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β-Adrenergic Over-Stimulation and Cardio-Myocyte Apoptosis: Two Receptors, One Organelle, Two Fates?

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Abstract: Neuro-hormonal regulation of cardiac function via cathecol-amines results in increased heart rate and contractility. A persistent adrenergic tone, however, is an insult to the heart, affecting its regular homeostasis, altering morphology and gene expression patterns, as well as inducing apoptosis of cardio-myocytes. At the same time as being the main oxygen consumers, mitochondria are also key to the energy production required for the heart to maintain its vital functions and to integrate a series of signaling pathways that define the life and death of the cell. As β-adrenergic receptors (β-AR) orchestrate multiple biochemical events that can either trigger or inhibit cell death, mitochondria can act as a referee in the entire process. In fact, β-AR subtypes β_1 and β_2 activate various down-stream pathways which differently modulate intracellular calcium levels and production of mitochondrial reactive oxygen species (ROS). The delicate balance between an adaptive (cardio-protective) response resulting in increased contractility and activation of survival pathways, *vs*. cell death caused by calcium and ROS-induced mitochondrial disruption, along with evidence of their clinical and potential therapeutic translations, are reviewed in this communication.

Keywords: Apoptosis, β-adrenergic receptors, calcium, cardio-myocyte, mitochondria, oxidative stress.

1. EXCESSIVE β-ADRENERGIC DRIVE AND CARDIAC PATHOLOGY

Heart failure represents one of the fastest-growing diseases, affecting 8/1000 men at the age group of 50-59 years, and up to 66/1000 men between 80-89 years [1]. It is caused by events that compromise heart function, such as cardiac damage or overload. One example is β-adrenergic over-stimulation following stress-induced release of epinephrine or norepinephrine (NE) to the bloodstream, that may contribute to the increase of fatty acids in the blood, which can then be deposited in arteries, contributing to the development of atherosclerosis [2, 3]. Since the hardening of blood vessels reduces both the wall diameter and the flow rate, an adaptive response through increased sympathetic nervous system (SNS) activity can initially contribute to increase ventricular contractility in order to sustain ejection performance. However, over time, the neuro-hormonal stimulation of heart rate initiates a process of dynamic and morphological alterations [4, 5]. When persistent, increased sympathetic nerve activity in the myocardium and accumulation of catecholamines may mechanistically explain pathogenesis of heart disease [6]. Often, the left ventricle develops hypertrophy associated with a complex set of alterations in the expression of structural and signaling proteins. This may lead to lack of oxygen and nutrients in specific areas of the heart muscle, resulting in the development of irreversible lesions. Commonly, catecholamine over-signaling downstream from β-adrenergic receptor (β-AR) stimulation can lead to cardiomyocyte death through the stimulation of stress responses which involve the activation of apoptotic pathways. Evidences concerning the mechanisms by which cardiac myocytes undergo apoptotic cell death show that mechanical conditions and elevated release of neuro-hormonal factors are stronger contributors (Fig. **1**) [7].

2. β-ADRENERGIC OVER-STIMULATION AND CARDIAC CELL DEATH

Cytotoxic effects of catecholamines on cardiomyocytes are mediated by their interaction with α- and β-AR. Adrenergic receptors belong to a family of G protein-coupled receptors (GPCRs) with seven-transmembrane domains, which play an important role on cellular signal transduction by specific agonists. Excess of circulating catecholamines are involved in many cardiac diseases by promoting cellular remodeling, especially when compensatory mechanisms are needed [8].

2.1. Two Receptors, Two Fates?

β-adrenergic receptors link the cardiovascular system with the SNS and can modulate cardioprotection/cardiotoxicity through a crosstalk with multiple signaling pathways.

