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The Adrenergic Nervous System in Heart Failure: Pathophysiology and Therapy

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Abstract

Heart failure (HF), the leading cause of death in the western world, develops when a cardiac injury or insult impairs the ability of the heart to pump blood and maintain tissue perfusion. It is characterized by a complex interplay of several neurohormonal mechanisms that get activated in the syndrome in order to try and sustain cardiac output in the face of decompensating function. Perhaps the most prominent among these neurohormonal mechanisms is the adrenergic (or sympathetic) nervous system (ANS), whose activity and outflow are enormously elevated in HF. Acutely, and if the heart works properly, this activation of the ANS will promptly restore cardiac function. However, if the cardiac insult persists over time, chances are the ANS will not be able to maintain cardiac function, the heart will progress into a state of chronic decompensated HF, and the hyperactive ANS will continue to "push" the heart to work at a level much higher than the cardiac muscle can handle. From that point on, ANS hyperactivity becomes a major problem in HF, conferring significant toxicity to the failing heart and markedly increasing its morbidity and mortality. The present review discusses the role of the ANS in cardiac physiology and in HF pathophysiology, the mechanisms of regulation of ANS activity and how they go awry in chronic HF, methods of measuring ANS activity in HF, the molecular alterations in heart physiology that occur in HF along with their pharmacological and therapeutic implications, and, finally, drugs and other therapeutic modalities used in HF treatment that target or affect the ANS and its effects on the failing heart.

Keywords

adrenergic nervous system; heart failure; cardiac myocyte; adrenal gland; catecholamine; adrenergic receptor; cardiac sympathetic nerve terminals; neurotransmission; hyperactivation; β–blockers

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Introduction

Heart failure (HF) is a clinical syndrome that develops in response to a cardiac injury or insult that causes decline in the pumping capacity (contractile function) of the heart. It is marked by a perpetual interplay between the underlying myocardial dysfunction and the compensatory neurohumoral mechanisms that are activated in an effort to maintain cardiac output in the face of declining heart function. Among these neurohormonal mechanisms, elevated activities of the adrenergic (or sympathetic) nervous system (ANS), of the renin-angiotensin-aldosterone system (RAAS), and of several cytokines, play central roles.[1,2] These systems are initially able to compensate for the depressed myocardial function and preserve cardiovascular homeostasis. Upon long-term presence of the initial insult to the heart muscle however, cardiac function ultimately succumbs to their deleterious effects on cardiac structure and performance, leading to cardiac decompensation and progressively worsening function, unable to sustain daily life activities. The present review will discuss the role of the ANS in HF pathophysiology and therapeutics, starting with a discussion of its role in normal cardiac function.

ANS & Cardiac Function

The ANS exerts a wide variety of cardiovascular effects, including heart rate acceleration (positive chronotropy, predisposing to arrhythmias), increase in cardiac contractility (positive inotropy), accelerated cardiac relaxation (positive lusitropy), decrease in venous capacitance, and constriction of resistance and cutaneous vessels (Fig. 1). All of these effects aim to increase cardiac performance to prepare and enable the body for the so-called “fight or flight response”. Conversely, the mirror branch of the autonomic nervous system, the parasympathetic (cholinergic) nervous system, slows the heart rate (bradycardia) through vagal nerve impulses, with minimal or no effect on cardiac contractility. This is because the cardiac ventricles, whose contraction is responsible for pumping the blood into the systemic and pulmonary circulations, receive almost exclusively adrenergic fiber innervations, whereas the cholinergic system fibers run with the vagus nerve subendocardially, after it crosses the atrioventricular groove, and reach mainly the atrial myocardium with minimal connections to the ventricular myocardium.[3,4] Therefore, whereas heart rate can be controlled (in opposing fashion) by both autonomic branches, cardiac contraction/relaxation is controlled practically solely by the ANS (Fig. 1).

The ventricular ANS innervation is characterized by a gradient from base to apex.[5] The cardiac neuronal system is composed of cell stations comprising afferent, efferent, and interconnecting neurons behaving as a control system.[6] The ANS outflow to the heart and to the peripheral circulation is regulated by cardiovascular reflexes. Afferent fibers project to the central nervous system by the autonomic nerves, whereas efferent impulses travel from the central nervous system to peripheral organs. The main reflex responses originate from the aortic arch and the carotid baroreceptors (ANS inhibition), cardiopulmonary baroreceptors (diverse reflexes including the Bezold-Jarisch reflex, ANS inhibition), cardiovascular low-threshold polymodal receptors (ANS activation), and peripheral chemoreceptors (ANS activation).[4,7]

ANS activation in the cardiovascular system translates into release of the two catecholamines that mediate its effects, i.e. norepinephrine (NE) (or noradrenaline) and epinephrine (Epi) (or adrenaline), and this can occur via the following mechanisms (Fig. 2): a) NE released by cardiac sympathetic nerve terminals located in the right stellate ganglion reaching the sinus and atrioventricular nodes (resulting in an increase in heart rate and shortening of atrioventricular conduction) and through the left stellate ganglion reaching the left ventricle (resulting in an increase in contractile strength), although NE release and

reuptake can occur throughout the heart, b) Epi (and to a much lesser extent NE) released into the circulation by the adrenal medulla, affecting both the myocardium and peripheral vessels, and, finally, c) local release of NE and Epi by various peripheral adrenergic nervous systems that can synthesize and release these catecholamines in an autocrine/paracrine manner and are located in blood vessels and in cardiac myocytes themselves.[8,9]

Adrenergic Receptors (ARs) in the cardiovascular system

Cardiac AR signaling and regulation

The ANS neurotransmitters NE and Epi mediate their effects in cells and tissues by binding to specific cell surface ARs, which belong to the superfamily of G protein-coupled receptors (GPCRs) or seven transmembrane-spanning receptors or heptahelical receptors (7TMRs). Approximately 80% of NE released by ANS nerve terminals is recycled by the NE transporter (NET) type 1, whereas the remainder spills over into the circulation.[10] The receptors for both ANS catecholamines are divided into three types and 9 total different subtypes, as follows: three α_1AR subtypes (α_{1A}, α_{1B}, α_{1D}), three α_2AR subtypes (α_{2A}, α_{2B}, α_{2C}), and three βAR subtypes (β_1, β_2, β_3).[11] All ARs primarily signal through heterotrimeric G proteins. The human heart contains all three βAR subtypes.[12] β_1AR is the predominant subtype in the (normal, healthy) myocardium, representing 75–80% of total βAR density, followed by β_2AR, which comprises about 15–18% of total cardiomyocyte βARs and the remaining 2–3% is β_3ARs (under normal conditions).[13] The principal role of βARs in the heart is the regulation of cardiac rate and contractility in response to NE and Epi. Stimulation of β_1ARs (mainly) and of β_2ARs (to a lesser extent) increases cardiac contractility (positive inotropic effect), frequency (positive chronotropic effect), and rate of relaxation (lusitropic effect) as well as accelerates impulse conduction through the atrioventricular node (positive dromotropic effect) and pacemaker activity from the sinoatrial node.[14] β_3ARs are predominantly inactive during normal physiologic conditions;[15] however, their stimulation seems to produce a negative inotropic effect opposite to that induced by β_1ARs and β_2ARs, involving the nitric oxide synthase (NOS) pathway, [16] thus acting as a “fuse” against cardiac adrenergic overstimulation.[17] Agonist-induced activation of βARs catalyzes the exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on the G_α subunit of heterotrimeric G proteins, resulting in the dissociation of the heterotrimer into active G_α and free $G_{\beta\gamma}$ subunits (always associated together, i.e. a heterodimer that functions essentially as a monomer) which can transduce intracellular signals independently of each other.[18] The most powerful physiologic mechanism to increase cardiac performance is activation of cardiomyocyte β_1ARs and β_2ARs, which, in turn, activates G_s proteins (stimulatory G proteins). G_s protein signaling stimulates the effector adenylate cyclase (AC), which converts adenosine triphosphate (ATP) to the second messenger adenosine 3′,5′-monophosphate or cyclic AMP (cAMP), which in turn binds to and activates the cAMP-dependent protein kinase (protein kinase A, PKA). PKA is the major effector of cAMP (there is also Epac, exchange protein directly activated by cAMP, which can be activated by cAMP independently of PKA), and, by phosphorylating a variety of substrates, it ultimately results in significant raise in free intracellular Ca^{2+} concentration, which is the master regulator of cardiac muscle contraction (Fig. 3).[19] Among the main targets of PKA phosphorylation in the cardiac myocyte are: the cell membrane-located L-type calcium channels (LTCC) and the sarcoplasmic reticulum (SR)-located ryanodine receptors (RyRs), both leading to an increase in Ca^{2+} entry into the cytoplasm;[19] phospholamban (PLB), a negative modulator of SERCA (Sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase), whose phosphorylation by PKA deinhibits SERCA, thus accelerating Ca^{2+} reuptake by the SR after contraction and increasing SR Ca^{2+} stores available for the next contraction;[19] hyperpolarization-activated cyclic nucleotide-gated channels, which generate the hyperpolarization-activated cation inward current (If) affecting the initiation and modulation of rhythmic activity in cardiac pacemaker cells;[20] troponin I

and myosin binding protein-C, which reduce myofilament sensitivity to Ca^{2+}, thereby accelerating the relaxation of myofilaments;[21] and, finally, phospholemman (PLM), closely associated with $Na^{+/}K^{+}$-ATPase and inhibiting its function, whose phosphorylation by PKA relieves this inhibition and stimulates the sodium pump, thereby accelerating cardiac muscle repolarization and relaxation (Fig. 3).[22] Moreover, PKA can phosphorylate the βARs themselves (and other GPCRs) in the heart, causing G protein uncoupling and functional desensitization of the receptor (heterologous or agonist-independent desensitization).[23]

