Chapter 2
The Adrenergic System of the Myocardium

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Cardiovascular homeostasis is guaranteed by the interplay of several neurohormonal systems in the body. Among them, the adrenergic (or sympathetic) nervous system (ANS) has a crucial role both in physiological and pathological conditions.

The major consequences of ANS stimulation on cardiovascular function can be summarized in the so-called fight or flight response, which manifests with heart rate acceleration (positive chronotropy), increased cardiac contractility (positive inotropy), reduction of venous capacitance, increased myocardial relaxation (positive lusitropy), acceleration of atrioventricular conduction (positive dromotropy), and constriction of resistance vessels. The mediators of these effects are the catecholamines epinephrine (Epi), or adrenaline, mainly released in the circulation by the adrenal medulla, and norepinephrine (NE), or noradrenaline, released by sympathetic nerve terminals. The effects of the ANS are counterbalanced by the parasympathetic (cholinergic) nervous system, which, through the vagal nerve terminals, is mainly responsible for heart rate reduction (negative chronotropy), with minimal inotropic effects [1].

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The pivotal role of ANS on cardiovascular function regulation is further emphasized by the fact that it constitutes one of the major therapeutic targets in cardiovascular diseases. Indeed, β-adrenergic receptor (β-AR) blockers are a cornerstone in the pharmacological treatment of myocardial ischemia, heart failure (HF), and hypertension [2].

In this chapter, we discuss the effects of ANS stimulation on cardiovascular function in both physiological and pathophysiological conditions, with a particular attention to the numerous evidence derived from preclinical and clinical studies on HF, a cardiovascular disease characterized by ANS hyperactivity.

The Adrenergic Receptors

The ARs belong to the superfamily of G-protein-coupled receptors (GPCRs) or seven transmembrane-spanning domain receptors (7TMRs), with an extracellular N-terminal region and an intracellular C-terminus. Their physiological agonists are the neurotransmitters NE and Epi.

To date, a total of three types and nine subtypes of ARs have been identified and classified into α1-AR (α1A, α1B, α1D), α2-AR (α2A, α2B, α2C), and β-AR (β1, β2, β3). All ARs primarily signal through heterotrimeric G proteins [3].

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 [2]. Recently, the existence of another subtype, the β4, has been postulated, but it is likely that the β4 represents a novel functional status of the β1 receptor [4].

Although β1-AR is the predominantly expressed AR subtype in the normal heart, accounting for 75–80 % of total β-ARs, cardiac cells express also β2 (15–18 %) and, to a lesser extent, β3 (2–3 % of total cardiac β-ARs) [5]. The most important function of cardiac -ARs is the regulation of heart rate and myocardial contractility in response to catecholamines. Indeed, β1-AR and, to a lesser extent, β2-AR stimulation increases cardiac contractility, heart rate, and rate of relaxation [6]. As for β3-ARs, recent data indicate that their stimulation has opposite effects compared with β1-ARs and β2-ARs, resulting in the negative inotropic effect [7].

Human heart also expresses α1-ARs, although at significantly lower levels (20 %) compared with β-ARs. It has been shown that the most predominant subtype in human cardiomyocytes is the α1A; however, the α1B is present in the left and right ventricles of both failing and nonfailing human myocardium. Little is known about the 1D-AR, instead, and its potential role in regulating cardiac contractility. Moreover, it is not clear whether α1-AR subtypes might have a differential expression in the various parts of the heart (endocardium and epicardium). However, the role of α1-AR in the regulation of blood flow by inducing vasoconstriction of the smooth muscle cells in arterial walls is well recognized [8].

Among the α2-AR subtypes, α2B-ARs are known to be present in vascular smooth muscle cells where they mediate vasoconstriction, while α2A-ARs act

as presynaptic inhibitory autoreceptors in the central nervous system, and their stimulation is able to lower systemic blood pressure [9]. Also α2C-AR subtype is a presynaptic receptor regulating NE release from cardiac sympathetic nerve terminals [10].