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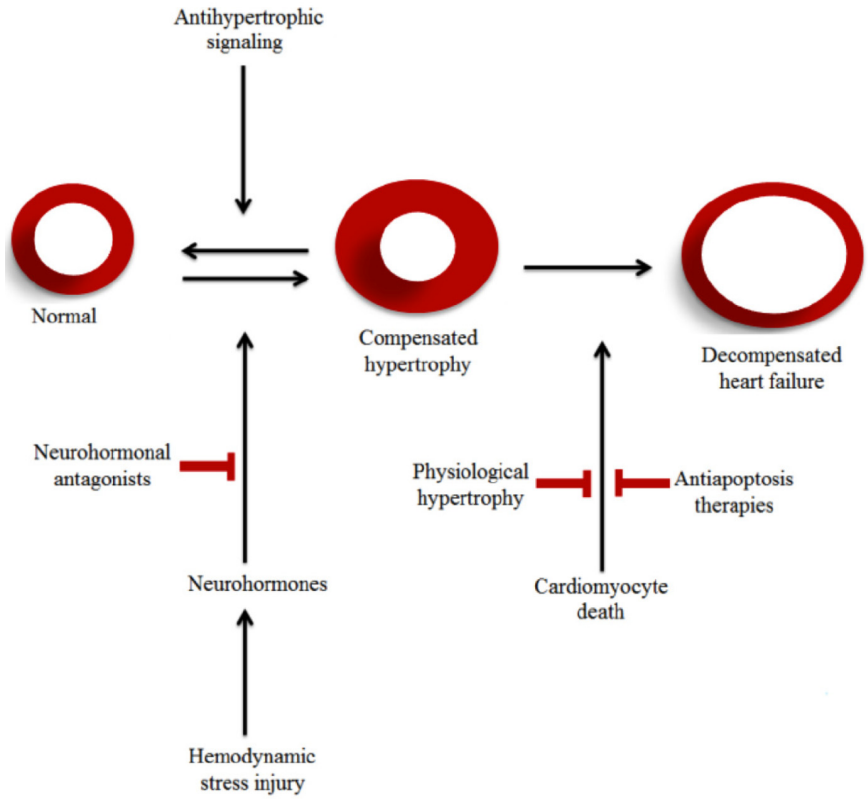

Fig. (1). Representative scheme of morphologic alterations induced by a stressor stimulus in the heart. Terminally differentiated cardiomyocytes adapt to increased work or hemodynamic stressors through an increase in heart rate. To compensate for new cardiac output demand, neurohormonal and cellular signaling cascades are activated, resulting in ventricular remodeling, including increased myocardial mass and ventricular walls thickness. Ultimately, myocardial hypertrophy leads to a progressive loss of cardiomyocytes, which has a causal role in the transition to end-stage disease (Figure adapted from [7, 97]).

Three different β-ARs subtypes are present in human cardiomyocytes, with the magnitude of expression following a specific order: β_1-AR > β_2-AR > β_3-AR [9]. Although being highly homologous, β_1- and β_2-AR receptors clearly play different roles in cardiac physiology and pathology. In fact, chronic stimulation of the two types of receptors has opposing effects on myocyte fate. However, the mechanistic links for their different downstream effects on cardiomyocyte apoptosis are still largely unknown. This agrees with the fact that hundreds of GPCRs use a fairly small pool of second messengers and still remain functionally different [10]. These receptors can be physically coupled to stimulatory (Gs) or inhibitory (Gi) heterotrimeric G proteins (Fig. **2**) [11]. Upon ligand binding, a conformational change induced by the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) in the G protein moiety occurs, leading to protein activation. Both β_1- and β_2-AR are coupled via G proteins to the effector enzyme adenylyl cyclase (AC), which converts the substrate Mg-ATP into the second messenger cyclic adenosine monophosphate (cAMP). Then, cAMP-dependent protein kinase A (PKA) mediates the phosphorylation of proteins involved in cardiomyocyte calcium (Ca^{2+}) handling [12-14], contributing to the regulation of multiple signaling pathways.

Despite its role as a pro-death receptor [15], stimulation of β_2-AR can also activate survival pathways via coupling to Gi proteins [15] that involves activation of phosphatidylinositol 3-kinase (PI3K) and Akt [16], and is able to inhibit the pro-apoptotic signaling axis AC-cAMP-PKA [17-19]. However, it still remains to be understood how cardiac myocytes activate the β_2-AR-based pathway and have distinctly variable biological consequences on cell death/survival. Clearly, downstream partners determine the cellular fate.

Transgenic rodents over-expressing cardiac β_1-AR-Gs presented increased cardiomyopathic phenotype, as well as increased basal rates of cardiomyocyte apoptosis [20]. Moreover, the pro-apoptotic role of β_1-receptor activation is also dependent on oxidative stress, while other mechanisms imply PKA-independent activation of Ca^{2+}/calmodulin-dependent protein kinase (CaMK) (Fig. **2**) [21]. However, it must be stressed that downstream effects of β-adrenergic signaling cannot be completely beneficial or deleterious, due to the complex mechanisms of molecular interactions. The tissue content in β-ARs is not constant and modulation of receptor expression levels is one of the dynamic mechanisms by which transmembrane β-AR signaling is achieved. The amount of β-ARs present in a tissue is modulated by a variety of drugs, hormones, physiological and pharmacological conditions [22]. Interestingly, β_1-AR antagonists, in combination with β_2-AR agonists, improved cardiac activity and inhibited deleterious cardiac remodeling, when compared with the single use of a β_1-AR antagonist in a heart failure rat model [23]. Also, downregulation of pro-apoptotic protein Bax and an increase in the anti-apoptotic protein Bcl-2 may contribute to the beneficial effects of the joint therapy in preventing cardiomyocyte apoptosis in these animals [24].

