefkowitz, 2002; Bathgate-Siryk et al., 2014). The specific subtypes of α2-ARs prevailing in the adrenal glands are still unknown and it seems there could be a species-specificity. Particularly in mice's adrenal gland α2C-AR subtype is the most important, while α2A-AR seems to be the most represented in rats (Lymperopoulos et al., 2007a). Thus, different expression of α2-AR subtypes reflects diverse neurotransmitter secretion in peripheral nerves and adrenal gland.

It is known that the major source for plasma NEpi are peripheral sympathetic nerve terminals while for Epi is the adrenal gland. The role of α2-AR in this story was clear when Brede et al showed that mice lacking the α2C-AR have twice plasmatic Epi levels compared to wild-type, whereas mice lacking the α2A-AR subtype presented higher NEpi levels of NEpi than wild-type (Brede et al., 2003).

In human adrenal the situation is controversial: α2A-AR is the most expressed but some authors reported that α2C-AR is present, too (Berkowitz et al., 1994). It is important to emphasize that human α2-AR subtypes dysfunction/deletion can influence SNS activation and heart function.

Patients with heart failure carrying a variant of the α2C-adrenoceptor with less function (α2C-Del322–325) showed reduced cardiac function (measured by echocardiography and cardiac catheterization) than patients with intact α2-adrenoceptor (Brede et al., 2002). Moreover, α2C-Del322–325 polymorphism in healthy people led to increased SNA and circulating CAs levels during supine rest and an augmented pharmacologically-induced NEpi and Epi secretion. On the other hand, human α2B-Del301–303 (consisting in a deletion of three glutamic acids) led to impaired agonist-promoted receptor phosphorylation and desensitization. Nguyen et al, showed that in α2B-transfected PC12 cells, this deletion produces an increased inhibitory function against nicotine-induced CAs secretion suggesting that some polymorphisms can confer a favorable phenotype in increased SNA–associated diseases as HF and hypertension (Nguyen et al., 2011).

Hence, further studies on α2-AR subtypes should help researchers to better understand pathophysiology of major cardiovascular diseases and then personalize their therapy.

CATECHOLAMINES SECRETION IN ADRENAL GLAND

The adrenal medulla is mainly constituted of groups of adrenergic and noradrenergic chromaffin cells and in minor part of ganglionic neurons. CAs derive from the amino acid tyrosine and are the principal hormones underlying the fight-or-flight response. Catecholamines from chromaffin cells are secreted after acetylcholine stimuli (from sympathetic ganglia) and their exocytosis is regulated by numerous membrane receptors (Becherer et al., 2012). Most of these receptors are GPCRs (G-Protein coupled receptor) comprehending ARs that exert their function as autoreceptors. In particular, βARs (primarily β2 subtype) stimulate CAs secretion (facilitatory autoreceptors) while the α2ARs inhibit CA secretion (inhibitory autoreceptors) (Foucart et al., 1988). ARs signaling and function are regulated by the family of GPCR kinases (GRKs), whose role has been well studied in HF (Rengo et al., 2009b, 2012b, 2014; Lymperopoulos et al., 2012; Salazar et al., 2013).

Circulating CAs originate from two major sources in the body: the sympathetic nerve endings, which secrete NEpi, and the chromaffin cells of the adrenal medulla, that liberate Epi (principally) and NEpi after acetylcholine stimulation of the nicotinic cholinergic receptors (nAChRs). Chromaffin cells act as a post-ganglionic sympathetic neuron and secrete different quantity of CAs in basal or stress conditions. However, circulating CAs levels have significant intraindividual and interindividual variations particularly after stressors as surgery (Sager et al., 1988). Basal percentages of adrenal CAs production are: 80% Epi (adrenal gland medulla is the chief source of Epi) o and 20% NEpi (Lymperopoulos et al., 2007a). Thus, we can summarize that cardiac β-ARs were bound by either Epi—deriving from the adrenal gland—than NEpi—from local sympathetic nerve terminals and in minor part from adrenal medulla. Anyway, in adrenal medulla there are other receptors that promote CAs secretion: muscarinic cholinergic receptors (mAChRs) (Zaika et al., 2004), angiotensin II receptors (Armando et al., 2004), and histaminergic receptors (Wallace et al., 2002). Furthermore, it has been shown that adenosine receptors act as inhibitory autoreceptors, though their real role and expression are not completely clarified (Tseng et al., 2001). ARs, including α2-AR and β-AR, undergo agonist-dependent desensitization and downregulation. These processes imply reduced receptor response and increased internalization due to constant or repetitive agonist binding (Reiter and Lefkowitz, 2006).

In particular, after ligand stimulation, receptor is phosphorylated by GPCR kinases (GRKs), with the subsequent binding of β-arrs to the GRK-phosphorylated receptor. Consequently, β-arrs uncouple the receptor from its related G-proteins, preventing its further binding to G-proteins and leading to downregulation (Reiter and Lefkowitz, 2006; Lymperopoulos et al., 2009, 2011).