Several pharmacogenomic studies have demonstrated that ARs present different polymorphic forms in humans, and some of them might affect therapeutic response to AR-modulating drugs (as β-blockers). Briefly, in the human β1-AR gene at least 12 single nucleotide polymorphisms (SNPs) have been described, but only two of them are actually clinically relevant. The first one is Ser49Gly, occurring in the N-terminus region, where it can be involved in receptor downregulation, as well as in intracellular trafficking. The second one is the Arg389Gly in the intracellular C-terminus, with the Arg389 variant showing higher activation both in basal conditions and after agonist stimulation [11].

In the β2-AR protein, three major variants have been identified: Arg or Gly16, Gln or Glu27, and Ile164. Among them, SNPs in positions 16 and 27 are more common, while Ile164 variant is very rare, and its clinical relevance is restricted to a small number of patients. Arg/Gly16 and Gln/Glu27 polymorphisms instead affect receptor downregulation after agonist stimulation [12].

In addition to that, an in-frame deletion of 12 nucleotides, leading to a loss of four aminoacids (Gly-Ala-Gly-Pro), has been described within the α2C-AR protein and associated with increased response to catecholamine stimulation [13].

Cardiac Adrenergic Signaling

As mentioned above, ARs belong to the superfamily of GPCRs; thus, their agonist-induced stimulation catalyzes the exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on the Gα subunit of heterotrimeric G proteins, resulting in the dissociation of the heterotrimer into Gα and Gβγ subunits, which can activate downstream intracellular pathways [14]. In cardiac myocytes, the stimulation of β1-ARs and β2-ARs results in the activation of stimulatory G (Gs) proteins. Gs signaling, in turn, stimulates adenylate cyclase (AC), which converts adenosine triphosphate (ATP) to the second messenger adenosine 3′,5′-monophosphate or cyclic AMP (cAMP), which binds and activates protein kinase A (PKA). PKA can, in turn, phosphorylate and activate several substrates, including L-type calcium channels and phospholamban, a calcium ATPase regulator localized on the sarcoplasmic reticulum, ultimately stimulating the increase of free intracellular Ca^{2+}, the regulator of cardiac contractility. The stimulation of β-ARs in the heart affects not only myocardial contractility but also other cellular functions such as gene transcription and cell growth, mainly through the activation of the mitogenic-activated protein kinase (MAPK) [15].

Persistent β1-AR stimulation can also induce the PKA-independent activation of the calmodulin-dependent kinase II, inducing cardiomyocyte hypertrophy [16].

Fig. 2.1 β-adrenergic receptor (*AR*) signaling in cardiac myocytes. The stimulation of β1-ARs and β2-ARs results in the activation of stimulatory G (*Gs*) proteins. Gs signaling, in turn, stimulates adenylate cyclase (*AC*), which converts adenosine triphosphate (*ATP*) to the second messenger cyclic adenosine monophosphate (*cAMP*), binding and activating protein kinase A (*PKA*). PKA can, in turn, phosphorylate and activate several substrates, including L-type calcium channels and phospholamban, ultimately stimulating the increase of free intracellular Ca^{2+}, the regulator of cardiac contractility. β2-ARs also couple to the inhibitory G (*Gi*) protein and may switch its coupling from Gs to Gi proteins. Differently, β3-AR signaling is associated with nitric oxide release via nitric oxide synthase (*NOS*) activation
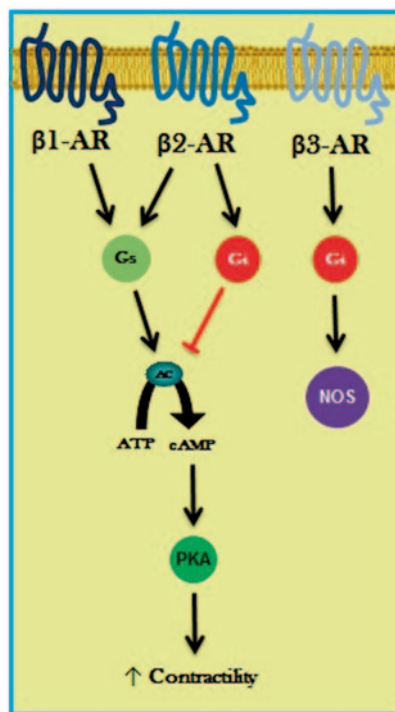