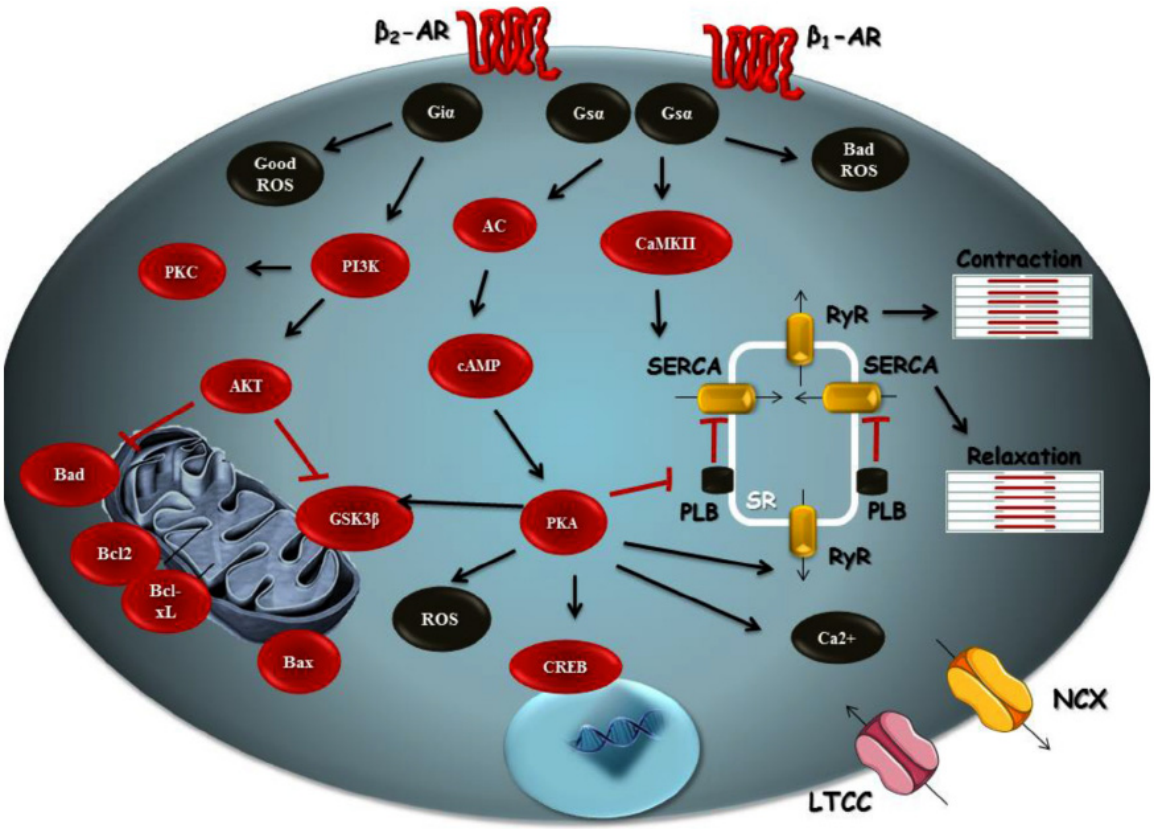

Fig. (2). **Integrative schematics of signaling pathways triggered by β-adrenergic receptors.** β_1 and β_2-adrenergic receptors modulate cellular behavior via several distinct signaling pathways. In this highly regulated system, maintenance of physiologic calcium levels and prevention of up-regulated pro-apoptotic elements is critical for cell death control. β_1-AR is reported as pro-apoptotic, being coupled to a stimulatory G protein which activates PKA via adenylate cyclase and cAMP. The activation of PKA initiates the influx of calcium by activating the L-type calcium channels (LTCC) in cell membranes. The increase in intracellular Ca^{2+} together with the concomitant stimulation of RyR by PKA, leads to Ca^{2+} release from the sarcoplasmic reticulum which stimulates cardiac contraction. PKA is also involved in the activation of SERCA which contributes to muscle relaxation by promoting calcium re-uptake from the cytosol. β_2-adrenergic receptor signals via both stimulatory and inhibitory G proteins and is associated with anti-apoptotic pathways, such as PI3K/Akt, contributing to inhibit apoptosis.