To date, GRK2, GRK3, and GRK5 are the most significant members among the GRKs because they are present ubiquitously in mammalian body (particularly in brain and cardiac tissue) and phosphorylate most of the GPCRs. Notably, GRK2 is upregulated in the heart and adrenal glands in HF and in vascular tissue during hypertension; strategies that inhibit or inactivate GRK2 in these diseases are very interesting for future human therapy (Gurevich et al., 2012).

It has been shown that human β1- and β2-ARs (*in vivo* and *in vitro*) and α2A- and α2B-ARs (*in vitro*) are phosphorylated by GRK2 but it isn't clear if α2C-AR is a GRK2 substrate, yet (Jewell-Motz and Liggett, 1996; Rengo et al., 2012c).

Besides, the role of GRK2 on α2C-ARs phosphorylation has been demonstrated in other species, prompting to similar hypothesis in humans (Lembo et al., 1999). Recently Cortez et al. showed that β1-, β2-, and β3-ARs are expressed in cultured human adrenal chromaffin cells and in particular β2- and β3-ARs stimulation determinate CAs release and β2- and β3-antagonists counteract nicotine-induced CAs secretion (Cortez et al., 2012). CAs secretion by chromaffin cells is also strongly regulated by adrenal gland cortex. In the whole adrenal gland, the medulla and the cortex, though with a diverse embryological development, are strictly linked and crosstalk in anatomical and functional ways, influencing each other.

In particular, Glucocorticoids (GCCs), among the steroids secreted by the adrenal cortex, determinate a multitude of effects on medullary chromaffin cells. The steroids, binding their nuclear receptors, activate some transcriptional factors that increase CAs production and release, upregulate Tyrosine hydroxylase and activate an alternative splicing of phenylethanolamine N-methyltransferase, a key enzyme in the transformation of NEpi in Epi. Moreover, GCCs influence chromaffin cell differentiation and characterization, determining the acquirement of adrenergic phenotype, particularly for the cell groups adjacent to adrenal cortex (Hodel, 2001). In addition, recent studies on knockout mice (in particular for the 21-hydroxylase or for the Corticotropin releasing hormone receptor 1 genes) confirmed that GCCs stimuli is necessary for the acquisition of the adrenergic but not the noradrenergic phenotype. It is also striking that chromaffin cell products as NEpi, Epi, Dopamine, VIP and Serotonin can enhance steroidogenesis of cortical hormones (Aldosterone, Cortisol, Androstendione, Deoxycorticosterone) in a paracrine way (Haase et al., 2011).

Of note, Flugge et al. demonstrated that GCCs determinate diverse expression of α2A-and α2C-ARs in brain during chronic stress. This finding suggests that adrenal cortex hormones could influence not only the adrenergic/noradrenergic phenotype but also the adrenal αAR expression/function thus cooperating in sympathetic overdrive-related diseases (Flugge et al., 2003).

ADRENAL GRK2 AND CARDIOVASCULAR PATHOPHYSIOLOGY

HF is characterized by elevated sympathetic tone with augmented levels of circulating and synaptic CAs. In the early phase of the disease increased SNA is an useful and compensatory mechanism to maintain cardiac output by increasing heart rate and cardiac contractility but, when β-ARs become disresponsive to CAs, this chronic stimulation determinates HF progression and its consequent detrimental systemic effects (Port and Bristow, 2001). Some studies in the last 10 years underline the critical inhibitory role of presynaptic α2-AR in peripheral nerve terminals and in adrenal medulla. This finding became clearer when mice with genetic deletions or knockout (KO) for α2-AR where studied. Particularly, α2A- or α2C-ARs KO mice that underwent HF after TAC-induced pressure overload presented an increase in circulating CAs with subsequently decreased cardiac parameters compared to control mice (Brede et al., 2002). In addition, double α2A/α2C-AR KO mice showed cardiomyopathy at 4 months of age, without surgery or other treatments (Brum et al., 2002). The crucial function of human α2-AR in HF development and progression was elucidated by studies on genetic polymorphism of this receptor. Small et al. demonstrated that α2CDel322–325 polymorphism is associated with high HF risk (Small et al., 2002; Davis and Johnson, 2011) probably because this variant was associated to increased α2-AR-related CAs secretion/outflow (as shown *in vitro*) (Small et al., 2000) and subsequent detrimental cardiotoxicity due to β-AR downregulation/desensitization. Our group demonstrated few years ago that adrenal hyperfunction is crucial for HF development and evolution (Lymperopoulos et al., 2007a). During HF there is an increase in CAs production, testified by enhanced tyrosine hydroxylase levels, and secretion