The effects of catecholamines on β2-ARs are different compared with those mediated by β1-ARs, since β2-ARs also couple to the inhibitory G (Gi) protein. In particular, it has been demonstrated that β2-AR-Gi coupling has a cardioprotective effect, while β1-ARs is proapoptotic [17]. However, the model of β2-AR dual coupling (Gi and Gs proteins) is not well clarified yet. It is likely that β2-AR–Gi signaling compartmentalizes the β2-AR-Gs–c-AMP signaling [18]. Also, β2-AR phosphorylation by different kinases (such as PKA) may switch receptor coupling from Gs to Gi proteins [15].

In preclinical studies, β2-AR stimulation and adenoviral-mediated β2-AR overexpression have protective effects in the heart, with improved cardiac function and reduced apoptosis. In contrast, hyperstimulation or overexpression of β1-AR has detrimental effects in the heart [19–21].

Differently, β3-AR signaling is associated with nitric oxide (NO) release via nitric oxide synthase (NOS) activation (Fig. 2.1) [3].

The α1-ARs, instead, couple to the subfamily of Gq heterotrimeric proteins, which activate phospholipase C (PLC)-β. PLC-β catalyzes the formation of the second messengers inositol trisphosphate (IP3) [1, 4, 5]and 2-diacylglycerol (DAG) from the phospholipid phosphatidylinositol-bisphosphate (PIP2). IP3 stimulates the release of Ca^{2+} from sarcoplasmic reticulum, while DAG activates protein kinase C (PKC), both contributing to increased myocardial contraction and vasoconstriction

Fig. 2.2 α-adrenergic receptor (*AR*) signaling in cardiac myocytes. The α1-ARs couple to the subfamily of Gq heterotrimeric proteins, which activate phospholipase C-β (*PLC-β*) that catalyzes the formation of the second messengers inositol trisphosphate (*IP3*) and 2-diacylglycerol (*DAG*) from phosphatidylinositol-bisphosphate (*PIP2*). *IP3* stimulates the release of Ca^{2+} from sarcoplasmic reticulum, while *DAG* activates protein kinase C (*PKC*), both contributing to increased myocardial contractility. The α2-ARs are coupled to the Gi/o family members of the G proteins; thus, its activation inhibits the effector enzyme adenylate cyclase (*AC*). *ATP* adenosine triphosphate, *cAMP* cyclic adenosine monophosphate
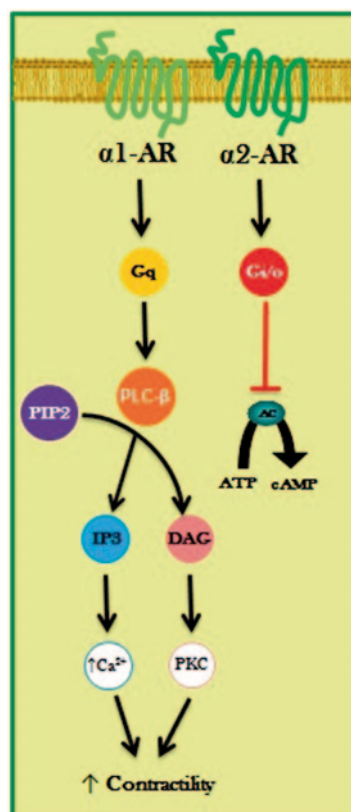
[1]. Finally, α2-ARs are coupled to the Gi/o family members of the G proteins; thus, its activation inhibits the effector enzyme AC (Fig. 2.2) [22].

Both α2- and β-ARs, like other GPCRs, are subject to complex regulatory mechanisms to protect the receptor from both acute and chronic stimulation, a process termed desensitization. In general, GPCR desensitization involves the following events: (1) receptor phosphorylation and uncoupling from G proteins, (2) internalization of membrane-bound receptors, and (3) downregulation through reduced receptor synthesis or increased degradation of internalized receptors [14].