In fact, our group has shown that a decrease in the Bcl-2/Bax ratio is associated with increased β-AR-induced cardiomyoblast death [25]. The effects of β-AR stimulation may also depend on the stage of cardiac cell differentiation. By using H9c2 cardiomyoblasts as an experimental model, our group demonstrated that the stimulation of β-AR by the non-selective agonist isoproterenol (ISO) leads to different cellular responses depending on the differentiation state of cells. Undifferentiated cells, unlike differentiated ones, developed higher protective stress responses to injury promoted by ISO [25]. When incubated with ISO, differentiated H9c2 muscle cells showed increased cytosolic Ca^{2+}, cAMP content and oxidative stress, as well as mitochondrial depolarization, increased levels of superoxide anion ($O_2^{\cdot-}$), loss of subunits from the mitochondrial respiratory chain, decreased Bcl-xL content, increased p53 and phosphorylated-p66Shc, as well as activated caspase-3. On the other hand, undifferentiated H9c2 cells incubated with ISO showed increased Bcl-xL protein and increased mitochondrial superoxide dismutase (SOD) expression, which may act as protective mechanisms. These results suggested that the differentiation of cardiomyoblasts is associated with differential regulation of stress responses, which impact the toxicity of several agents, namely those acting through β-AR and resulting in mitochondrial disruption in differentiated cells only [25]. These results also suggest that multiple cell signaling pathways, including those linked to β-AR, modulate cell fate by up or down-regulating mitochondrial processes, including oxidative stress and calcium signaling.

2.2. From Receptor Activation to Cell Fate: The Role of Calcium and ROS Signaling

Reactive oxygen species (ROS) are formed as a natural byproduct of oxygen metabolism, and regulate various biological responses such as cell proliferation, tumor progression, hypertrophy, and apoptosis, among others. Since the majority of oxygen consumption for cellular metabolism occurs through oxidative phosphorylation, mitochondria are described as important sources of ROS in the cell [26], predominately under pathological conditions [27].

Studies in animal models showed that the addition of exogenous oxidants scavengers provided strong anti-apoptotic action in cardiac cells, demonstrating that oxidative stress may be, at least partially, involved in myocyte degeneration [28].

At low levels, ROS generation has an important role in signaling functions in cardiac cells by mediating cell proliferation, differentiation and survival pathways [29]. During cardiomyogenesis, mitochondrial ROS formation has an important role in establishing the metabolic mechanisms that allow undifferentiated myoblasts drive to a cardiac-specific energetic requirement [30-32]. Our recent study demonstrated that differentiated H9c2 cells generate higher content of mitochondrial $O_2^{\cdot-}$ [30], supporting the hypothesis that redox signaling alterations are required for myoblasts differentiation [33]. In the heart, the excitation-contraction coupling (ECC) is a physiological process essential for cardiomyocyte function (contraction and relaxation), and involves redox signaling [34]. Modulation of ECC is one of the adaptive processes in which low-level production of ROS may be involved [35].

Mitochondrial sources of $O_2^{\cdot-}$ include the respiratory chain, and the enzymes dehydrolipoamide dehydrogenase (DLD), monoamine oxidase (MAO), aconitase, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) [36, 37]. In the respiratory chain, complexes I and III have been described as important sources of $O_2^{\cdot-}$ [38]. At both complexes, superoxide anion is formed on Fe-S clusters, upstream from ubiquinone reduction. The generation of this radical is increased by the presence of rotenone and antimycin A, complex I and III inhibitors, respectively, thus demonstrating the role of these two complexes for mitochondrial ROS generation [39].

Particularly relevant to β-AR-induced overstimulation and intracellular effects are two mitochondrial ROS generation enzymatic generators: MAO and NOX. Two isoforms of MAO exist bound to the outer mitochondrial membrane (MAO-A and MAO-B). These oxidases are also flavoproteins and catalyze the oxidation of amines to molecular oxygen, leading to the formation of aldehydes and hydrogen peroxide (H_2O_2) [40]. These enzymes are involved in catecholamine catabolism, particularly the MAO-A isoform, and were described to be important ROS sources, as byproducts of the catabolism of neurotransmitters such as norepinephrine and dopamine, leading to H_2O_2 formation [35]. A decrease in oxidative stress, inhibition of contractile dysfunction, inhibition of matrix metalloproteinase and caspase-3 activation were observed in mice with pressure overload-induced heart failure when treated with the MAO-A inhibitor, clorgyline, or in a dominant-negative MAO-A model [35, 41].