(both NEpi and Epi) by hypertrophic adrenal glands (**Figure 1**). To better understand if this mechanism was peculiar of HF, we tested two different models of HF for etiology and pathology. Particularly, we evaluated rats that developed congestive HF 10 weeks after myocardial infarction induced by surgical ligation of left anterior descending coronary artery and transgenic mice with cardiac overexpression of sarcoplasmic reticulum calcium-binding protein calsequestrin that underwent progressive HF in a short time (3 months) and commonly died when they become 4 months old. In both our models of HF (independently from the reason that determinates this disease) we found adrenal GRK2-related α2-AR desensitization and downregulation that lead to enhanced circulating CAs levels. Furthermore, Schneider et al. demonstrated adrenal GRK2 upregulation in a model of cardiac hypertrophy due to pressure overload (obtained by TAC surgery), too. As expected, the degree of cardiac hypertrophy was significantly associated with adrenal weight and adrenal CAs production (Schneider et al., 2011). Of note, GRK2 increase results in α2-AR phosphorylation and subsequently in loss of inhibitory feedback (**Figure 1**). This ends up in the increase of Epi and NEpi release incisively contribute to SNS overdrive. The main function of adrenal GRK2 in sympathetic overactivity and consequent progression of HF became more evident when we tried to contrast GRK2 increase by direct adrenal injection of its inhibitor βARKct in HF rats (this peptide is the C-terminal part of GRK2 that doesn't contain the phosphorylation portion but competes with GRK2 for G-proteins βγ subunits binding). In particular we used an Adenovirus codifying for βARKct and 1 week after gene delivery we performed the *in vivo* and *in vitro* evaluations. βARKct was able, by inhibiting GRK2, to restore α2AR membrane levels/function and subsequently have a sympatholytic effect lowering plasma CAs levels. This permits to counteract CA cardiotoxic effects by decreasing cardiac β-AR downregulation/desensitization and thus ameliorate heart dilatation and function as attested by echocardiography and *in vivo* cardiac hemodynamic.

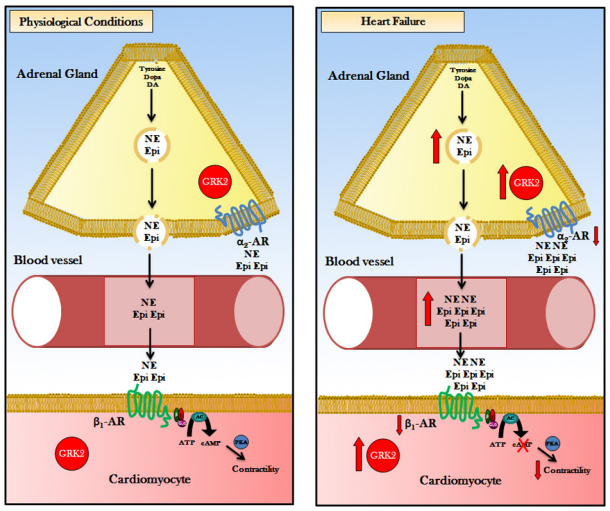

FIGURE 1 | Representation of the pathophysiologic role of GRK2 in adrenal CA-production/secretion: Body's major source of CAs is the adrenal medulla, the central part of the adrenal gland, where the chromaffin cells secrete approximately 20% NEpi and 80% Epi. Physiological conditions: G-protein-coupled receptor kinase 2 (GRK2) regulates ARs: (1) in chromaffin cell of adrenal gland GRK2 phosphorylate α2ARs that exert a tonic sympathoinhibitory function. (2) in cardiomyocytes GRK2 phosphorylate β1-AR regulate cardiac contractility by AC-PKA pathway activation. Heart Failure: G-protein-coupled receptor kinase 2 (GRK2) is upregulated in chromaffin cell and in cardiac myocyte. In the adrenal chromaffin cell, augmented GRK2 levels determinate an hyper-phosphorylation and desensitization of α2ARs, causing increased levels of Epi/NE production and secretion. Increasing in amounts of circulating CAs led to hyper-stimulation of β1-AR and GRK2 overactivation. Cardiac GRK2 upregulation results in phosphorylation and desensitization/downregulation of β1-ARs leading to reduction of contractility. Consequently, double inhibition of GRK2 (pharmacological or gene therapy) in the heart and in the adrenal gland could have impressive therapeutic effect in heart failure enhancing cardiac contractility and reducing plasmatic CAs levels. Acronyms: CAs, Catecholamines; DA, Dopamine; NE, Norepinephrine; Epi, Epinephrine; GRK2, G protein-coupled Receptor Kinase 2; ARs, Adrenergic Receptor; α2-AR, α2-Adrenergic Receptor; β1-AR, β1-Adrenergic Receptor; ATP, Adenosine Tri-Phosphate; AC, Adenylyl Cyclase; cAMP, cyclic Adenosine Mono-Phosphate; PKA, Protein Kinase A.