The desensitization process is orchestrated by three families of proteins: second-messenger-dependent PKs, GRKs, and arrestins. Initially, GRKs (G-protein-coupled receptors kinases) recognize and phosphorylate agonist-bound receptors, and then they promote the association of cytosolic cofactor proteins called arrestins, which target GPCRs for endocytosis and activate G-protein-independent signaling [23].

The GRKs are a family of cytosolic serine/threonine kinases consisting of seven isoforms with structural and functional similarities. They are classified into three subfamilies: (1) the rhodopsin kinase GRK1 and visual pigment kinase GRK7, (2) the β-AR kinases (or GRK2 and GRK3); and (3) the GRK4 group (GRK4–6).

Across mammalian species, GRK2 and GRK5 are the most important members of the GRK family due to their ubiquitous expression; in fact, they are particularly abundant in neuronal tissues and in the heart [24, 25].

GRK2 was first identified as β-AR kinase-1 (β-ARK-1); it is the most expressed GRK subtype in the heart. As a consequence of agonist stimulation, Gβγ subunits interact with GRK2, thus mediating its translocation to the plasma membrane where it initiates receptor desensitization. The importance of GRK2 relies also on the fact that in the last decades, much evidence has suggested a key role for GRK2 in the development of myocardial dysfunction [26, 27].

Cardiac Adrenergic System in Heart Failure

HF is a chronic clinical syndrome characterized by reduced pumping capacity of the myocardium (systolic HF), which can develop in response to several cardiac insults [28]. Regardless of the initial cause, the failing heart usually ends up in a vicious cycle of progressive functional decline, with the increased activity of neurohormonal systems aiming to compensate for the reduced blood pressure and cardiac output [1, 29]. In congestive HF, both the activities of the sympathetic nervous system and the renin-angiotensin system (RAS) are increased [30, 31]. In the long term, however, myocardial exposure to high levels of circulating catecholamines and angiotensin increases cardiac workload ultimately leading to maladaptive cardiac remodeling and myocyte death [32, 33].

In HF, ANS hyperactivity is highlighted by increased plasma NE levels, central sympathetic outflow, and NE plasma spillover from activated sympathetic nerve fibers. It has been demonstrated that in patients with HF, cardiac NE spillover is increased by 50-fold, similar to levels found in healthy subjects during maximal exercise [34]. It has also been observed that patients with HF present decreased neuronal density, which can be assessed with 123I-labeled meta-iodobenzylguanidine (MIBG) imaging. MIBG is an analogue of NE, sharing the same uptake, storage, and release processes. As a result, scintigraphic images obtained with MIBG depict the status of catecholamine storage at the level of the myocardial sympathetic presynaptic fibers [35]. Recent MIBG studies have also shown that diabetic patients with HF have lower cardiac sympathetic activity than nondiabetic patients with HF [36].

The ANS hyperactivity observed in HF can also be ascribed to abnormalities in cardiovascular reflexes. In patients with HF, the arterial baroreceptor reflex is largely suppressed, while the sympathoexcitatory reflexes, including the cardiac sympathetic afferent reflex and the arterial chemoreceptor reflex, are augmented [37].

Moreover, other neurohormonal mechanisms, through their interaction with the ANS, can contribute to the progression of cardiac dysfunction in HF. For instance, it has been demonstrated that angiotensin-II can initiate a feedback mechanism leading to the increased sympathetic outflow through the upregulation of the angiotensin-II type 1 receptor and NO inhibition [1].

From the molecular point of view, typical alterations have been described in failing cardiomyocytes, mainly affecting the adrenergic signaling pathway.