Of note, β_2AR also mediates the effects of catecholamines in the heart, but in a qualitatively different manner from β_1AR, as it can also couple to the AC inhibitory G protein (G_i). In fact, this switching of β_2AR signaling from G_s to G_i proteins is postulated to be induced by the phosphorylation of the β_2AR by PKA.[23] Nonetheless, it is now generally accepted that in the heart, β_2AR signals and functions in a substantially different way compared to β_1AR.[24–26] Importantly, whereas β_1AR activation enhances cardiomyocyte apoptosis, β_2AR exerts antiapoptotic effects in the heart.[24–27] This essential difference between the two receptor subtypes is ascribed to the signal of β_2AR through G_i proteins.[25] Studies using transgenic mice, β_2AR-selective stimulation and adenoviral-mediated β_2AR overexpression, have demonstrated the protective effects of β_2AR signaling in the myocardium, including improved cardiac function and decreased apoptosis. Conversely, hyperstimulation or overexpression of β_1AR has detrimental effects in the heart.[27,28]

Both α_2- and βARs, like the majority of GPCRs, are subject to agonist-promoted (homologous) desensitization and downregulation, a regulatory process that diminishes receptor response to continuous or repeated agonist stimulation.[29,30] At the molecular level, this process is initiated by receptor phosphorylation by a family of kinases, termed GPCR kinases (GRKs), followed by binding of βarrestins (βarrs) to the GRK-phosphorylated receptor (see below). The βarrs then uncouple the receptor from its cognate G proteins, sterically hinder its further binding to them (functional desensitization) and subsequently target the receptor for internalization.[29,30] Across all mammalian species, GRK2 and GRK5 are the most physiologically important members of the GRK family because they are expressed ubiquitously and regulate the vast majority of GPCRs. They are particularly abundant in neuronal tissues and in the heart.[31,32]

Of note, the differences between the two predominant cardiac βARs, i.e. β_1AR & β_2AR, in terms of their signaling properties, might take a quite different shape and have a much bigger bearing on pathophysiologic implications in the setting of human HF: for instance, and as discussed in more detail in subsequent sections, β_1AR is selectively downregulated (i.e. functional receptor number reduced) in human HF, thus shifting the above mentioned stoichiometry of β_1AR: β_2AR towards 50:50 in the failing heart from ~75%:~20% in the normal, healthy heart.[33,34] However, β_2AR is also non-functional and does not signal properly in the failing heart.[32–34] In addition, emerging evidence suggests that β_2AR signaling in the failing heart is quite different from that in the normal heart, i.e. is more diffuse and non-compartmentalized and resembles more the pro-apoptotic “diffuse” cAMP signaling pattern of the β_1AR.[35] Therefore, this stoichiometric shift in favor of the supposedly “good” β_2AR in HF appears unable to help the heart improve its structure and function.

The human heart also expresses α_{1A}- and α_{1B}ARs, albeit at much lower levels than βARs (~20% of total βARs).[36] How important a role cardiac α_1ARs play in cardiac physiology is still a matter of debate. In contrast, their role in regulation of blood flow by inducing constriction in the smooth muscle wall of major arteries (e.g. aorta, pulmonary arteries, mesenteric vessels, coronary arteries, etc.) is well known and indisputable.[37] The α_1ARs couple to the $G_{q/11}$ family of heterotrimeric G proteins, thereby activating phospholipase C

(PLC)-β. PLCβ generates the second messengers inositol [1,4,5]-trisphosphate (IP_3) and 2-diacylglycerol (DAG) from the cell membrane component phospholipid phosphatidylinositol (4,5)-bisphosphate (PIP_2). IP_3 binds specific receptors in the SR membrane which cause release of Ca^{2+} from intracellular stores, whereas DAG activates protein kinase C (PKC) and transient receptor potential (TRPV) channels (Fig. 3). The end result is again raised intracellular [Ca^{2+}], which leads to contraction (vasoconstriction) (Fig. 3).

Finally, regarding α_2AR subtypes, α_{2B}ARs are known to be present in vascular smooth muscle causing constriction of certain vascular beds, while centrally located α_{2A}ARs can inhibit sympathetic outflow (presynaptic inhibitory autoreceptors) and thus lower systemic blood pressure.[38,39] The release of NE from cardiac sympathetic nerve terminals is controlled by both presynaptic α_{2A}- and α_{2C}ARs,[40] and genetic deletion of both of these α_2AR subtypes leads to cardiac hypertrophy and heart failure due to chronically enhanced cardiac NE release, as well as enhanced NE and Epi secretion from the adrenal medulla.[8,41,42]

AR polymorphisms & cardiac function

There are some very important genetic polymorphisms in human β- and αAR genes, which have been associated with heart failure phenotypes and interaction with β-blocker therapy, a mainstay of HF standard of care (see below), and can significantly influence cardiac function. Thus, it would be useful here to briefly discuss these polymorphisms and their functional consequences for the heart. The Arg389Gly human β_1AR gene polymorphism is probably the best studied AR gene polymorphism to date; it is associated with significantly elevated AC/PKA activities and hence β_1AR-stimulated cardiac contractility in the Arg389 variant carriers (compared to Gly389 carriers).[43] Another human β_1AR polymorphism is the Ser49Gly variation, associated with increased agonist-promoted receptor downregulation for the Gly49 over the Ser49 variant.[44] A lower prevalence of ventricular arrhythmias has been associated with the Gly389 allele.[45] Of note, African-American HF patients with an enhanced activity mutation (Leu41) of GRK5, the second (after GRK2) major cardiac GRK (see above), demonstrate improved survival (explained by this GRK5 mutant acting as a "genetic β-blocker").[46] Additionally, the Gly49 allele of the human β_1AR gene has been shown to confer enhanced survival benefit in response to β-blocker treatment[47] and significant reduction in left ventricular end-diastolic diameter[48] compared to Ser49 homozygous carriers. Ameliorated cardiac adverse remodeling has been reported for Arg389 homozygous HF patients treated with the β-blocker carvedilol[49] and for Arg389Gly heterozygous carriers.[50] There are also negative studies however, reporting no associations of these polymorphisms with any cardiac outcome.[51–53] Further adding to the confusion, a dependency of whether the Ser49Gly polymorphism has any bearing on dilated cardiomyopathy outcomes on the dose of the β-blocker has been reported, according to which the Gly49 allele associates with worse outcomes than Ser49 allele homozygosity at low β-blocker doses, but at higher doses, genotype apparently has no effect.[54] On the other hand, HF patients carrying the Arg389 genotype had a greater agonist-promoted ventricular contractility and improved age-, sex-, and race-adjusted survival than Gly389 carriers in a BEST trial subcohort.[55]

The human β_2AR gene is known to display two variations that are in linkage disequilibrium (and thus constitute a haplotype): Gly16Arg and Gln27Glu, which affect receptor downregulation, and a third polymorphism, Thr164Ile, which confers impaired receptor-G protein coupling and reduced AC-mediated signaling.[56] Finally, a four amino acid deletion in the human α_{2C}AR gene (Del322–325) leads to increased NE release from ANS cardiac nerve terminals[57] and this polymorphism, in combination with the β_1AR Arg389Gly polymorphism, were recently used to stratify patients according to the clinical response to

the β-blocker bucindolol into "very favorable," "favorable," and "unfavorable" response genotypes.[58] The latter polymorphism (β_1AR Arg389Gly) belongs also to a set of three genetic polymorphisms that were recently shown to serve as predictors of appropriate implantable cardioverter-defibrillator (ICD) shock therapies in HF patients.[59] In conclusion, human AR genetic polymorphisms may prove to be very useful tools in guiding the individual "tailoring" ("personalization") of HF therapy in the future.[60–62]

Assessment of ANS activity

Plasma NE measurements represent the usual, crude method to assess ANS function/activity levels, since the latter depends on the rate of immediate NE reuptake as well as NE clearance from the circulation.[63] Two state-of-the-art techniques to quantify ANS activity in humans are radiotracer measurements of regional NE spillover and microneurography (microelectrode direct measurements of post-ganglionic nerve activity). These techniques can discern central from peripheral contributions of increased plasma NE levels and facilitate precise assessment of the regional (e.g. cardiac) sympathetic nerve function, both under physiological and pathological conditions. Cardiac neuronal distribution and function can be imaged with standard gamma-cameras and positron emission tomography (PET) scanners using radiolabeled NE analogs.[64] Cardiac ANS activity or its pharmacological inhibition can also be non-invasively assessed with [^{123}I]-labeled metaiodobenzylguanidine (MIBG), an analogue of NE.[65] β-blockade and RAAS inhibition are associated with an increase in [^{123}I]-labeled MIBG uptake and a reduced washout. [^{123}I]-labeled MIBG cardiac imaging has also been shown to carry independent prognostic information for risk stratification of HF patients in a complementary manner to more commonly used biomarkers such as left ventricular ejection fraction and B-type natriuretic peptide (BNP).[66]

Regulation of ANS outflow & activity in health and in chronic HF

Cardiac sympathetic efferent nerves

There are several mechanisms by which the ANS controls cardiac function. The first one to be documented historically is through the aortic arch and carotid sinus (high pressure) and cardiopulmonary (low pressure) baroreceptor reflexes.[67] Aside from these baroreceptor inputs, additional factors that act within the central nervous system play a role in regulation of cardiac ANS activity. In particular, suprabulbar subcortical monoaminergic neurons and brainstem angiotensin II have attracted interest courtesy of their ability to regulate ANS outflow in HF (Fig. 2). NE turnover in subcortical regions in HF is significantly higher than that in the cortex and than in healthy subjects.[68] Moreover, the rate of subcortical NE release correlates well with global ANS activity, as measured by total body NE plasma spillover. Angiotensin II-dependent ANS activation plays an important role in adverse hemodynamic and left ventricular remodeling responses to myocardial infarction, possibly through superoxide formation.[69,70] Thus, part of the benefit of RAAS modulators in HF might derive from centrally-mediated suppression of ANS activity (see below).