Increased NOX-induced ROS production and progressive ventricular dysfunction were reported to occur in β_2-AR overexpressing transgenic mice, leading to activated p38 mitogen-activated protein kinase (p38 MAPK), and significantly contributing to cardiac inflammation, remodeling and failure [42]. The NOX family enzymes catalyze formation of ROS and protons [34] derived from electron transfer from NADPH to NOX flavin domains. These enzymes are key regulators of redox signaling in several organisms, including during cell proliferation and differentiation. In particular, heart-expressing isoforms NOX2 and NOX4 contribute to $O_2^{\cdot-}$ and H_2O_2 production, respectively [34, 43, 44]. Interestingly, NOX4 was shown to be located in intracellular space of cardiomyocytes in various sites, including the endoplasmic reticulum, nucleus and mitochondria [34] and to be a major source of mitochondrial oxidative stress in cardiomyocytes [45]. Several molecular targets can be regulated by free radicals in cardiomyocytes, such as protein phosphatases, kinases, ion transporters, transcription factors and receptors [34].

Besides ROS, Ca^{2+} is also important in the regulation of several intracellular pathways in cardiac cells, namely in the control of cardiac beating and other signaling pathways. Calmodulin-dependent kinase II (CaMKII) activity is stimulated during pro-oxidant conditions, promoting the phosphorylation of cardiac Ca^{2+}-handling proteins, leading to modulation ECC, apoptosis and gene transcription [34]. After oxidation and formation of disulfide bonds, PKA acquires higher affinity for binding to A-kinase anchoring proteins (AKAPs) [34, 46], allowing the localization of PKA in particulate membranes and cellular organelles [47]. Mitochondria have a specific subset of AKAPs, including AKAP121 [48]. Some studies demonstrate that the complex AKAP121-PKA inhibits the mitochondrial apoptotic pathway by phosphorylation of the pro-apoptotic protein BAD, blocking its association with Bcl-2 [49]. Therefore, upon β-AR stimulation, the cAMP-PKA pathway can be activated leading to mitochondrial protection. More recently, the existence of a cAMP signaling microdomain in the outer mitochondrial membrane (OMM) with higher PKA activity pointed out the mitochondrial localization of this holoenzyme [50]. These findings, although controversial, suggest that cAMP-triggered activity in mitochondria is not only due to PKA interaction with specific AKAPs, but may also be due to this compartmentalization. Moreover, PKA mediates the phosphorylation of several substrates, contributing to the regulation of several cell signaling pathways. For instance, β-AR-regulated Ca^{2+} handling in cardiac cells is regulated by mitochondrial ROS production, since these modulate ryanodine receptors (RyRs) through phosphorylation and oxidation, leading to increased Ca^{2+} leakage and to inhibition of sarcoplasmic reticulum (SR) Ca^{2+} uptake [12, 51]. Protein kinase A can phosphorylate phospholamban, a protein that regulates cardiac SR Ca-ATPase (SERCA), which is indirectly redox regulated. Moreover, it can also be directly regulated through thiol oxidation [34].

Under normal conditions, mitochondrial metabolism can be stimulated by Ca^{2+}, providing an increase in reduced nicotinamide adenine dinucleotide (NADH) and adenosine triphosphate (ATP) production [52]. On the other hand, Ca^{2+} overload can lead to mitochondrial dysfunction and increased $O_2^{\cdot-}$ production, leading to mitochondrial permeability transition pore (MPTP) induction and triggering of cell death [53]. Several studies indicate an association between cytosolic Ca^{2+} and ROS generation, not only at the mitochondrial level but also in other organelles. In addition, ROS can stimulate the rise in intracellular Ca^{2+} concentration [54].

In mitochondria, excessive Ca^{2+} uptake is one of the mechanisms that may lead to ROS generation, resulting from high levels of intracellular Ca^{2+} [54, 55]. Nevertheless, heart mitochondria have the capacity to store large amounts of Ca^{2+} without triggering the MPTP [56], but when the heart tissue suffers an insult, such as during cardiac reperfusion, oxidative stress largely increases, and lower mitochondrial Ca^{2+} concentrations can lead to disrupted function [57].