The principal features are the decrease in β1-AR density and mRNA levels, uncoupling of β1-AR from Gs and impaired compartmentalization of cAMP/PKA signaling [38, 39]. As previously mentioned, β-AR desensitization and downregulation are predominantly protective mechanisms, which follow the increase in NE plasma levels with consequent receptor overstimulation. β1-AR abnormalities have been attributed to the recruitment of GRK2 to the agonist-bound receptor [40, 41]. In the past decades, it has been demonstrated that an inverse correlation exists between β-AR density and GRK levels in cardiomyocytes [42], and in transgenic mouse models overexpressing the GRK2 inhibitor β-ARKct, myocardial contractility is increased, and HF development is prevented [43]. In 2005, Iaccarino et al. found an inverse correlation between β-ARs expression and GRK2 levels in patients with HF, with lymphocyte levels mirroring myocardial levels. Interestingly, GRK2 levels also correlated with disease severity, suggesting the importance of GRK2 as a potential biomarker for HF [44]. Recently, the data of a prospective study have been published, indicating that in patients with HF undergoing exercise training, GRK2 levels decrease and are also able to predict survival [45].

Further data on the importance of GRK2 in HF came from preclinical studies using gene therapy approaches. A study was performed in which long-term cardiac gene therapy with an adeno-associated vector encoding for -ARKct resulted in sustained improvement of cardiac function, reversal of remodeling, and normalization of the neurohormonal signaling axis in a rodent model of HF [46].

The role of β2-ARs in HF has not been clearly defined yet. In the failing heart, levels of β2-ARs do not change significantly, and studies in transgenic animals have demonstrated that while only fivefold overexpression of β1-AR results in cardiomyopathy, even a 100-fold increase of β2-ARs in the mouse heart does not have any detrimental consequences but, instead, significantly increases cardiac contractile force [20]. This probably needs to be ascribed to the fact that β2-ARs also signal through Gi proteins, which can activate a protective antiapoptotic pathway. Recently, the role of β2-AR has also been studied in a murine postischemic model of HF. Adenoviral-mediated β2-AR overexpression resulted in improved angiogenesis and enhanced coronary reserve and myocardial blood flow in HF mice. This was associated with the activation of the proangiogenic pathway mediated by the vascular endothelial growth factor (VEGF)/protein kinase B (PKB)/endothelial NOS (eNOS) [47, 48].

The role of β3-ARs in HF has not been elucidated. Some evidence suggest that in HF there is an excess of β3-AR signaling, which exerts a negative inotropic effect by increasing NO production and inhibiting calcium transients [49].

α1-ARs, which are involved in cardiomyocyte growth and pathological hypertrophy, may also play a compensatory role in HF in order to preserve cardiac inotropy. In particular, the α1A-subtype has shown to produce prosurvival effects and to protect from maladaptive remodeling both in models of pressure overload and acute myocardial infarction [50].

As for the role of α2-AR in HF, it has been extensively investigated in studies evaluating the contribution of the adrenal gland function in HF, which will be discussed below.

Adrenergic Signaling in Adrenal Gland During Heart Failure

The ANS hyperactivity has been known as a peculiarity during HF [29, 51]. Sympathetic overdrive is strictly implied in the establishment and development of cardiac dysfunction, determines higher risk of arrhythmias, and contributes to worsen the prognosis in patients with HF (increase in plasmatic concentration of catecholamines leads to significant higher mortality) [52].

Indeed, HF is characterized by elevated sympathetic tone, which involves increased levels of circulating and synaptic catecholamines. Cardiac sympathetic nerve activity is significantly augmented in animal models of HF as well as muscle sympathetic nerve activity in patients with HF [53].

Simultaneously, in the adrenal gland, there is an increase in catecholamine output and secretion attested by higher tyrosine hydroxylase levels that is a key enzyme in the production of both NE and Epi.

Chromaffin cells of adrenal medulla are the major source of circulating catecholamines and secrete into the blood 80 % Epi and 20 % NE [54, 55]. The adrenal gland should be considered a specialized sympathetic ganglion with the distinguishing characteristic to excrete its neurohormones directly into the bloodstream [56]. Catecholamines are secreted from chromaffin cells after acetylcholine stimulation of the nicotinic cholinergic receptors; this secretion is regulated by many receptors, among which we mention β2-ARs and α2-ARs that have, respectively, a facilitatory and an inhibitory role [29].