As the heart becomes progressively unresponsive to the stimulatory effects of catecholamines, chronic stimulation of cardiac ANS nerve terminals leads to chronically elevated NE release from the heart (increased NE spillover). Presynaptic α_2ARs present on cardiac ANS nerve terminals and acting as NE release-inhibiting autoreceptors play a crucial role in regulation of cardiac NE release from sympathetic nerves.[38,39] Indeed, knockout (KO) mice lacking either the α_{2A}- or α_{2C}AR subtype show significantly enhanced cardiac ANS activity and circulating catecholamine levels, as well as significantly worse heart function and clinical indices, during the course of surgical pressure overload (by means of transverse aortic constriction, TAC)-induced HF compared with age-matched wild-type HF mice.[40,41] Moreover, double α_{2A}/α_{2C}AR KO mice exhibit even worse cardiac phenotypes

than single α_{2A}AR KO mice and, by 4 months of age, they spontaneously develop cardiomyopathy (without stress or any specific insult).[71] In HF patients, the expected inhibitory effects of α_2AR stimulation on NE spillover are markedly blunted, thereby contributing to the increase in cardiac NE spillover observed in chronic HF.[72] In addition, the human α_{2C}AR Del322–325 variant that displays impaired signaling and sympatho-inhibitory function (see above) is alone associated with increased risk of HF in homozygous African-American carriers, especially when co-carried with the hyperfunctional cardiac β_1AR Arg389 variant, with the most probable mechanism being attenuated autoinhibitory feedback of, and thus enhanced NE release from the cardiac sympathetic nerves.[57] In fact, even in healthy humans, the α_{2C}AR Del322–325 variant is associated with increased sympathetic nervous and adrenomedullary hormonal activities, during both supine rest and pharmacologically evoked catecholamine release.[73] Thus, presynaptic inhibitory α_2-adrenergic autoreceptors crucially regulate ANS cardiac nerve activity and NE release into the heart and any dysfunction of these receptors either due to genetic polymorphisms or enhanced desensitization/downregulation (see below) translate into increased morbidity and mortality in chronic HF (Fig. 2). Perhaps the crucial role of presynaptic α_2ARs in regulating NE release from cardiac ANS nerves stems from the fact that they are the only presynaptic ARs that can inhibit NE release; presynaptic βARs (of the β_2AR subtype, mainly) are facilitatory autoreceptors enhancing NE release at sympathetic nerve terminals,[74] a phenomenon whose inhibition may contribute to the therapeutic benefit of β-blockers in HF (see below) (Fig. 2).

Adrenal glands

Circulating Epi and NE derive from two major sources in the body: the cardiac sympathetic nerve endings, which release NE directly onto the cardiac muscle, and the adrenal medulla, whose chromaffin cells synthesize, store and release Epi (mainly) and NE upon acetylcholine stimulation of the nicotinic cholinergic receptors (nAChRs) present on their cell membranes (Fig. 2).[8] Epi represents approximately 80% of the total adrenal catecholamine secretion under normal conditions, with NE the rest ~20%.[75] However, these percentages vary widely depending on the physiological condition of the adrenal gland and of the whole body. Thus, all of the Epi in the body and a significant amount of circulating NE derive from the adrenal medulla, and the total amount of catecholamines presented to cardiac ARs at any given time is composed of these circulating NE & Epi plus NE released locally from sympathetic nerve terminals within the heart.[8] The secretion of catecholamines from the adrenal glands is regulated in a complex manner by a variety of cell membrane receptors present in chromaffin cells. Many of these receptors are GPCRs, including, similarly to cardiac ANS nerve endings, α_2ARs that inhibit secretion (inhibitory presynaptic autoreceptors), and βARs that enhance it (facilitatory presynaptic autoreceptors) (Fig. 2).[8,38,41,42] Of note, although various presynaptic auto- and heteroreceptors, facilitate (increase) adrenal catecholamine secretion, e.g. βARs, muscarinic cholinergic receptors (mAChRs), angiotensin II-ergic, histaminergic, and adrenomedullin receptors, the α_2ARs are the only receptor type reported to date to inhibit adrenal catecholamine secretion.[8,42,76]

An increase in GRK2 expression and activity (see above) has been documented in several cardiovascular diseases, including increased cardiac expression in HF[77–79] and increased expression in some vascular beds in hypertension.[80] A few years ago, we reported that GRK2 expression and activity are increased also in the adrenal medulla during HF.[81] Specifically, our studies over the past few years have established that adrenal GRK2 upregulation is responsible for severe adrenal α_2AR dysfunction in chronic HF, which causes a loss of the sympathoinhibitory function of these receptors in the adrenal gland, and catecholamine secretion is thus chronically elevated (Fig. 2).[81–85] This emerging crucial role for adrenal GRK2 in HF is underlined by the fact that its specific inhibition, via adenoviral-

mediated βARKct adrenal gene delivery (see below), leads to a significant reduction in circulating catecholamine levels, restoring not only adrenal, but also cardiac function in HF.[81] Additional evidence for the crucial role of adrenal GRK2-regulated α_2ARs in regulating adrenal ANS tone in HF comes from the phenylethanolamine-N-methyl transferase (PNMT)-driven GRK2 KO mice.[82] These mice, which do not express GRK2 in their adrenal medullae from birth, display decreased ANS outflow and circulating catecholamines in response to myocardial infarction, which translates into preserved cardiac function and morphology over the course of the ensuing HF.[82] Of note, elevated GRK2-dependent α_2AR dysfunction during HF might also occur in other peripheral sympathetic nerve terminals of the heart (Fig. 2) and of other organs, thus contributing to the increased NE release and spillover, as well as to the presynaptic α_2AR dysfunction in ANS neurons observed in chronic HF (see above).[72,86]

Effects of ANS overactivity in chronic HF

Myocardial systolic dysfunction is associated with neurohormonal hyperactivity as a compensatory mechanism to maintain cardiac output in the face of declining cardiac function. The neuronal part of this response is represented by ANS cardiac nerve terminals, whereas the hormonal (or humoral) part is represented by increased secretion, and elevated circulating levels of certain hormones, the most prominent being Epi & NE, along with the RAAS hormones (i.e. angiotensin II & aldosterone).[87] ANS hyperactivity is evidenced by increased plasma NE & Epi levels, elevated (central) sympathetic outflow, and heightened NE spillover from activated cardiac sympathetic nerve terminals into the circulation.[88] Cardiac NE spillover in untreated HF patients can reach up to 50-fold higher levels than those of healthy individuals under maximal exercise conditions.[89] The information on chronic ANS activation in HF with preserved left ventricular ejection fraction (i.e. diastolic HF) is very limited. In patients with hypertension, ANS hyperactivity may contribute to the development of left ventricular diastolic dysfunction and thus increase HF risk.[90] In systolic HF, patients may actually have decreased ANS neuronal density & function, resulting in decreased NE concentration within the heart, in addition to decreased postsynaptic βAR density, due to depletion of cardiac ANS neuronal NE stores and decreased NE presynaptic reuptake secondary to NE transporter downregulation.[91,92]

Effects on cardiac ARs

The elevated ANS outflow and NE and Epi levels in chronic HF lead to chronically elevated stimulation of the cardiac βAR system which has detrimental repercussions for the failing heart. Extensive investigations over the past three decades have helped delineate the molecular alterations afflicting the cardiac βAR system that occur during HF, and it is now well known that in chronic human HF, cardiomyocyte βAR signaling and function are significantly deranged and the adrenergic reserve of the heart is diminished.[33,34,93] Cardiac βAR dysfunction in human HF is characterized at the molecular level by selective reduction of β_1AR density at the plasma membrane (downregulation) and by uncoupling of the remaining membrane β_1ARs and β_2ARs from G proteins (functional desensitization).[33] Importantly, myocardial levels and activities of the most important, versatile and ubiquitous GRKs, GRK2 and GRK5, are elevated, both in humans and in animal models of HF.[32,77–79,94] The current consensus is that in chronic HF, the excessive amount of ANS-derived catecholamines “hitting” cardiac βARs extracellularly triggers the GRK2 upregulation inside the cardiomyocytes, thus leading to a reduction in cardiac βAR density and responsiveness and resulting in cardiac inotropic reserve depletion.[95,96] This GRK2 elevation possibly serves as a homeostatic protective mechanism aimed at defending the heart against excessive catecholaminergic toxicity. However, several studies soon refuted this assumption, demonstrating that GRK2 upregulation is detrimental for the heart and causes the functional uncoupling of βARs in vivo.[94] This finding prompted investigations of

the role GRK2 plays in cardiac function, which revealed that cardiac GRK2 is an absolutely critical regulator of cardiac βAR-dependent contractility and function. Specifically, cardiomyocyte-restricted overexpression of GRK2 to the same level of upregulation found in human HF (i.e. 3–4 fold) markedly attenuated βAR signaling and contractile reserve, showing that GRK2 is the main culprit for the functional desensitization of cardiac βARs in HF (Fig. 3).[97] The proof for this was provided by studies of the in vivo inhibition of cardiomyocyte GRK2, which were enabled by the development of the βARKct mini-gene, which blocks cell membrane translocation and hence activation of GRK2, and its cardiomyocyte-specific expression in vivo in transgenic mice by virtue of the αMHC (myosin heavy chain) gene promoter.[97] Indeed, GRK2 inhibition in vivo in the heart with βARKct (or its partial genetic deletion) enhances cardiac contractility both at baseline and after adrenergic stimulation, reverses the contractile and βAR dysfunctions, and preserves or even augments cardiac function and survival in HF.[97–102] On the down side, loss of cardiac GRK2 can predispose the heart to catecholamine toxicity, exactly because it works, in essence, as a positive inotropic therapy.[103] In summary, elevated ANS activity in chronic HF causes enhanced GRK2-mediated cardiac β_1- and β_2AR desensitization and β_1AR downregulation, which leads to the progressive loss of the adrenergic and inotropic reserves of the heart, the hallmark molecular abnormality of this disease (Fig. 3).[104]

With regards to the other major AR type expressed in the heart, α_1ARs in HF may function in a compensatory fashion to maintain cardiac inotropy, but their involvement in cardiac pathophysiology appears limited to situations of cardiac hypertrophy that ultimately lead to HF.[105] For instance, in the presence of pressure overload, cardiac α_{1A}ARs get activated and promote cardiomyocyte survival (i.e. block apoptosis), protecting against adverse remodeling and decompensation to HF.[106,107]