The interaction between Ca^{2+} and oxidative stress regulates other stress pathways involved in cell death, such as the p66Shc pathway [30]. p66Shc is known to regulate oxidative stress responses and apoptosis [58]. The phosphorylation of p66Shc on serine 36 by protein kinase Cβ increases during cellular stress, leading to an amplification of mitochondrial oxidative stress and apoptotic signaling [59]. In a previous study, we demonstrated that the β-AR agonist ISO increases the ratio between Ser36-phosphorylated p66Shc and the total p66Shc content in differentiated cardiac cells [30], suggesting a pro-apoptotic/pro-oxidant effect.

The dual effects of mitochondrial ROS on β-AR-induced modulation of cardiomyocyte function are well described. As mentioned above, mitochondria are an important source of intracellular ROS and complexes I and III have been described as potential sources of ROS, including $O_2^{\cdot-}$ [60]. Therefore, one hypothesis is that there may be an association between β-AR pathways at the sarcolemma and ROS generation at the mitochondrial level. In this regard, if Ca^{2+} influx resulting from persistently activated β-AR, or if Ca^{2+} removal systems are compromised, as during chronic β-AR stimulation, Ca^{2+} overload can occur [61, 62] leading to excessive mitochondrial Ca^{2+} uptake [63]. When Ca^{2+} exceeds a certain threshold, the mitochondrial buffering capacity is overloaded and a substantial mitochondrial Ca^{2+} accumulation occurs. In certain tissues, such as in muscle, calcium over-accumulation, accompanied by oxidative stress, facilitates MPTP opening and forces mitochondrial electron transfer to accelerate, contributing to higher ROS generation by the respiratory chain [64].

Despite this, moderate β-AR-induced ROS release from the respiratory chain regulates Ca^{2+} transient amplitude, contraction and L-type Ca^{2+} current densities, while persistent β-adrenergic stress compromises cardiac viability, most likely by persistently inducing mitochondrial oxidative stress [65]. In fact, long-term (24 h) β-AR stimulation induced mitochondrial membrane depolarization and apoptosis in adult rat cardiomyocytes [66, 67]. Induction of cell death was inhibited by a SOD/catalase mimetic and by the overexpression of catalase, which indicated that the apoptotic signaling induced by β-ER stimulation involved increased ROS production [66].

Activation of PKA can also induce a fast and reversible increase in mitochondrial ROS generation in rat cardiomyocytes [68]. In a similar study using mouse ventricular cardiomyocytes, the β-AR agonist ISO induced mitochondrial ROS production via cAMP–PKA and stimulated cytoplasmic Ca^{2+} transients, which were blunted by pre-incubation with antioxidants [65]. In addition, mice hearts perfused with ISO showed increased mitochondrial ROS production, which was independent of mitochondrial Ca^{2+} accumulation or membrane depolarization [65], but resulted instead from cAMP–PKA signaling pathway stimulation. Moreover, increased mitochondrial ROS production plays a critical role in the β-adrenergic inotropic effect since ISO-induced Ca^{2+} transients are diminished in the presence of the antioxidant N-acetyl-cysteine (NAC), as well as by the mitochondria-targeted antioxidant SS3 [65]. These approaches do not exclude that other pathways, linking β-AR signaling to specific proteins in mitochondria (such as MAO) may be occurring.

3. THE β-ADRENERGIC SYSTEM AND DOWNSTREAM MITOCHONDRIAL SIGNALING AS A THERAPEUTIC TARGET

3.1. A Protective Role for NOS?

Besides ROS, reactive nitrogen species (RNS) also contribute to oxidative stress. Nitric oxide synthase (NOS) is a cytochrome P450 reductase-like enzyme that catalyzes flavin-mediated electron transfer from NADPH to a prothestic heme group. This reaction leads to the generation of nitric oxide (NO), which is a relatively stable radical, with an half-life of up to 10 s. The simultaneous production of mitochondrial NO and $O_2^{\cdot-}$ results in the production of peroxynitrite, a very damaging agent [69]. However, NO also plays an important role in the regulation of cardiovascular function, including the regulation of protein trafficking in the cardiovascular system [70]. Besides activating the cGMP-dependent pathway, NO can also regulate cell function through protein S-nitrosylation, a reversible, redox dependent, posttranslational protein modification that involves the binding of NO to a protein sulfhydryl group. S-nitrosylation of proteins seems to play an important role in cardioprotection, leading to changes in protein structure and function, and also preventing further irreversible oxidative/nitrosative modification of the modified thiol groups [71, 72]. Protein S-nitrosylation also has an important role of in modulating mitochondrial respiration. Mitochondrial S-nitrosylated proteins (including complex I) are associated with mainly protective effects, as in inflammatory and ischemia/reperfusion syndromes, but also in some pathological effects, as in neurodegenerative diseases [73]. However, it remains to be known if local mitochondrial NO effects are important in the context of cell protection.