Recently, we decided to investigate if GRK2 inhibition before HF onset can determinate any advantage in development and progression of this invaliding disease. For this purpose we used Cre/loxP technology to obtain tissue-specific GRK2 KO mice. In particular, GRK2 was deleted only in chromaffin cells of adrenal medulla by the use of mice expressing Cre recombinase under the control of the phenylethanolamine N-methyl transferase (PNMT) gene promoter (PNMT-driven GRK2 KO mice) (Lymperopoulos et al., 2010). PNMT is the enzyme that catalyses the trasformation of NEpi into Epi and this function is peculiar in chromaffin cells. According to our results, adrenal GRK2 pre-HF deletion allows for a significant attenuation of adrenal hypertrophy and reduction of *in vivo* plasmatic CAs in post-MI HF mice. Decreased systemic cathecolaminergic stimulation that is usually detrimental for HF establishment, determinates lower cardiac β-AR downregulation/desensitization (GRK2 decreasing-mediated), with a consequent better heart function and enhanced cardiac inotropic reserve. Significantly, the PNMT-driven GRK2 KO mice showed a characteristic basal phenotype: reduced CAs production (lower Thirosine Hydroxylase protein levels) and adrenal dimensions. All these findings suggest that GRK2 could be a significant adrenal trophic element in physiologic conditions and in HF in particular, being a crucial CAs production regulator (directly acting on biosynthetic enzymes or indirectly by β2-AR mediated CAs secretion stimulation). In addition, our group has recently shown that adrenal GRK2 is also a physiological regulator of adrenal CAs production/secretion and thereby of SNA. In particular, in healthy rats, adrenal GRK2 adenovirus-mediated (Ad-GRK2) gene delivery led to increased plasmatic levels of Epi and NEpi whereas Ad-βARKct adrenal gene transfer determined a significant decrease of the same levels. Of note, despite NEpi was only the 20% of the total CAs secreted by adrenal medulla, gene delivery influencing GRK2 activity is able to change its levels. These results were confirmed by *in vitro* chromaffin cells experiments that also showed, as expected, that physiological adrenal GRK2 action is α2-AR mediated (Lymperopoulos et al., 2008). Moreover, adrenal GRK2 has a significant role

on beneficial sympatholytic effects of β-blockers and exercise training during HF (Rengo et al., 2010, 2012a; Femminella et al., 2013a). Importantly, training and beta-blocker therapy are know to have several biological effects and to improve survival in HF. Effectively, both these therapies led to a reduction of adrenal GRK2 levels/activity which conducts to decrease and normalization of CAs biosynthesis and production through restoration of α2-AR density/signaling. In our study on β-blockers effects on adrenal gland activity, we evaluated bisoprolol (a β1-AR selective blocker) to exclude any involvement of facilitatory pre-synaptic β2-ARs (see above). Of note, bisoprolol effects on left ventricular reverse remodeling preceded adrenal GRK2 downregulation and α2AR restoration. Consequently, these two treatments could exert a complementary neurohormonal action in contrasting the detrimental consequence of autonomic overdrive that affects HF patients. Furthermore, α2-AR dysfunction during HF may have important therapeutic implications because it could explain the failure of MOXSE and MOXCON trials (Swedberg et al., 2002). These trials were interrupted for the excessive mortality in the treated group and one of the possible explanations could be the dysfunction of α2-AR in adrenal medulla and peripheral nerve terminals that could not permit the drug to exert its beneficial consequences. Therefore, β-blockers and exercise training treatments taking advantage of their adrenal α2-ARs effects could potentially impact on moxonidine efficacy during HF.

However, the complete mechanism through which adrenal CAs overdrive occurs in HF is still unclear. In this regard some studies in dogs showed that bilateral adrenal denervation significantly reduced heart dysfunction after cardiac pressure overload (Womble et al., 1980).

Accordingly, it has recently been shown that unilateral denervation of the adrenal gland from the preganglionic cholinergic nerves, did not permit adrenal hypertrophy and rising of CAs production during cardiac pressure overload (Schneider et al., 2011). Hence, cholinergic innervation of the adrenal gland by nicotinic receptors and a Ca2++/calmodulin-dependent signaling is crucial to determinate adrenal hypertrophy, increase GRK2 levels and raise NEpi and Epi storage.

Of note, in isolated adrenal gland with undamaged splanchnic nerves, cholinergic stimulation caused release of cortisol and aldosterone (Ehrhart-Bornstein et al., 1995). These findings, together with the strict adrenocortical linkage (treated above), suggest that adrenal activation could be triggered by preganglionic cholinergic nerves stimulation through release of corticosteroids hormones.

CONCLUSIONS

CAs levels are a powerful prognostic factor of morbidity and mortality in HF (Cohn et al., 1984). GRK2 has a multiorgan pivotal role: in adrenal medulla and in cardiac nerve terminals this kinase regulates NEpi/Epi production and secretion through α2-ARs, whereas in heart it mediates cardiac effects of CAs by β-ARs regulation. In particular, adrenal GRK2-dependent α2-AR dysregulation seems to be crucial in enhanced CAs secretion from the adrenal gland during HF, contributing to detrimental sympathetic cardiotoxic effects. Consequently, restoration of adrenal α2-AR signaling through the inhibition of GRK2 may be a novel sympatholytic therapeutic strategy for HF. Decreasing CAs levels would permit restoration of cardiac β-AR downregulation/desensitization via cardiac GRK2 downregulation and ameliorate some critical aspects of failing heart such as adverse remodeling, arrhythmias and cardiac arrest. Of note, several therapeutic strategies, as β-blockers and exercise training, can exert their beneficial effects on HF also by decreasing sympathetic overdrive through adrenal GRK2 inhibition (probably also in sympathetic nerve terminals).