ANS cardiotoxicity

ANS cardiac toxicity is well documented. For instance, intravenous infusion of isoproterenol (a standard non-subtype selective βAR full agonist) or NE results in acute contraction band lesions attributed to relative hypoxia, increased sarcolemmal permeability, calcium overload, elevation of cAMP, activation of α- and βARs, and formation of oxidative catecholamine metabolites.[108,109] Chronic catecholamine administration in rats causes interstitial fibrosis, reduces βAR–mediated inotropic responses (adrenergic inotropic reserve), promotes cardiac apoptosis, and induces contractile dysfunction primarily through left ventricular dilatation.[110,111] Moreover, NE induces cardiac apoptosis via both β_1AR- and ROS–tumor necrosis factor (TNF)–caspase-mediated signaling pathways. [112,113] A perfect example of catecholamine-induced cardiac toxicity leading to cardiac dysfunction is found in stress (Takotsubo) cardiomyopathy: high circulating levels of Epi trigger a form of "myocardial stunning", which includes the signaling switch of the cardiac β_2AR from G_s to G_i proteins, especially in the region of the apical myocardium, where βAR density is highest,[114] thus negatively affecting inotropy.[115]

ANS therapeutics in HF

AR blockers

β-blockers—β-blockers (Table 1) can be broadly classified into generations: first generation, which are non-subtype selective and competitively block both the β_1- and β_2ARs (propranolol, nadolol, timolol); second generation, with much higher affinity for the β_1- than for the β_2AR (atenolol, metoprolol, bisoprolol); and third generation, which may be subtype-selective (celiprolol, nebivolol) or non-selective (bucindolol, carvedilol, labetalol). The latter ones can also block α_1ARs, thereby causing peripheral vasodilation (bucindolol, carvedilol, labetalol). Celiprolol possesses also β_2AR agonist properties, while nebivolol can also

induce nitric oxide (NO) synthesis.[116] Cardioselectivity (i.e. selectivity for the β_1-over the β_2AR subtype) is dose-dependent and decreases with increasing dosage. Both subtype-selective and non-selective agents have negative chronotropic and inotropic effects. β_1AR-selective agents have a lesser inhibitory effect on the β_2AR and thus are less likely to cause peripheral vasoconstriction (and bronchoconstriction).[116] Exercise performance may be impaired to a lesser extent by β_1AR-selective agents, since they spare the β_2AR which increases skeletal muscle blood flow (via vasodilation) in response to exercise. Finally, some β-blockers are mixed βAR agonists/antagonists (or partial agonists), i.e. at low concentrations antagonize the receptors but at high concentrations they actually activate βARs (act as agonists) causing cardiac stimulation. These β-blockers possess (the so-called) "intrinsic sympathomimetic activity" (ISA), e.g. pindolol, alprenolol, oxprenolol, and inhibit the effects of catecholamines through the high affinity binding state of the myocardial β_1AR, while mimicking catecholamines when binding to the low affinity state of the cardiomyocyte β_1AR.[117] The β-blockers with ISA have a high propensity for arrhythmias and should not be used for chronic HF treatment. The majority of β-blockers are partially or completely metabolized by CYP2D6, a gene with considerable genetic variability.[118] All β-blockers are approved for chronic HF treatment (Table 1).[119] Conversely, they are all contraindicated in acute HF (due to the acute drop in cardiac output they cause).[119] Chronic β-blocker therapy reverses left ventricular remodeling, reduces risk of hospitalization, improves survival, reduces risk of arrhythmias (sudden cardiac death), improves coronary blood flow to the heart (relieves angina), and protects the heart against cardiotoxic overstimulation by the catecholamines. All of these effects result in a decrease in the oxygen/energy and metabolic demands of the heart (cardiac workload is decreased) and in an increase in its oxygen/energy supply, thereby improving, in the long run, left ventricular function and performance. Various molecular mechanisms underlying these effects have been postulated: 1) direct antagonism of catecholaminergic cardiotoxic effects; 2) cardiac βAR upregulation and restoration of their signaling and function (i.e. increase in adrenergic and inotropic reserves of the heart), partly via cardiac GRK2 downregulation;[120] 3) suppression of the elevated cardiotoxic, adverse remodeling-promoting, and pro-apoptotic neurohormonal systems (RAAS, endothelin); 4) coronary blood flow enhancement (as a result of diastolic prolongation); and 5) restoration of the reflex controls on the heart and the circulation.[121] In addition, restoration of adrenal GRK2-α_2AR-catecholamine secretion axis and suppression of NE release from cardiac ANS endings might contribute to the beneficial effects of β-blockers in chronic HF, as well.[74,85]

α-blockers—HF patients receiving the α_1-blocker prazosin experienced worse outcomes than did those receiving the combined vasodilator therapy of hydralazine and isosorbide dinitrate (BiDil).[122] Prazosin has been reported to increase catecholamine levels in a feedback manner, thus diminishing any potential benefit reaped from vascular smooth muscle α_1AR inhibition-induced vasodilation (Table 1).[123] Adding to the inappropriateness of α_1AR blockers for HF therapy, the doxazosin arm in the ALLHAT clinical trial was terminated early because of higher HF incidence (Table 1).[124]

Centrally acting sympatholytic agents

Central α_2ARs inhibit ANS outflow via an autocrine negative feedback mechanism.[39] Clonidine is a centrally acting α_2AR agonist that significantly attenuates cardiac and renal sympathetic tones in HF patients (sympatholytic). It exerts marked sympathoinhibitory effects without clinical deterioration (Table 1).[125] Large clinical trials, however, are still needed to evaluate its true place in the chronic HF therapeutic armamentarium. Another centrally acting sympatholytic agent, moxonidine, also an imidazoline derivative like clonidine, has been used in clinical trials for HF. Moxonidine is also an α_2AR agonist as well as an agonist at the putative imidazoline receptors.[126,127] It causes marked reductions

in plasma NE,[128] and it failed in clinical trials as it was found to increase HF-related mortality (Table 1).[129] As a possible explanation for this, excessive sympatholysis to a point that was incompatible with life was postulated. However, another explanation might have been the reported α_2AR desensitization and downregulation that accompanies HF,[72] which renders α_2ARs dysfunctional, raising sympathetic outflow in HF and limiting efficacy of α_2AR sympatholytic agonists (see above) (Table 1).

RAAS modulating drugs

Hyperactivation of the RAAS is another neurohormonal hallmark of chronic HF and the degree of its activation correlates with prognosis. Angiotensin II enhances the release and inhibits the reuptake of NE at ANS nerve endings (Fig. 2).[130,131] Angiotensin-converting enzyme (ACE) inhibitors, by decreasing angiotensin II and aldosterone levels, increase plasma renin activity, while they also decrease circulating catecholamines and vasopressin thanks to the hemodynamic improvements they bring about.[132] Plasma aldosterone levels may be elevated as high as 20-fold in HF patients, primarily due to increased production by the adrenal glands following stimulation by the high plasma angiotensin II concentrations.[133–135] Our lab has also recently identified another mechanism for the enhanced cardiotoxic aldosterone production by the adrenal cortex in HF: enhanced activity of adrenal βarrestin1, a co-factor of GRKs in receptor desensitization (see above), at the AT_1R angiotensin II receptor.[136,137] In addition to its electrolyte, hemodynamic and metabolic effects, aldosterone has several direct detrimental effects on the myocardium, promoting cardiac adverse remodeling and HF progression, and also mediates several of the cardiotoxic effects of angiotensin II in the cardiac muscle, e.g. myocardial fibrosis, increased oxidative stress, inflammation, etc.[133–135] With regards to the ANS in HF, aldosterone, like angiotensin II, can decrease NE reuptake from ANS presynaptic neurons, thereby contributing to the enhanced ANS outflow in chronic HF (Fig. 2).[131,133] Some of the beneficial effects of aldosterone antagonists, such as spironolactone and eplerenone, in human HF may thus derive from suppression of this effect of aldosterone on the ANS (i.e. partial sympatholysis) (Table 1).[138,139]

Sympathomimetics (AR agonists)

Dopamine, dobutamine and milrinone (its congener amrinone has been withdrawn from the market) represent the most commonly used sympathomimetic drugs (adrenergic agonists) used as positive inotropes for acute HF (Table 1). All positive inotropes lead to cAMP accumulation inside cardiomyocytes, which increases contractility via elevation of intracellular free Ca^{2+} concentration (Fig. 3). Dopamine and dobutamine achieve that by binding to and activating cardiac β_1ARs, whereas milrinone blocks phosphodiesterase type 3 (PDE3, cAMP-specific phosphodiesterase), thereby preventing cAMP degradation.[140] Of course, the elevation of intracellular free Ca^{2+} inside cardioymocytes predisposes to arrhythmias (major adverse effect of positive inotropes). All three inotropes produce a vasodilatory effect and can cause a reduction in blood pressure; this is especially the case for milrinone, since there are no β_1ARs in vascular smooth muscle (β_2AR is the βAR subtype there) (Table 1).[141] Finally, the effects of dobutamine and dopamine are blunted when the patient is already on β-blocker therapy.[142] In that case, non-βAR-related inotropes are preferred, such as milrinone or glucagon (which can also increase cAMP in cardiomyocytes through its own G_s protein-coupled receptor). Despite the straightforward rationale for using positive inotropes in HF (drop in cardiac output, hence administration of an agent that will directly increase it), clinical trials have clearly demonstrated that sympathomimetics significantly increase mortality in chronic HF.[143] Therefore, they are reserved only for treatment of acute episodes of HF, characterized by clinically evident hypoperfusion or shock, or as a bridge to more definitive treatment, such as revascularization or cardiac transplantation (Table 1).

Another therapeutic strategy involving sympathomimetics in HF is combined β_1AR blockade with simultaneous β_2AR stimulation with clenbuterol (a β_2AR-selective agonist), which has been shown to help reverse severe HF in selected patients requiring left ventricular assist devices (LVADs).[144] The rationale for this approach is based on studies demonstrating that clenbuterol is able to improve left ventricular function at the whole heart and cellular levels by affecting cell morphology, excitation-contraction coupling, and myofilament sensitivity to calcium.[145] However, a recent small, randomized controlled trial showed that clenbuterol was associated with a significant increase in both lean mass and lean/fat ratio as well as in muscle strength, and an increase in exercise duration in chronic HF patients (presumably via enhanced vascular smooth muscle β_2AR-dependent vasodilation which increases skeletal blood flow), and it is, in fact, used in sports medicine as a performance enhancing drug (PED).[146] Therefore, determination of the ultimate role of clenbuterol in HF therapy requires further investigations in larger prospective trials.