3.2. Mitochondria as a Drug Target

The data described above imply that a stress response to β-AR stimulation often involves an increased generation of ROS by mitochondria, which may initially serve as an adaptive feature, aiming at improving the antioxidant network and contraction. On the other hand, continued mitochondrial ROS production can result in organelle degeneration and induction of cell death. However, there are still open questions, especially regarding the mechanisms on how mitochondria are stimulated to generate more ROS. Nevertheless, the way in which cardiomyocyte oxidative stress is managed following β-adrenergic stimulation depends on the type of receptor involved. For example, β_2-AR activation was shown to afford cardioprotective effects during oxidative stress induced by doxorubicin (DOX) in cardiomyocytes. Although the cardiotoxicity exerted by this anti-neoplastic agent is mediated in part through Ca^{2+}-dependent opening of the MPTP [74], the signals linking β_2-AR signaling to the prevention of DOX toxicity through this pathway are unclear. Although DOX administration to wild type mice resulted in no acute mortality, 85% of β_2-AR $^{-/-}$ mice died within 30 min after DOX administration [75]. β_2-AR activation of pro-survival kinases appears to be essential for mitochondrial preservation. Likewise, knock-down of those receptors negatively regulates pro-survival kinases, increases intracellular Ca^{2+} levels, potentiating mitochondrial dysfunction by induction of oxidative stress and MPTP opening, which represent

a critical step on cardiotoxic events [75]. Another study using ventricular myocytes isolated from wild type (C57BL/6) and NOS1 knockout NOS1$^{-/-}$ mice, demonstrated that scavenging $O_2^{\cdot-}$ and increasing NO levels to an adequate $^{\cdot}NO/O_2^{\cdot-}$ balance improved myocyte Ca^{2+} transients in response to β-adrenergic stimulation [76]. In fact, moderate ROS production enhances Ca^{2+} fluxes in cardiomyocytes, resulting in more vigorous contraction. However, elevated $O_2^{\cdot-}$ production affects a variety of proteins involved in ECC, leading to contractile dysfunction [77].

It is known that β-AR blockers improve cardiac contractility and reduce mortality in patients with heart failure [78]. However, it is still unclear how blocking a pathway that increases contractility in normal hearts can improve the function of a failing heart. Although poorly understood, the competition for NE receptors may attenuate cardiomyocyte overstimulation, with Ca^{2+} playing a pivotal role [79]. As described above, β-AR stimulation activates both AC-cAMP-PKA and CaMKII pathways [80]. Similarly to PKA signaling, CaMKII is upregulated in failing hearts [81].

By using a knockout mice for type 5 AC (AC5KO), a major cardiac isoform, a long-term ISO treatment (7 to 14 days) did not result in further increase of left ventricular ejection fraction (LVEF) as observed in wild type animals. Instead, ISO treatment in AC5KO animals resulted in a greater degree of AC signaling downregulation, an improvement on myocyte viability and increased Bcl-2 protein expression and Akt/GSK signaling, potentially elucidating a novel approach to the therapy of heart failure and a protective mechanism of mitochondrial integrity [82]. In this regard, several studies are focused on cellular checkpoints that provide 'rescue' opportunities for cardiomyocytes. The control of pro-apoptotic regulatory mechanisms protects cells from complete execution of the apoptotic program. Studies in experimental animals have shown that Bcl-2 affords strong anti-apoptotic effects and overexpression of this protein provided cardioprotection [83]. Similar results were also obtained in studies using exogenous oxidants scavengers [28].