Significantly, systemic GRK2 inhibition during HF might be impressive because of its well-known positive cardiac effects and its ability to thwart the chronic activation of the renin–angiotensin–aldosterone (GRK2 inhibition could counteract phosphorylation and desensitization of Angiotensin II receptor type 1) and endothelin (GRK2 inhibition could prevent endothelin-induced insulin resistance) systems (Rockman et al., 1996; Zolk et al., 1999; Anavekar and Solomon, 2005; Usui et al., 2005). Furthermore it is interesting that GRK2 inhibition could be obtained by both systemic administration of a pharmaceutical GRK2 inhibitor molecule (Piao et al., 2012) or by local (cardiac or eventually adrenal) and systemic gene therapy delivery (Zincarelli et al., 2008, 2010).

In addition, GRK2 inhibitors could be useful as adjunctive therapy in HF, thus reducing the dosage and consequently the adverse effects of β-blockers.

As discussed above, α2-AR agonists are able to increase α2-AR inhibitory activity and thus to determinate sympatholysis in HF due to peripheral and adrenal α2-AR downregulation/desensitization. Importantly, our group evidenced that the therapeutic effects of moxonidine on decreasing CAs *in vivo* in rats with HF were enhanced with GRK2 inhibition via adrenal gene therapy. Of note, this combined therapy led to lower Epi levels, a non-typical phenomenon for moxonidine alone (Lymperopoulos et al., 2007a).

Adrenal GRK2 inhibition could be also positive and valuable as a therapy for other diseases characterized by sympathetic hyperactivity as hypertension (Schlaich et al., 2004), hyperthyroidism (Foley et al., 2001), pheochromocytoma (Roghi et al., 2011) or some cognitive, and psychiatric disorders as depression (Hausberg et al., 2007; Femminella et al., 2013b).

To summarize, cardiac and adrenal GRK2 inhibition represents an important therapeutic target during HF. However, further studies would be necessary to better understand the underlying complete mechanism and to allow potential and innovative specific peptides or gene delivery techniques to become part of common HF therapy.

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Effects of exercise training on cardiovascular adrenergic system

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In heart failure (HF), exercise has been shown to modulate cardiac sympathetic hyperactivation which is one of the earliest features of neurohormonal derangement in this syndrome and correlates with adverse outcome. An important molecular alteration related to chronic sympathetic overstimulation in HF is represented by cardiac β-adrenergic receptor (β-AR) dysfunction. It has been demonstrated that exercise reverses β-AR dysfunction by restoring cardiac receptor membrane density and G-protein-dependent adenylyl cyclase activation. In particular, several evidence indicate that exercise reduces levels of cardiac G-protein coupled receptor kinase-2 (GRK2) which is known to be involved in both β1-AR and β2-AR dysregulation in HF. Similar alterations of β-AR system have been described also in the senescent heart. It has also been demonstrated that exercise training restores adrenal GRK2/α-2AR/catecholamine (CA) production axis. At vascular level, exercise shows a therapeutic effect on age-related impairment of vascular reactivity to adrenergic stimulation and restores β-AR-dependent vasodilatation by increasing vascular β-AR responsiveness and reducing endothelial GRK2 activity. Sympathetic nervous system overdrive is thought to account for >50% of all cases of hypertension and a lack of balance between parasympathetic and sympathetic modulation has been observed in hypertensive subjects. Non-pharmacological, lifestyle interventions have been associated with reductions in SNS overactivity and blood pressure in hypertension. Several evidence have highlighted the blood pressure lowering effects of aerobic endurance exercise in patients with hypertension and the significant reduction in sympathetic neural activity has been reported as one of the main mechanisms explaining the favorable effects of exercise on blood pressure control.

Keywords: exercise, heart failure, adrenergic system, aging process, systemic hypertension

INTRODUCTION

It has been generally accepted that regular physical activity is associated with beneficial effects on the cardiovascular system (Belardinelli et al., 1999; Hambrecht et al., 2000; Piepoli et al., 2004; Rinaldi et al., 2006; Flynn et al., 2009; Giallauria et al., 2013). In fact, the idea that exercise maintains cardiovascular health is evident by the direct links between a sedentary lifestyle and the risk of cardiovascular and other disease states. Cardiovascular diseases, such as heart failure (HF) and hypertension, and impairment of cardiovascular reserve observed with aging, are often associated with SNS overactivity (Francis and Cohn, 1986; Brodde et al., 1995a; Kaye et al., 1995; Davies et al., 1996; Julius and Nesbitt, 1996; Ferrara et al., 1997a,b; Xiao et al., 1998; Seals and Esler, 2000; Kilts et al., 2002; Schlaich et al., 2004). Conversely, exercise has been shown to decrease elevated SNS activity in HF, hypertension and in the aging heart and vasculature. Although somewhat controversial in humans, evidence from animal studies also indicates that exercise training reduces baroreflex-mediated and other forms of sympathoexcitation in normal individuals. Collectively, these data are consistent with the hypothesis that physical activity may decrease the incidence of cardiovascular disease and improve cardiac outcome via alterations in SNS activity. Despite the important clinical implications of this possibility, the mechanisms by which exercise alters control of SNS activity remain to be fully elucidated. The aim of this review is to focus on the pathophysiological mechanisms by which exercise can modulate SNS overactivity and exert its favorable effect on the onset and progression of cardiovascular diseases.