Non-drug therapeutic modalities affecting ANS in HF

Exercise training—Exercise intolerance is a major symptom of chronic HF, and skeletal myopathies contribute to the reduced functional capacity in HF.[147] ANS activation serves as a coordinator of the heart and muscle vasculature to maintain adequate blood pressure during exercise.[148] However, ANS overactivity leads to skeletal myopathies in HF, because ANS-mediated vasoconstriction at rest and during exercise limits muscle blood flow and arteriolar elasticity, leading to hypoperfusion/ischemia, release of ROS, and chronic inflammation.[149] On the other hand, exercise training has been shown to improve central hemodynamics, peripheral muscle function and symptoms, and to actually reduce ANS activity in HF patients, alone or in conjunction with β-blocker therapy, resulting in reductions of all-cause and cardiovascular mortalities (Table 1).[150,151] The postulated mechanisms for these beneficial effects of exercise training in HF include improvements in arterial and chemoreflex controls, significant reduction in central ANS outflow, correction of central nervous system abnormalities, increases in peripheral blood flow and reduction in circulating pro-inflammatory cytokines.[152] Additionally, studies from our laboratory and others in experimental HF animals have shown that exercise training improves cardiac βAR signaling and function, increases adrenergic and inotropic reserves of the heart ameliorating cardiac contractility and function and helping restore normal ANS activity/outflow and circulating catecholamine levels, partly via normalization of GRK2 activity and restoration of sympathoinhibitory α_2AR function in the adrenal gland (Table 1).[81,153,154]

Cholinergic system modulation—There is a complex interplay between the parasympathetic (cholinergic) nervous system and the ANS. Indeed, vagus nerve afferent activation from the periphery can modulate efferent adrenergic and cholinergic function centrally and at the baroreceptors. Moreover, cholinergic neurons exert tonic inhibition of adrenergic neuron activation and of NE release from presynaptic ANS nerve terminals. The well known cardiovascular effects of the parasympathetic nervous system, i.e. heart rate reduction (bradycardia, indirectly via inhibition of the ANS and directly by hyperpolarization and pacemaker activity suppression of the sinoatrial node) and vasorelaxation (indirectly via NO synthesis)[155] are significantly attenuated in chronic HF, which leads, among other consequences, also to lifting of the “harness” of ANS activation which the cholinergic system normally imposes, and thus, (indirect) enhancement of ANS outflow (Table 1).[156] Clinical and experimental data suggest that β-blockade augments reflex vagal nerve control of heart rate in HF, via suppression of the cardiac sympathetic presynaptic β_2AR-facilitated NE release.[157] Additionally, muscarinic cholinergic M_2 receptors (M_2 mAChRs) are upregulated in the left ventricular free wall, resulting in reduced heart rate variability.[158,159] Finally, vagus nerve stimulation therapy, combined with chronic

β-blocker therapy, has been shown to further improve left ventricular function and reverse remodeling beyond what is achieved with β-blockers alone (Table 1).[160,161]

Conclusions/Future perspectives

A vast number of studies over the past few decades have established the crucial role of activated ANS in the compensatory response of the circulation to retain its hemodynamic stability in the face of a cardiac insult, and when this fails, its excessive activation that accelerates HF progression and poses severe toxicity on the chronically failing heart. Additionally, the benefits of β-blockers and other therapeutic modalities that mitigate or protect the heart against this ANS hyperactivity are also well documented nowadays. Several recent developments in the basic cardiovascular research field that are at various stages of preclinical testing to ultimately reach the bedside in HF therapeutics also aim at reducing the activity and/or the detrimental effects of the ANS on the failing heart. Among these are sympatholytic agents (α_2AR agonists), polymorphic variants of cardiac ARs that confer better prognosis in HF or better responses to current HF treatments, new sympathomimetics that seek to augment the function of the seemingly "cardioprotective" β_2AR while simultaneously blocking the "cardiotoxic" β_1AR (e.g. clenbuterol), activation of the cardiac parasympathetic nervous system, and, last but not least, augmentation of cardiac βAR-dependent function without the accompanying elevation of ANS activity/outflow. The latter is pursued with the very promising GRK2 inhibition therapeutic approach, which poses to improve cardiac adrenergic and inotropic reserves by restoring cardiac βAR signaling and function (i.e. to provide positive inotropy), while keeping the ANS outflow at bay by restoring or augmenting central, cardiac and adrenal sympathoinhibitory α_2AR function. Further understanding of the mechanisms of ANS activation and of the repercussions this has on regulation of cardiac function and structure in chronic HF is most certainly bound to provide the clinicians of the future with some, currently very desperately needed, newer and better weapons in the battle against this devastating disease.

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Non-standard abbreviations and acronyms

HF	Heart failure
ANS	Adrenergic Nervous System
RAAS	Renin-angiotensin-aldosterone system
NE	Norepinephrine
Epi	Epinephrine
AR	Adrenergic receptor
GPCR	G protein-coupled receptor
NET	Norepinephrine transporter
NOS	NO synthase
AC	Adenylyl cyclise

ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
PKA	Protein kinase A (cAMP-dependent protein kinase)
LTCC	L-type calcium channel
RyR	Ryanodine receptor
PLB	Phospholamban
SERCA	Sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase
SR	Sarcoplasmic reticulum
PLM	Phospholemman
G_s	stimulatory G protein
$G_{i/o}$	inhibitory or other G protein
GRK	GPCR kinase
PLC	Phospholipase C
PIP_2	Phosphatidylinositol (4,5)-bisphosphate
PKC	Protein Kinase C
DAG	2-diacylglycerol
TRPV	Transient Receptor Potential Vanilloid
MIBG	Metaiodobenzylguanidine
KO	Knockout
TAC	Transverse aortic constriction
βARKct	βAR kinase carboxyl terminal
ROS	Reactive oxygen species
TNF	Tumor necrosis factor
CYP	Cytochrome P450 enzyme
ACE	Angiotensin converting enzyme
PDE	Phosphodiesterase
LVAD	Left ventricular assist device
PED	Performance enhancing drug
NO	Nitric oxide

References

1. Mann DL, Bristow MR. Mechanisms and models in heart failure: the biomechanical model and beyond. Circulation. 2005; 111:2837–2849. [PubMed: 15927992]
2. Mudd JO, Kass DA. Tackling heart failure in the twenty-first century. Nature. 2008; 451:919–928. [PubMed: 18288181]
3. Zipes DP. Heart-brain interactions in cardiac arrhythmias: role of the autonomic nervous system. Cleve Clin J Med. 2008; 75:S94–S96. [PubMed: 18540155]

4. 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; 54:1747–1762. [PubMed: 19874988]
5. Pierpont GL, DeMaster EG, Reynolds S, Pederson J, Cohn JN. Ventricular myocardial catecholamines in primates. J Lab Clin Med. 1985; 106:205–210. [PubMed: 4020248]
6. Armour JA. Cardiac neuronal hierarchy in health and disease. Am J Physiol Regul Integr Comp Physiol. 2004; 287:R262–R271. [PubMed: 15271675]
7. Malliani A, Pagani M, Pizzinelli P, Furlan R, Guzzetti S. Cardiovascular reflexes mediated by sympathetic afferent fibers. J Auton Nerv Syst. 1983; 7:295–301. [PubMed: 6875194]
8. Lymperopoulos A, Rengo G, Koch WJ. Adrenal adrenoceptors in heart failure: fine-tuning cardiac stimulation. Trends Mol Med. 2007 Dec.13:503–511. [PubMed: 17981507]
9. Lymperopoulos A. Ischemic emergency? endothelial cells have their own “adrenaline shot” at hand. Hypertension. 2012; 60:12–14. [PubMed: 22665125]
10. Leineweber K, Wangemann T, Giessler C, Bruck H, Dhein S, Kostelka M, Mohr FW, Silber RE, Brodde OE. Age-dependent changes of cardiac neuronal noradrenaline reuptake transporter (uptake1) in the human heart. J Am Coll Cardiol. 2002; 40:1459. [PubMed: 12392837]
11. Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR Jr, Trendelenburg U. International Union of Pharmacology nomenclature of adrenoceptors. Pharmacol Rev. 1994; 46:121–136. [PubMed: 7938162]
12. Lymperopoulos A, Rengo G, Koch WJ. GRK2 inhibition in heart failure: something old, something new. Curr Pharm Des. 2012; 18:186–191. [PubMed: 22229578]
13. Brodde OE. Beta-adrenoceptors in cardiac disease. Pharmacol Ther. 1993; 60:405–430. [PubMed: 7915424]
14. Colucci WS, Wright RF, Braunwald E. New positive inotropic agents in the treatment of congestive heart failure. Mechanisms of action and recent clinical developments. N Engl J Med. 1986; 314:290–299. [PubMed: 2867470]
15. Skeberdis VA, Gendviliene V, Zablockaite D, Treinys R, Macianskiene R, Bogdelis A, Jurevicius J, Fischmeister R. Beta3-adrenergic receptor activation increases human atrial tissue contractility and stimulates the L-type Ca^{2+} current. J Clin Invest. 2008; 118:3219–3227. [PubMed: 18704193]
16. Gauthier C, Leblais V, Kobzik L, Trochu JN, Khandoudi N, Bril A, Balligand JL, Le Marec H. The negative inotropic effect of beta3-adrenoceptor stimulation is mediated by activation of a nitric oxide synthase pathway in human ventricle. J Clin Invest. 1998; 102:1377–1384. [PubMed: 9769330]
17. Rozec B, Erfanian M, Laurent K, Trochu JN, Gauthier C. Nebivolol, a vasodilating selective beta(1)-blocker, is a beta(3)-adrenoceptor agonist in the nonfailing transplanted human heart. J Am Coll Cardiol. 2009; 53:1532– 1538. [PubMed: 19389564]
18. Lohse MJ, Engelhardt S, Eschenhagen T. What is the role of beta-adrenergic signaling in heart failure? Circ Res. 2003; 93:896–906. [PubMed: 14615493]
19. Bers DM. Calcium cycling and signaling in cardiac myocytes. Annu Rev Physiol. 2008; 70:23–49. [PubMed: 17988210]
20. Ludwig A, Zong X, Jeglitsch M, Hofmann F, Biel M. A family of hyperpolarization-activated mammalian cation channels. Nature. 1998; 393:587–591. [PubMed: 9634236]
21. Sulakhe PV, Vo XT. Regulation of phospholamban and troponin-I phosphorylation in the intact rat cardiomyocytes by adrenergic and cholinergic stimuli: roles of cyclic nucleotides, calcium, protein kinases and phosphatases and depolarization. Mol Cell Biochem. 1995; 149:103–126. [PubMed: 8569720]
22. Despa S, Bossuyt J, Han F, Ginsburg KS, Jia LG, Kutchai H, Tucker AL, Bers DM. Phospholemman-phosphorylation mediates the beta-adrenergic effects on Na/K pump function in cardiac myocytes. Circ Res. 2005; 97:252–259. [PubMed: 16002746]
23. Daaka Y, Luttrell LM, Lefkowitz RJ. Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. Nature. 1997; 390:88–91. [PubMed: 9363896]
24. Communal C, Singh K, Sawyer DB, Colucci WS. Opposing effects of β1- and β2-aadrenergic receptors on cardiac myocyte apoptosis: role of a pertussis toxin-sensitive G protein. Circulation. 1999; 100:2210–2212. [PubMed: 10577992]