The development of therapies aiming at increasing mitochondrial biogenesis can be useful for cell repair and/or regeneration. In fact, by using adult feline cardiomyocytes exposed to the β-AR agonist formoterol, increased mitochondrial biogenesis was observed. [84]. At least in adipocytes, nebivolol, a third-generation β-AR blocker stimulates mitochondrial biogenesis, increasing mitochondrial DNA copy number, protein levels and the expression of transcription factors involved in mitochondrial biogenesis, such as PPAR-γ coactivator-1α (PGC-1α), Sirtuin 3 (Sirt3), mitochondrial transcription factor A (Tfam) and nuclear related factor 1 (Nrf1) [85], suggesting the use and development of β_2-AR ligands for therapeutic mitochondrial biogenesis. Also, the β-AR blocker carvedilol demonstrated cardiac protection in a variety of settings [86]. Although the hemodynamic effects result mostly from non-selective β-AR blockage and selective alpha-receptor blockage, carvedilol also prevents cardiac mitochondrial deterioration resulting from oxidative stress [87-92]. It is thought that antioxidant protection afforded by carvedilol may be originated from mechanisms ranging from iron chelation [93] to a sub-toxic generation of ROS which acts to precondition the heart tissue against deleterious insults [94]. It appears that the anti-apoptotic effects of carvedilol can have multiple effects besides direct anti-oxidant protection. Novel mechanisms include up-regulation of anti-apoptotic miRNA [95] and regulation of phosphatidylinositol 3-kinase and mitogen-activated protein-kinase kinase pathways [96]. How blockage of β-AR triggers these effects or whether those are completely independent events still remains to be determined.

CONCLUSION

We hereby provide evidence that mitochondria play a key role in β-AR downstream signaling namely regarding the regulation of Ca^{2+} fluxes, ROS production and integration of pro- and anti-apoptotic signals. Nevertheless, it is still far from being understood how the different pathways cross-link/divide downstream from the two receptors to generate distinct effects on mitochondria and cell viability. Therapeutics aimed at preventing mitochondrial degeneration during β-AR over-stimulation in various cardiac conditions may be a useful strategy, namely using antioxidants targeted to mitochondria, although still little work has been done in this regard.

ABBREVIATIONS

AC	=	Adenylate cyclase
AC5KO	=	Knockout mice for type 5 AC
AKAPs	=	A-kinase anchoring proteins
ATP	=	Adenosine triphosphate
Bad	=	Bcl-2-associated death promoter
Bax	=	Bcl-2-associated X protein
Bcl-2	=	B-cell lymphoma 2
Bcl-xL	=	B-cell lymphoma-extra-large
Ca^{2+}	=	Calcium
CaMK	=	Ca^{2+}/calmodulin-dependent protein kinase
CaMKII	=	Ca^{2+}/calmodulin-dependent protein kinase
cAMP	=	Cyclic adenosine monophosphate
cGMP	=	Cyclic guanosine monophosphate
CREB	=	cAMP response element-binding protein
DLD	=	Dehydrolipoamide dehydrogenase
DOX	=	Doxorubicin
ECC	=	Excitation-contraction coupling
GDP	=	Guanosine diphosphate
Gi	=	Guanine nucleotide-binding protein inhibitory subunit
GPCRs	=	G protein-coupled receptors
Gs	=	Guanine nucleotide-binding protein stimulatory subunit
GSK3β	=	Glycogen synthase kinase 3β
GTP	=	Guanosine triphosphate

H_2O_2 = Hydrogen peroxide
ISO = Isoproterenol
LEVF = Left ventricular ejection fraction
LTCC = L-type calcium channel
MAO = Monoamine oxidase
MPTP = Mitochondrial permeability transition pore
NAC = N-acetyl-cysteine
NADH = Nicotinamide adenine dinucleotide
NADPH = Nicotinamide adenine dinucleotide phosphate
NCX = Sodium-calcium exchanger
NE = Norepinephrine
NO = Nitric oxide
NOS = Nitric oxide synthase
NOX = NADPH oxidases
Nrf-1 = Nuclear-related factor 1
$O_2^{\bullet-}$ = Superoxide anion
OMM = Outer mitochondrial membrane
p38 = p38 mitogen-activated protein kinases
PGC1α = Peroxisome proliferator-activated receptor-γ co-activator 1α
PI3-K = Phosphoinositide 3-kinase
PKA = Protein kinase A
PKC = Protein kinase C
PLB = Phospho-lamban
RNS = Reactive nitrogen species
ROS = Reactive oxygen species
RyRs = Ryanodine receptors
SERCA = SR Ca-ATPase
Sirt3 = Sirtuin 3
SNS = Sympathetic nervous system
SOD2 = Superoxide dismutase
SR = Sarcoplasmic reticulum
Tfam = Mitochondrial transcription factor A
β-AR = β-adrenergic receptor

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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