EFFECTS OF EXERCISE ON SNS HYPERACTIVITY IN HEART FAILURE

Sympathetic activation has been shown to be one of the earliest features of neurohormonal rearrangement in HF where the prolonged exposure to pathological levels of catecholamines (CAs) are associated with adverse outcome (Kaye et al., 1995; Marciano et al., 2012; Paolillo et al., 2013; Rengo et al., 2013a; Savarese et al., 2013). In this process, cardiac β-adrenergic receptor (β-AR) dysfunction seems to be crucial (Bristow et al., 1986; Ungerer et al., 1993; Brodde et al., 1995b; Xiao et al., 1999; Rockman et al., 2002; Giallauria et al., 2010; Rengo et al., 2012a; Femminella et al., 2013), in particular downregulation/desensitization of β1-AR,

and prevalent desensitization/uncoupling of β2-AR. In particular, the receptors dysfunction seem to be related to increased levels of cardiac G-protein coupled receptor kinase-2 (GRK2). GRK2 is a kinase that phosphorylates intracellular domains of activated receptors, leading to the recruitment of arrestins to the receptors and the attenuation of intracellular G protein-dependent signaling. Therefore, GRK2 phosphorylates receptors and uncouples them from the adenylyl cyclase effector system (Rengo et al., 2012a). The relevance of cardiac GRK2 up-regulation in failing myocardium is supported by the observation of the therapeutic effect exerted by its inhibition (Salazar et al., 2013). Interestingly, GRK2 inhibition reverses left ventricular (LV) remodeling and improves myocardial contractility in the failing heart (Raake et al., 2008; Rengo et al., 2009, 2011, 2012a,b,c; Ciccarelli et al., 2011; Lymperopoulos et al., 2012).

Since it has been demonstrated that myocardial GRK2 levels and activity mirror those measured in peripheral lymphocytes in HF patients (Iaccarino et al., 2005), it could be possible to monitor the efficacy of different therapies using circulating white blood cells (Rengo et al., 2013b).

Exercise training in patients with stable HF, can relieve symptoms, improve exercise capacity and quality of life, and reduce disability, hospitalization, and mortality (Piepoli et al., 2004; van Tol et al., 2006). Physical inactivity can thus be considered a major cardiovascular risk factor and current treatment guidelines recommend exercise training in patients with HF in NYHA functional classes II and III (Hunt et al., 2005; Rinaldi et al., 2006). Exercise training is associated with numerous pulmonary, cardiovascular, and skeletal muscle metabolic adaptations that are beneficial.

The crucial role of β-AR dysregulation in the pathophysiology of HF is well established. GRK2, which plays a key role in the regulation of β-AR, is significantly elevated in human and experimental HF (Ungerer et al., 1993; Gros et al., 2000; Rockman et al., 2002; Petrofski and Koch, 2003; Iaccarino et al., 2005). Moreover, molecular manipulations of β-AR utilizing GRK2 inhibitors, such as the peptide known as the βARKct, restore β-AR signaling in the heart and increase cardiac function (Koch et al., 1995; Rockman et al., 1998; Harding et al., 2001; Shah et al., 2001). A significant reduction of cardiac GRK2 expression has been also recognized as a potential mechanism by which selective and non-selective β-AR blockade may positively affect β-AR signaling (Iaccarino et al., 1998; Leosco et al., 2007; Cannavo et al., 2013a; Rengo et al., 2013c). Previous works have shown that exercise is able to decrease GRK2 myocardial levels and improve β-AR signaling and responsiveness in spontaneously hypertensive rats (SHR) (MacDonnell et al., 2005) as well as in the aged heart (Leosco et al., 2007). More recently, it has been demonstrated that training evokes similar effects on β-AR system also in the post-ischemic hypertrophied failing myocardium leading to an enhanced cardiac inotropic state at the adrenergic stimulation (Leosco et al., 2008). Similar observation also have been reported in post-MI exercised mice (de Waard et al., 2007) that show increased cardiac β-AR protein and cAMP levels in the exercise animal group but these findings are not associated with significant changes of GRK2 protein levels. In this vein, it has been demonstrated that the mechanisms of β-AR desensitization may be GRK2- dependent (MacDonnell et al., 2005) or GRK2-independent (Xiao et al., 1994). Importantly, the observation of improved β-AR responses also in intact cardiomyocytes of post-infarcted failing hearts indicate that training may restore receptor signaling alterations in remote non-infarcted myocardium contributing to LV dysfunction (Leosco et al., 2008; Cannavo et al., 2013b). It still remains an unresolved issue why exercise does not seem to affect basal LV contractility in failing hearts (Musch et al., 1986; Gaudron et al., 1994) despite the improved β-AR function. Consistent with this is the observation that basal cAMP production, which remains still depressed in exercised HF animals, is unchanged, since adenylyl cyclase activity and cardiac contraction via protein kinase A mediated downstream effects are closely interlinked (Georget et al., 2002). This finding strongly supports the importance of downstream cellular events in the improvement of β-AR signaling and responsiveness in the failing heart.