25. Chesley A, Lundberg MS, Asai T, Xiao RP, Ohtani S, Lakatta EG, Crow MT. The β2-adrenergic receptor delivers an antiapoptotic signal to cardiac myocytes through Gi-dependent coupling to phosphatidylinositol 3-kinase. Circ Res. 2000; 87:1172–1179. [PubMed: 11110775]
26. Zhu WZ, Zheng M, Koch WJ, Lefkowitz RJ, Kobilka BK, Xiao RP. Dual modulation of cell survival and cell death by β2-adrenergic signalling in adult mouse cardiomyocytes. Proc Natl Acad Sci USA. 2001; 98:1607–1612. [PubMed: 11171998]
27. Dorn GW II, Tepe NM, Lorenz JN, Koch WJ, Liggett SB. Low- and high-level transgenic expression of β2-adrenergic receptors differentially affect cardiac hypertrophy and function in Gaq-overexpressing mice. Proc Natl Acad Sci USA. 1999; 96:6400–6405. [PubMed: 10339599]
28. Liggett SB, Tepe NM, Lorenz JN, Canning AM, Jantz TD, Mitarai S, Yatani A, Dorn GW 2nd. Early and delayed consequences of beta(2)-adrenergic receptor overexpression in mouse hearts: critical role for expression level. Circulation. 2000; 101:1707–1714. [PubMed: 10758054]
29. Reiter E, Lefkowitz RJ. GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. Trends Endocrinol Metab. 2006; 17:159–165. [PubMed: 16595179]
30. Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev. 2001; 53:1–24. [PubMed: 11171937]
31. Arriza JL, Dawson TM, Simerly RB, Martin LJ, Caron MG, Snyder SH, Lefkowitz RJ. The G-protein-coupled receptor kinases betaARK1 and betaARK2 are widely distributed at synapses in rat brain. J Neurosci. 1992; 12:4045–4055. [PubMed: 1403099]
32. Rockman HA, Koch WJ, Lefkowitz RJ. Seven-transmembrane-spanning receptors and heart function. Nature. 2002; 415:206–212. [PubMed: 11805844]
33. Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P, Jamieson S, et al. β1-and β2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β1-receptor down-regulation in heart failure. Circ Res. 1986; 59:297–309. [PubMed: 2876788]
34. Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC, Stinson EB. Decreased catecholamine sensitivity and β-adrenegic receptor density in failing human hearts. N Engl J Med. 1982; 307:205–211. [PubMed: 6283349]
35. Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H, Lohse MJ, Korchev YE, Harding SE, Gorelik J. Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation. Science. 2010; 327:1653–1657. [PubMed: 20185685]
36. Woodcock EA, Du XJ, Reichelt ME, Graham RM. Cardiac alpha 1-adrenergic drive in pathological remodelling. Cardiovasc Res. 2008; 77:452– 462. [PubMed: 18032391]
37. Shannon R, Chaudhry M. Effect of alpha1-adrenergic receptors in cardiac pathophysiology. Am Heart J. 2006; 152:842–850. [PubMed: 17070143]
38. Philipp M, Hein L. Adrenergic receptor knockout mice: distinct functions of 9 receptor subtypes. Pharmacol Ther. 2004; 101:65–74. [PubMed: 14729393]
39. Philipp M, Brede M, Hein L. Physiological significance of alpha(2)-adrenergic receptor subtype diversity: one receptor is not enough. Am J Physiol Regul Integr Comp Physiol. 2002; 283:R287–R295. [PubMed: 12121839]
40. Hein L, Altman JD, Kobilka BK. Two functionally distinct alpha2-adrenergic receptors regulate sympathetic neurotransmission. Nature. 402:181–184. [PubMed: 10647009]
41. Brede M, Wiesmann F, Jahns R, Hadamek K, Arnolt C, Neubauer S, Lohse MJ, Hein L. Feedback inhibition of catecholamine release by two different alpha2-adrenoceptor subtypes prevents progression of heart failure. Circulation. 2002; 106:2491–2496. [PubMed: 12417548]
42. Brede M, Nagy G, Philipp M, Sorensen JB, Lohse MJ, Hein L. Differential control of adrenal and sympathetic catecholamine release by alpha2-adrenoceptor subtypes. Mol Endocrinol. 2003; 17:1640–1646. [PubMed: 12764077]
43. Sandilands AJ, O'Shaughnessy KM, Brown MJ. Greater inotropic and cyclic AMP responses evoked by noradrenaline through Arg389 beta1-adrenoceptors versus Gly389 beta1-adrenoceptors in isolated human atrial myocardium. Br J Pharmacol. 2003; 138:386–292. [PubMed: 12540530]

44. Levin MC, Marullo S, Muntaner O, Andersson B, Magnusson Y. The myocardium-protective Gly-49 variant of the beta 1-adrenergic receptor exhibits constitutive activity and increased desensitization and down-regulation. J Biol Chem. 2002; 277:30429–30435. [PubMed: 12034720]
45. Biolo A, Clausell N, Santos KG, Salvaro R, Ashton-Prolla P, Borges A, Rohde LE. Impact of beta1-adrenergic receptor polymorphisms on susceptibility to heart failure, arrhythmogenesis, prognosis, and response to beta-blocker therapy. Am J Cardiol. 2008; 102:726–732. [PubMed: 18773997]
46. Liggett SB, Cresci S, Kelly RJ, Syed FM, Matkovich SJ, Hahn HS, Diwan A, Martini JS, Sparks L, Parekh RR, Spertus JA, Koch WJ, Kardia SL, Dorn GW 2nd. A GRK5 polymorphism that inhibits beta-adrenergic receptor signaling is protective in heart failure. Nat Med. 2008; 14:510–517. [PubMed: 18425130]
47. Borjesson M, Magnusson Y, Hjalmarson A, Andersson B. A novel polymorphism in the gene coding for the beta(1)-adrenergic receptor associated with survival in patients with heart failure. Eur Heart J. 2000; 21:1853– 1858. [PubMed: 11052857]
48. Terra SG, Hamilton KK, Pauly DF, Lee CR, Patterson JH, Adams KF, Schofield RS, Belgado BS, Hill JA, Aranda JM, Yarandi HN, Johnson JA. Beta1-adrenergic receptor polymorphisms and left ventricular remodeling changes in response to beta-blocker therapy. Pharmacogenet Genomics. 2005; 15:227–234. [PubMed: 15864115]
49. Mialet Perez J, Rathz DA, Petrashevskaya NN, Hahn HS, Wagoner LE, Schwartz A, Dorn GW, Liggett SB. Beta1-adrenergic receptor polymorphisms confer differential function and predisposition to heart failure. Nat Med. 2003; 9:1300–1305. [PubMed: 14502278]
50. Chen L, Meyers D, Javorsky G, Burstow D, Lolekha P, Lucas M, Semmler AB, Savarimuthu SM, Fong KM, Yang IA, Atherton J, Galbraith AJ, Parsonage WA, Molenaar P. Arg389Gly-beta1-adrenergic receptors determine improvement in left ventricular systolic function in nonischemic cardiomyopathy patients with heart failure after chronic treatment with carvedilol. Pharmacogenet Genomics. 2007; 17:941–949. [PubMed: 18075464]
51. Sehnert AJ, Daniels SE, Elashoff M, Wingrove JA, Burrow CR, Horne B, Muhlestein JB, Donahue M, Liggett SB, Anderson JL, Kraus WE. Lack of association between adrenergic receptor genotypes and survival in heart failure patients treated with carvedilol or metoprolol. J Am Coll Cardiol. 2008; 52:644–651. [PubMed: 18702968]
52. de Groote P, Helbecque N, Lamblin N, Hermant X, Mc Fadden E, Foucher-Hossein C, Amouyel P, Dallongeville J, Bauters C. Association between beta-1 and beta-2 adrenergic receptor gene polymorphisms and the response to beta-blockade in patients with stable congestive heart failure. Pharmacogenet Genomics. 2005; 15:137–142. [PubMed: 15861037]
53. White HL, de Boer RA, Maqbool A, Greenwood D, van Veldhuisen DJ, Cuthbert R, Ball SG, Hall AS, Balmforth AJ. MERIT-HF Study Group. An evaluation of the beta-1 adrenergic receptor Arg389Gly polymorphism in individuals with heart failure: a MERIT-HF sub-study. Eur J Heart Fail. 2003; 5:463– 468. [PubMed: 12921807]
54. Magnusson Y, Levin MC, Eggertsen R, Nyström E, Mobini R, Schaufelberger M, Andersson B. Ser49Gly of beta1-adrenergic receptor is associated with effective beta-blocker dose in dilated cardiomyopathy. Clin Pharmacol Ther. 2005; 78:221–231. [PubMed: 16153393]
55. Liggett SB, Mialet-Perez J, Thaneemit-Chen S, Weber SA, Greene SM, Hodne D, Nelson B, Morrison J, Domanski MJ, Wagoner LE, Abraham WT, Anderson JL, Carlquist JF, Krause-Steinrauf HJ, Lazzeroni LC, Port JD, Lavori PW, Bristow MR. A polymorphism within a conserved beta(1)-adrenergic receptor motif alters cardiac function and beta-blocker response in human heart failure. Proc Natl Acad Sci USA. 2006; 103:11288–11293. [PubMed: 16844790]
56. Johnson JA, Liggett SB. Cardiovascular pharmacogenomics of adrenergic receptor signaling: clinical implications and future directions. Clin Pharmacol Ther. 2011; 89:366–378. [PubMed: 21289619]
57. Small KM, Wagoner LE, Levin AM, Kardia SL, Liggett SB. Synergistic polymorphisms of beta1- and alpha2C-adrenergic receptors and the risk of congestive heart failure. N Engl J Med. 2002; 347:1135–1142. [PubMed: 12374873]
58. O'Connor CM, Fiuzat M, Carson PE, Anand IS, Plehn JF, Gottlieb SS, Silver MA, Lindenfeld J, Miller AB, White M, Walsh R, Nelson P, Medway A, Davis G, Robertson AD, Port JD, Carr J, Murphy GA, Lazzeroni LC, Abraham WT, Liggett SB, Bristow MR. Combinatorial

pharmacogenetic interactions of bucindolol and β1, a2C adrenergic receptor polymorphisms. PLoS One. 2012; 7:e44324. [PubMed: 23071495]