Recently, some studies have shown the crucial inhibitory role of presynaptic α2-AR in peripheral nerve terminals and in adrenal medulla during HF. In particular, α2A- or α2C-AR knockout (KO) mice that underwent HF after transverse aortic constriction manifested an increase in circulating catecholamines and a worst cardiac function compared with control mice [57], while, more interestingly, double α2A/α2C-AR KO mice developed cardiomyopathy at 4 months of age, without any treatment [58]. Moreover, this finding was corroborated by comparable results in human polymorphisms of α2C-AR: α2CDel322–325 in healthy people determines an increase in ANS activity and in plasmatic catecholamine levels during supine rest and an improved pharmacologically induced NE and Epi secretion; besides, the same polymorphisms associated with high HF risk influence the therapeutic effects of the -blocker bucindolol in patients with HF [59–61].

Few years ago, it has been shown that adrenal GRK2 is a physiological regulator of catecholamine production/secretion [62], and its upregulation is fundamental for α2-AR desensitization/downregulation in animal models of HF [55, 63, 64]. In adrenal gland, overexpressed GRK2 phosphorylates α2-ARs determining their

dysfunction and leading to their incapability to inhibit catecholamine production during HF. A gene therapy that inhibits adrenal GRK2 levels (through the peptide β-ARKct) is able to restore α2-AR membrane levels/function and consequently to decrease plasmatic catecholamine levels. Therefore, this sympatholytic therapy contrasts the detrimental cardiac effects of catecholamines and allows re-sensitizing cardiac β-ARs, to decrease left ventricle dilatation and to importantly improve systolic function. Moreover, adrenal GRK2 downregulation has an important role on beneficial sympatholytic effects of β-blockers and exercise training during HF [65, 66].

Hence, since adrenal GRK2 is crucial for α2-AR dysregulation and subsequent increase of circulating catecholamine secretion during HF, its inhibition (through gene therapy, small molecules, or peptides) may represent an innovative sympatholytic therapeutic strategy for HF [67].

Conclusions

Adrenergic system is a key regulator of cardiac activity both in physiologic and in pathological conditions. Indeed, the consequences of sympathetic hyperactivity (and mainly of β-ARs) during HF have been demonstrated to play a fundamental role in the progression of this disease, thus overturning the therapeutic strategies used in the treatment of this and other cardiovascular diseases. Therefore, efforts have been made to further understand adrenergic signaling influencing heart activity, focusing not only on the heart and β-ARs but also on different "players" in the pathogenesis of HF, thus looking at HF as a "systemic" disease involving a dysregulation of various tissues and systems. In this vein, new potential pivotal regulators of sympathetic systems, such as adrenal presynaptic α2-AR or cardiac and adrenal GRK2, have been identified in the last years; such new targets might represent additional, useful tools to increase the therapeutic strategy in the treatment of cardiovascular diseases.

References

1. Triposkiadis F, Karayannis G, Giamouzis G, Skoularigis J, Louridas G, Butler J. The sympathetic nervous system in heart failure physiology, pathophysiology, and clinical implications. J Am Coll Cardiol. 2009;54:1747–62.
2. Barrese V, Taglialatela M. New advances in beta-blocker therapy in heart failure. Front Physiol. 2013;4:323.
3. Lymperopoulos A. Physiology and pharmacology of the cardiovascular adrenergic system. Front in Physiol. 2013;4:240.
4. Granneman JG. The putative beta4-adrenergic receptor is a novel state of the beta1-adrenergic receptor. Am J Physiol Endocrinol Metab. 2001;280:E199–202.
5. Brodde OE. Beta-adrenoceptors in cardiac disease. Pharmacol Ther. 1993;60:405–30.

6. Colucci WS, Wright RF, Braunwald E. New positive inotropic agents in the treatment of congestive heart failure. Mechanisms of action and recent clinical developments. 1. N Engl J Med. 1986;314:290–9.

7. Skeberdis VA, Gendviliene V, Zablockaite D, et al. beta3-adrenergic receptor activation increases human atrial tissue contractility and stimulates the L-type Ca2 + current. J Clin Invest. 2008;118:3219–27.