EFFECTS OF EXERCISE ON ADRENAL α2-ARs DYSREGULATION IN HEART FAILURE

As mentioned above, exercise training appears to reduce autonomic derangement and neurohumoral excitation at rest in HF. The effects of exercise training on adrenergic hyperactivation in HF patients have not been completely clarified. Recently, an important molecular mechanism has been identified that contributes to the sympathetic overdrive of the failing heart. This mechanism involves the upregulation of GRK2 in adrenal medulla of HF animals, which leads to downregulation and G protein uncoupling of the α2-ARs present in the chromaffin cell membranes of the adrenal gland that normally exert negative feedback control on CA turnover (Lymperopoulos et al., 2007a; Rengo et al., 2012a,b,c,d,e). Thus, dysfunction of these receptors results in chronically elevated CA secretion and circulating levels in HF (Lymperopoulos et al., 2007a). More recently, a novel molecular neurohormonal mechanism has been reported to explain the effects of exercise training on counterbalancing sympathetic overactivation and the enhanced circulating CA levels of chronic HF that significantly increase the morbidity and mortality of this devastating disease. This mechanism involves lowering and restoration of adrenal GRK2 levels/activity, which results in marked reduction of adrenal CA production and secretion via decreased adrenal α2-AR desensitization/downregulation and normalization of circulating CA levels (Rengo et al., 2010). This finding is of particular importance because several studies have reported that exercise training counteracts the catecholaminergic activation of chronic HF in humans and in several animal models of the disease (Gademan et al., 2007; Lymperopoulos et al., 2007a,b; Rengo et al., 2009); however, essentially no evidence has been provided to mechanisms mediating this beneficial effect of this modality in HF. Circulating CAs originate from the adrenal medulla in the form of Epinephrine and Norepinephrine, which are secreted at a ratio of 80–20%, respectively, under normal conditions (Lymperopoulos et al., 2007b). Spilled-over Norepinephrine produced at sympathetic nerve endings also contributes to the total circulating amount of CA. Adrenal CA production is under tight regulation by sympathoinhibitory α2-ARs, which are expressed in the adrenal medulla and inhibit CA release (Lymperopoulos et al., 2007b). α2-AR function in turn is regulated by GRK2,

which phosphorylates and desensitizes the α2-AR, thus suppressing its function (Petrofski and Koch, 2003). By reducing GRK2 activity on adrenal α2-ARs, exercise training appears to restore adrenal α2-AR number and CA feedback inhibition, and this represents a mechanism whereby it reduces circulating CA levels in chronic HF.

EFFECTS OF EXERCISE ON AGE-RELATED CARDIAC AND VASCULAR β-AR DYSREGULATION

Noteworthy, alterations of β-AR system, similar to those observed in HF, have been described also in the senescent heart (Davies et al., 1996; Ferrara et al., 1997a,b; Rengo et al., 2012a,b,c,d,e). With aging, sympathetic activity is increased and cardiac neuronal uptake of CA is decreased. Although alterations in Gs-coupled receptors in the failing and aging human heart are quite comparable, GRK2 activity seems to be not affected by age (Xiao et al., 1998). In this vein, animal studies demonstrated that the positive inotropic effects after both β1- and β2-AR stimulation are markedly decreased in myocardium of aged rats as a consequence of a lower density of receptors and a diminished adenylyl cyclase activity. There have been conflicting reports about the effect of age on cardiac inhibitory G protein (Gi) levels in both humans and rodents. In one study of human heart, Gi levels were measured in atrial appendages received from surgical patients, and it was found that Gi expression increased with age (Brodde et al., 1995a,b). Accordingly, age-dependent Gi upregulation has been documented in animal models. This observation is particularly relevant since β2-AR signaling couples to Gi proteins as well as to stimulatory G proteins (Gs) (Kilts et al., 2002). In contrast, some authors reported that neither GRKs nor Gi proteins appear to contribute to the age-related reduction in cardiac β-AR responsiveness (Xiao et al., 1998). This evidence can be the consequence of a delayed progression of sympathetic activity dysfunction in the elderly, while in HF it develops much more rapidly (Brodde et al., 1995a,b; Kilts et al., 2002). Thus, time course and intensity of increase in sympathetic activity can explain the different behavior of GRK activity in the aging and failing human heart.

Exercise has been shown to modulate GRK2 levels/activity by reducing levels of this kinase in the heart and, consequently, inducing β-AR "resensitization." It has been previously demonstrated in rats that both exercise and β-blockers reverse β-AR dysfunction by restoring cardiac receptor membrane density and G-protein-dependent adenylyl cyclase activation (Leosco et al., 2007). Of note, although cardiac GRK2 levels were not upregulated in old sedentary rats compared to young sedentary rats, exercise resulted in a significant reduction of GRK2 activity even at lower levels than those observed in young controls. This latter phenomena represents a further demonstration of the beneficial effects of physical activity on β-AR signaling. Furthermore, Böhm et al. have demonstrated that exercise can partially reverse depression in cAMP production due to age-dependent Gi alpha increased expression (Böhm et al., 1993).