59. Chemello D, Rohde LE, Santos KG, Silvello D, Goldraich L, Pimentel M, Rosa PR, Zimerman L, Clausell N. Genetic polymorphisms of the adrenergic system and implantable cardioverter-defibrillator therapies in patients with heart failure. Europace. 2010; 12:686–691. [PubMed: 20185488]
60. Dorn GW 2nd. Adrenergic signaling polymorphisms and their impact on cardiovascular disease. Physiol Rev. 2010; 90:1013–1062. [PubMed: 20664078]
61. Cresci S, Kelly RJ, Cappola TP, Diwan A, Dries D, Kardia SL, Dorn GW 2nd. Clinical and genetic modifiers of long-term survival in heart failure. J Am Coll Cardiol. 2009; 54:432–444. [PubMed: 19628119]
62. Cresci S, Dorn GW 2nd, Jones PG, Beitelshees AL, Li AY, Lenzini PA, Province MA, Spertus JA, Lanfear DE. Adrenergic-pathway gene variants influence beta-blocker-related outcomes after acute coronary syndrome in a race-specific manner. J Am Coll Cardiol. 2012; 60:898–907. [PubMed: 22703928]
63. Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G. Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. Physiol Rev. 1990; 70:963–985. [PubMed: 1977182]
64. Wieland DM, Brown LE, Tobes MC, Rogers WL, Marsh DD, Mangner TJ, Swanson DP, Beierwaltes WH. Myocardial imaging with a radioiodinated norepinephrine storage analog. J Nucl Med. 1981; 22:22–31. [PubMed: 7452352]
65. Sisson JC, Shapiro B, Meyers L, Mallette S, Mangner TJ, Wieland DM, Glowniak JV, Sherman P, Beierwaltes WH. Metaiodobenzylguanidine to map scintigraphically the adrenergic nervous system in man. J Nucl Med. 1987; 28:1625–1636. [PubMed: 3655915]
66. Jacobson AF, Lombard J, Banerjee G, Camici PG. 123I-MIBG scintigraphy to predict risk for adverse cardiac outcomes in heart failure patients: design of two prospective multicenter international trials. J Nucl Cardiol. 2009; 16:113–121. [PubMed: 19152136]
67. Kaye D, Esler M. Sympathetic neuronal regulation of the heart in aging and heart failure. Cardiovasc Res. 2005; 66:256–264. [PubMed: 15820194]
68. Aggarwal A, Esler MD, Lambert GW, Hastings J, Johnston L, Kaye DM. Norepinephrine turnover is increased in suprabulbar subcortical brain regions and is related to whole-body sympathetic activity in human heart failure. Circulation. 2002; 105:1031–1033. [PubMed: 11877349]
69. Wang H, Huang BS, Ganten D, Leenen FHH. Prevention of sympathetic and cardiac dysfunction after myocardial infarction in transgenic rats deficient in brain angiotensinogen. Circ Res. 2004; 94:843–849. [PubMed: 15061159]
70. Lindley TE, Doobay M, Sharma R, Davisson R. Superoxide is involved in the central nervous system activation and sympathoexcitation of myocardial infarction induced heart failure. Circ Res. 2004; 94:402–409. [PubMed: 14684626]
71. Brum PC, Kosek J, Patterson A, Bernstein D, Kobilka B. Abnormal cardiac function associated with sympathetic nervous system hyperactivity in mice. Am J Physiol Heart Circ Physiol. 2002; 283:H1838–H1845. [PubMed: 12384461]
72. Aggarwal A, Esler MD, Socratous F, Kaye DM. Evidence for functional presynaptic alpha-2 adrenoceptors and their down-regulation in human heart failure. J Am Coll Cardiol. 2001; 37:1246–1251. [PubMed: 11300430]
73. Neumeister A, Charney DS, Belfer I, Geraci M, Holmes C, Sharabi Y, Alim T, Bonne O, Luckenbaugh DA, Manji H, Goldman D, Goldstein DS. Sympathoneural and adrenomedullary functional effects of alpha2C-adrenoceptor gene polymorphism in healthy humans. Pharmacogenet Genomics. 2005; 15:143–149. [PubMed: 15861038]
74. Docherty JR. Age-related changes in adrenergic neuroeffector transmission. Auton Neurosci. 2002; 96:8–12. [PubMed: 11911505]
75. Eaton MJ, Duplan H. Useful cell lines derived from the adrenal medulla. Mol Cell Endocrinol. 2004; 228:39–52. [PubMed: 15541571]

76. Moura E, Afonso J, Hein L, Vieira-Coelho MA. Alpha2-adrenoceptor subtypes involved in the regulation of catecholamine release from the adrenal medulla of mice. Br J Pharmacol. 2006; 149:1049–1058. [PubMed: 17075569]
77. Rengo G, Perrone-Filardi P, Femminella GD, Liccardo D, Zincarelli C, de Lucia C, Pagano G, Marsico F, Lymperopoulos A, Leosco D. Targeting the β-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; 5:385–391. [PubMed: 22589366]
78. Lymperopoulos A, Bathgate A. Pharmacogenomics of the heptahelical receptor regulators G protein-coupled receptor kinases and arrestins: the known and the unknown. Pharmacogenomics. 2012; 13:323–341. [PubMed: 22304582]
79. Rengo G, Lymperopoulos A, Leosco D, Koch WJ. GRK2 as a novel gene therapy target in heart failure. J Mol Cell Cardiol. 2011; 50:785–792. [PubMed: 20800067]
80. Penn RB, Pronin AN, Benovic JL. Regulation of G protein-coupled receptor kinases. Trends Cardiovasc Med. 2000; 10:81–89. [PubMed: 11150735]
81. Lymperopoulos A, Rengo G, Funakoshi H, Eckhart AD, Koch WJ. Adrenal GRK2 upregulation mediates sympathetic overdrive in heart failure. Nat Med. 2007; 13:315–323. [PubMed: 17322894]
82. Lymperopoulos A, Rengo G, Gao E, Ebert SN, Dorn GW II, Koch WJ. Reduction of sympathetic activity via adrenal-targeted GRK2 gene deletion attenuates heart failure progression and improves cardiac function after myocardial infarction. J Biol Chem. 2010; 285:16378–16386. [PubMed: 20351116]
83. Lymperopoulos A, Rengo G, Zincarelli C, Soltys S, Koch WJ. Modulation of adrenal catecholamine secretion by in vivo gene transfer and manipulation of G protein-coupled receptor kinase-2 activity. Mol Ther. 2008; 16:302–307. [PubMed: 18223549]
84. Rengo G, Leosco D, Zincarelli C, Marchese M, Corbi G, Liccardo D, Filippelli A, Ferrara N, Lisanti MP, Koch WJ, Lymperopoulos A. Adrenal GRK2 lowering is an underlying mechanism for the beneficial sympathetic effects of exercise training in heart failure. Am J Physiol Heart Circ Physiol. 2010; 298:H2032–H2038. [PubMed: 20304818]
85. Rengo G, Lymperopoulos A, Zincarelli C, Femminella G, Liccardo D, Pagano G, de Lucia C, Cannavo A, Gargiulo P, Ferrara N, Perrone Filardi P, Koch W, Leosco D. Blockade of β-adrenoceptors restores the GRK2-mediated adrenal a(2) -adrenoceptor-catecholamine production axis in heart failure. Br J Pharmacol. 2012; 166:2430–2440. [PubMed: 22519418]
86. Lang CC, Stein CM, Nelson RA, He HB, Belas FJ, Blair IA, Wood M, Wood AJ. Sympathoinhibitory response to clonidine is blunted in patients with heart failure. Hypertension. 1997; 30:392–397. [PubMed: 9314422]
87. Dzau VJ, Colucci WS, Hollenberg NK, Williams GH. Relation of the renin-angiotensin-aldosterone system to clinical state in congestive heart failure. Circulation. 1981; 63:645–651. [PubMed: 7006851]
88. Pepper GS, Lee RW. Sympathetic activation in heart failure and its treatment with beta-blockade. Arch Intern Med. 1999; 159:225–234. [PubMed: 9989534]
89. Morris MJ, Cox HS, Lambert GW, Kaye DM, Jennings GL, Meredith IT, Esler MD. Region-specific neuropeptide Y overflows at rest and during sympathetic activation in humans. Hypertension. 1997; 29:137–143. [PubMed: 9039093]
90. Hogg K, McMurray J. Neurohumoral pathways in heart failure with preserved systolic function. Prog Cardiovasc Dis. 2005; 47:357–366. [PubMed: 16115515]
91. Regitz V, Leuchs B, Bossaller C, Sehested J, Rappolder M, Fleck E. Myocardial catecholamine concentrations in dilated cardiomyopathy and heart failure of different origins. Eur Heart J. 1991; 12 (Suppl D):171–174. [PubMed: 1915450]
92. Backs J, Haunstetter A, Gerber SH, Metz J, Borst MM, Strasser RH, Kübler W, Haass M. The neuronal norepinephrine transporter in experimental heart failure: evidence for a post-transcriptional downregulation. J Mol Cell Cardiol. 2001; 33:461–472. [PubMed: 11181015]
93. Port JD, Bristow MR. Altered beta-adrenergic receptor gene regulation and signaling in chronic heart failure. J Mol Cell Cardiol. 2001; 33:887–905. [PubMed: 11343413]

94. Ungerer M, Böhm M, Elce JS, Erdmann E, Lohse MJ. Altered expression of beta-adrenergic receptor kinase and beta 1-adrenergic receptors in the failing human heart. Circulation. 1993; 87:454–463. [PubMed: 8381058]

95. Rengo G, Lymperopoulos A, Koch WJ. Future g protein–coupled receptor targets for treatment of heart failure. Curr Treatment Options Cardiovasc Med. 2009; 11:328–338.