8. Shannon R, Chaudhry M. Effect of alpha1-adrenergic receptors in cardiac pathophysiology. Am Heart J. 2006;152:842–50.

9. Philipp M, Hein L. Adrenergic receptor knockout mice: distinct functions of 9 receptor subtypes. Pharmacol Ther. 2004;101:65–74.

10. Hein L, Altman JD, Kobilka BK. Two functionally distinct alpha2-adrenergic receptors regulate sympathetic neurotransmission. Nature. 1999;402:181–4.

11. Johnson JA, Liggett SB. Cardiovascular pharmacogenomics of adrenergic receptor signaling: clinical implications and future directions. Clin Pharmacol Ther. 2011;89:366–78.

12. Shin J, Johnson JA. Beta-blocker pharmacogenetics in heart failure. Heart Fail Rev. 2010;15:187–96.

13. Small KM, Forbes SL, Rahman FF, Bridges KM, Liggett SB. A four amino acid deletion polymorphism in the third intracellular loop of the human alpha 2 C-adrenergic receptor confers impaired coupling to multiple effectors. J Biol Chem. 2000;275:23059–64.

14. Rengo G, Perrone-Filardi P, Femminella GD, et al. Targeting the beta-adrenergic receptor system through G-protein-coupled receptor kinase 2: a new paradigm for therapy and prognostic evaluation in heart failure: from bench to bedside. Circ Heart Fail. 2012;5:385–91.

15. Ferrara N, Komici K, Corbi G, et al. beta-adrenergic receptor responsiveness in aging heart and clinical implications. Front Physiol. 2014;4:396.

16. Morisco C, Zebrowski D, Condorelli G, Tsichlis P, Vatner SF, Sadoshima J. The Akt-glycogen synthase kinase 3beta pathway regulates transcription of atrial natriuretic factor induced by beta-adrenergic receptor stimulation in cardiac myocytes. J Biol Chem. 2000;275:14466–75.

17. Hall RA, Premont RT, Chow CW, et al. The beta2-adrenergic receptor interacts with the Na+/H+-exchanger regulatory factor to control Na+/H + exchange. Nature. 1998;392:626–30.

18. Xiao RP, Ji X, Lakatta EG. Functional coupling of the beta 2-adrenoceptor to a pertussis toxin-sensitive G protein in cardiac myocytes. Mol Pharm. 1995;47:322–9.

19. Dorn GW 2nd, Tepe NM, Lorenz JN, Koch WJ, Liggett SB. Low- and high-level transgenic expression of beta2-adrenergic receptors differentially affect cardiac hypertrophy and function in Galphaq-overexpressing mice. Proc Natl Acad Sci U S A. 1999;96:6400–5.

20. Liggett SB, Tepe NM, Lorenz JN, et al. Early and delayed consequences of beta(2)-adrenergic receptor overexpression in mouse hearts: critical role for expression level. Circulation. 2000;101:1707–14.

21. Salazar NC, Vallejos X, Siryk A, et al. GRK2 blockade with betaARKct is essential for cardiac beta2-adrenergic receptor signaling towards increased contractility. Cell Comm Signal. 2013;11:64.

22. De Lucia C, Femminella GD, Gambino G, et al. Adrenal adrenoceptors in heart failure. Front Physiol. 2014;5:246.

23. Bathgate-Siryk A, Dabul S, Pandya K, et al. Negative impact of beta-arrestin-1 on post-myocardial infarction heart failure via cardiac and adrenal-dependent neurohormonal mechanisms. Hypertension. 2014;63:404–12.

24. Rengo G, Lymperopoulos A, Leosco D, Koch WJ. GRK2 as a novel gene therapy target in heart failure. J Mol Cell Cardiol. 2011;50:785–92.

25. Femminella GD, Rengo G, Pagano G, et al. beta-adrenergic receptors and G protein-coupled receptor kinase-2 in Alzheimer's disease: a new paradigm for prognosis and therapy? J Alzheimer's Dis. 2013;34:341–7.

26. Rengo G, Lymperopoulos A, Koch WJ. Future g protein-coupled receptor targets for treatment of heart failure. Curr Treat Opt Cardiovasc Med. 2009;11:328–38.

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