At vascular level, studies conducted in the aorta and carotid arteries of old rats have shown a reduced β-AR-dependent vasorelaxation (Chapman et al., 1999; Schutzer et al., 2001; Leosco et al., 2003). Importantly, β-AR dysfunction observed in the aorta and carotids of old rats is mainly due to GRK2 upregulation that seems to have a crucial pathogenic role in age-related vascular β-AR dysfunction. Importantly, exercise shows a therapeutic effect on age-related impairment of vascular reactivity to adrenergic stimulation and restores β-AR-dependent vasodilatation by increasing vascular β-AR responsiveness and by reducing endothelial GRK2 activity (Leosco et al., 2003).

In old healthy subjects, it has been demonstrated that physical training ameliorates age-related deterioration of cardiac function in terms of enhanced LV inotropic response to CA (Ehsani et al., 1991; Stratton et al., 1994; Spina et al., 1998). Contrasting data have been reported by other authors who described unchanged LV systolic performance (Stratton et al., 1992) in response to adrenergic stimulation after training in the elderly. However, it is important to underline that exercise training also enhances vagal tone (Levy et al., 1966), which could mask the favorable effect of exercise on cardiac β-adrenergic responsiveness.

EFFECTS OF EXERCISE ON NEURAL REGULATION OF BLOOD PRESSURE

The most common form of hypertension is neurogenic hypertension that is associated with sympathetic overdrive, loss of parasympathetically mediated cardiac variability, and excessive angiotensin II activity (Esler, 2010). Evidence from studies in both patients and animal models of hypertension strongly implicate the chronic sympathetic neural activation in the etiology and progression of hypertension (Anderson et al., 1989; Smith et al., 2002, 2004; Simms et al., 2009; Esler, 2010). Studies in adult SHR have also identified a reduced cardiac parasympathetic nerve activity (Friberg et al., 1988), elevated SNS activity and increased noradrenaline release (Judy and Farrell, 1979; Lundin et al., 1984). Notably, neonatal sympathectomy prevents the SHR from developing hypertension (Cabassi et al., 1998), and SNS is elevated in young SHR prior to the development of hypertension (Korner et al., 1993). In humans, it has been estimated that a neurogenic component is observed in 40–65% of hypertensive patients and different studies report an increase of 100–200% of SNS activity in the brain, heart, kidneys, and skeletal muscle vasculature (Esler et al., 1988; Grassi et al., 1998a; Huggett et al., 2004; Lambert et al., 2007). The magnitude of the elevation in SNS is related to the magnitude of hypertension (Grassi, 1998; Grassi et al., 1998b). Indeed, it has been described that the increase in blood pressure from control subjects to mildly hypertensive, and to more severely hypertensive patients is associated with a parallel increase in muscle SNS activity (Grassi et al., 1998b).

Clinical interventions showing impressive blood pressure lowering effects by targeting reductions in SNS activation (Krum et al., 2009, 2011; Wustmann et al., 2009; Esler et al., 2010) have contributed to a better understanding of the central sympathetic regulatory pathways altered in hypertension, and have stressed the importance in the control of the sympathetic nervous system in hypertension and its utility as a clinical target. Exercise has been shown to reduce SNS hyperactivity and blood pressure in hypertension. There are important mechanistic data from animal studies to show that exercise training limits sympathoexcitation and favors sympathoinhibition in the brainstem cardiovascular centers (Mueller, 2007). Previous studies have highlighted the

blood pressure lowering effects of aerobic endurance exercise training in patients with hypertension (Esler et al., 2010). Indeed, regular, moderate intensity training is associated with a 10 mmHg average fall in systolic and diastolic blood pressure in hypertensive patients (American College of Sports Medicine, 1993). In this regard, it has been reported that a 4-month programme of aerobic exercise training reduces of ∼37% muscle SNS activity and blood pressure in never-treated hypertensive patients (Laterza et al., 2007).

Exercise training could elicit adaptations in the adrenergic system, since SNS is activated during each bout of exercise and repeated activation of SNS may result in an attenuation of sympathetic activity (Grassi et al., 2000). Animal studies suggested that nitric oxide decreased overall sympathetic excitability within the brainstem and possibly through actions in higher brain regions (Goodson et al., 1994; Patel et al., 1996). It is unclear whether the increased release of nitric oxide during exercise training has a central sympathoinhibitory effect in humans. Previous studies demonstrated that hyperinsulinemia and insulin resistance were associated with hypertension and sympathetic activation (Julius et al., 1991; Baron et al., 1993). Training-dependent improvement of insulin sensitivity in normotensive and hypertensive individuals (Kohno et al., 2000; Henriksen, 2002) could contribute to attenuate insulin mediated sympathetic activation.

CONCLUSIONS

SNS overactivity is common in many cardiovascular disease states and is related to a higher incidence of morbidity and mortality. It is widely accepted that exercise training is associated with reductions in SNS activity, whether at rest or during conditions that produced sympathoexcitation, and this effect may represent an important mechanism by which exercise may contribute to long term cardiovascular health. Future studies are needed to further identify the molecular mechanisms that are involved in physical activity dependent changes in the control of SNS activity.

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