96. Floras JS. The “unsympathetic” nervous system of heart failure. Circulation. 2002; 105:1753–1755. [PubMed: 11956112]

97. Koch WJ, Rockman HA, Samama P, Hamilton RA, Bond RA, Milano CA, Lefkowitz RJ. Cardiac function in mice overexpressing the β-adrenergic receptor kinase or a βARK inhibitor. Science. 1995; 268:1350–1353. [PubMed: 7761854]

98. Akhter SA, Eckhart AD, Rockman HA, Shotwell K, Lefkowitz RJ, Koch WJ. In vivo inhibition of elevated myocardial β-adrenergic receptor kinase activity in hybrid transgenic mice restores normal β-adrenergic signaling and function. Circulation. 1999; 100:648–653. [PubMed: 10441103]

99. Rockman HA, Chien KR, Choi DJ, Iaccarino G, Hunter JJ, Ross J Jr, Lefkowitz RJ, Koch WJ. Expression of a beta-adrenergic receptor kinase 1 inhibitor prevents the development of myocardial failure in gene-targeted mice. Proc Natl Acad Sci USA. 1998; 95:7000–7005. [PubMed: 9618528]

100. Rengo G, Lymperopoulos A, Zincarelli C, Donniacuo M, Soltys S, Rabinowitz JE, Koch WJ. Myocardial adeno-associated virus serotype 6-betaARKct gene therapy improves cardiac function and normalizes the neurohormonal axis in chronic heart failure. Circulation. 2009; 119:89–98. [PubMed: 19103992]

101. Rockman HA, Choi DJ, Akhter SA, Jaber M, Giros B, Lefkowitz RJ, Caron MG, Koch WJ. Control of myocardial contractile function by the level of β-adrenergic receptor kinase 1 in gene-targeted mice. J Biol Chem. 1998; 273:18180–18184. [PubMed: 9660778]

102. Raake PW, Schlegel P, Ksienzyk J, Reinkober J, Barthelmes J, Schinkel S, Pleger S, Mier W, Haberkorn U, Koch WJ, Katus HA, Most P, Müller OJ. AAV6.βARKct cardiac gene therapy ameliorates cardiac function and normalizes the catecholaminergic axis in a clinically relevant large animal model of heart failure. Eur Heart J. 2013 in press.

103. Matkovich SJ, Diwan A, Klanke JL, Hammer DJ, Marreez Y, Odley AM, Brunskill EW, Koch WJ, Schwartz RJ, Dorn GW 2nd. Cardiac-specific ablation of G-protein receptor kinase 2 redefines its roles in heart development and beta-adrenergic signaling. Circ Res. 2006; 99:996–1003. [PubMed: 17008600]

104. Eschenhagen T. Beta-adrenergic signaling in heart failure-adapt or die. Nat Med. 2008; 14:485–487. [PubMed: 18463653]

105. Knowlton KU, Michel MC, Itani M, Shubeita HE, Ishihara K, Brown JH, Chien KR. The alpha 1A-adrenergic receptor subtype mediates biochemical, molecular, and morphologic features of cultured myocardial cell hypertrophy. J Biol Chem. 1993; 268:15374–15380. [PubMed: 8393439]

106. Du XJ, Gao XM, Kiriazis H, Moore XL, Ming Z, Su Y, Finch AM, Hannan RA, Dart AM, Graham RM. Transgenic alpha1A-adrenergic activation limits post-infarct ventricular remodeling and dysfunction and improves survival. Cardiovasc Res. 2006; 71:735–743. [PubMed: 16859660]

107. Huang Y, Wright CD, Merkwan CL, Baye NL, Liang Q, Simpson PC, O’Connell TD. An alpha1A-adrenergic extracellular signal-regulated kinase survival signaling pathway in cardiac myocytes. Circulation. 2007; 115:763–772. [PubMed: 17283256]

108. Todd GL, Baroldi G, Pieper GM, Clayton FC, Eliot RS. Experimental catecholamine-induced myocardial necrosis. II. Temporal development of isoproterenol-induced contraction band lesions correlated with ECG, hemodynamic and biochemical changes. J Mol Cell Cardiol. 1985; 17:647–656. [PubMed: 4020881]

109. Mann DL, Kent RL, Parsons B, Cooper GT. Adrenergic effects on the biology of the adult mammalian cardiocyte. Circulation. 1992; 85:790–804. [PubMed: 1370925]

110. Brouri F, Hanoun N, Mediani O, Saurini F, Hamon M, Vanhoutte PM, Lechat P. Blockade of beta 1- and desensitization of beta 2-adrenoceptors reduce isoprenaline-induced cardiac fibrosis. Eur J Pharmacol. 2004; 485:227–234. [PubMed: 14757145]

111. Osadchii OE, Norton GR, McKechnie R, Deftereos D, Woodiwiss AJ. Cardiac dilatation and pump dysfunction without intrinsic myocardial systolic failure following chronic beta-adrenoreceptor activation. Am J Physiol Heart Circ Physiol. 2007; 292:H1898–H1905. [PubMed: 17158652]

112. Communal C, Singh K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of the beta-adrenergic pathway. Circulation. 1998; 98:1329–1134. [PubMed: 9751683]

113. Fu YC, Chi CS, Yin SC, Hwang B, Chiu YT, Hsu SL. Norepinephrine induces apoptosis in neonatal rat cardiomyocytes through a reactive oxygen species-TNF alpha-caspase signaling pathway. Cardiovasc Res. 2004; 62:558–567. [PubMed: 15158148]

114. Mori H, Ishikawa S, Kojima S, Hayashi J, Watanabe Y, Hoffman JI, Okino H. Increased responsiveness of left ventricular apical myocardium to adrenergic stimuli. Cardiovasc Res. 1993; 27:192–198. [PubMed: 8386061]

115. Lyon AR, Rees PS, Prasad S, Poole-Wilson PA, Harding SE. Stress (Takotsubo) cardiomyopathy—a novel pathophysiological hypothesis to explain catecholamine-induced acute myocardial stunning. Nat Clin Pract Cardiovasc Med. 2008; 5:22–29. [PubMed: 18094670]

116. López-Sendón J, Swedberg K, McMurray J, Tamargo J, Maggioni AP, Dargie H, Tendera M, Waagstein F, Kjekshus J, Lechat P, Torp-Pedersen C. Task Force On Beta-Blockers of the European Society of Cardiology. Expert consensus document on beta-adrenergic receptor blockers. Eur Heart J. 2004; 25:1341–1362. [PubMed: 15288162]

117. Kaumann AJ, Molenaar P. The low-affinity site of the beta1-adrenoceptor and its relevance to cardiovascular pharmacology. Pharmacol Ther. 2008; 118:303–336. [PubMed: 18501968]

118. Sistonen J, Sajantila A, Lao O, Corander J, Barbujani G, Fuselli S. CYP2D6 worldwide genetic variation shows high frequency of altered activity variants and no continental structure. Pharmacogenet Genomics. 2007; 17:93–101. [PubMed: 17301689]

119. Bristow M. Antiadrenergic therapy of chronic heart failure: surprises and new opportunities. Circulation. 2003; 107:1100–1102. [PubMed: 12615784]

120. Iaccarino G, Tomhave ED, Lefkowitz RJ, Koch WJ. Reciprocal in vivo regulation of myocardial G protein-coupled receptor kinase expression by b-adrenergic receptor stimulation and blockade. Circulation. 1998; 98:1783–1789. [PubMed: 9788834]

121. Adamson PB, Gilbert EM. Reducing the risk of sudden death in heart failure with beta-blockers. J Card Fail. 2006; 12:734–746. [PubMed: 17174236]

122. Cohn JN, Archibald DG, Ziesche S, Franciosa JA, Harston WE, Tristani FE, Dunkman WB, Jacobs W, Francis GS, Flohr KH, et al. Effect of vasodilator therapy on mortality in chronic congestive heart failure. Results of a Veterans Administration Cooperative Study. N Engl J Med. 1986; 314:1547–1552. [PubMed: 3520315]

123. Colucci WS, Williams GH, Braunwald E. Increased plasma norepinephrine levels during prazosin therapy for severe congestive heart failure. Ann Intern Med. 1980; 93:452–453. [PubMed: 7436166]

124. ALLHAT Collaborative Research Group. Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). J Am Med Assoc. 2000; 283:1967–1975.

125. Giles TD, Thomas MG, Quiroz A, Rice JC, Plauche W, Sander GE. Acute and short-term effects of clonidine in heart failure. Angiology. 1987; 38:537–548. [PubMed: 3304028]

126. Ernsberger P, Meeley MP, Mann JJ, Reis DJ. Clonidine binds to imidazole binding sites as well as alpha 2-adrenoceptors in the ventrolateral medulla. Eur J Pharmacol. 1987; 134:1–13. [PubMed: 3030779]

127. Ernsberger P, Meeley MP, Reis DJ. An endogenous substance with clonidine-like properties: selective binding to imidazole sites in the ventrolateral medulla. Brain Res. 1988; 441:309–318. [PubMed: 2834001]

128. Lang CC, Rayos GH, Chomsky DB, Wood AJ, Wilson JR. Effect of sympathoinhibition on exercise performance in patients with heart failure. Circulation. 1997; 96:238–245. [PubMed: 9236440]

129. Cohn JN, Pfeffer MA, Rouleau J, Sharpe N, Swedberg K, Straub M, Wiltse C, Wright TJ. MOXCON Investigators, Adverse mortality effect of central sympathetic inhibition with sustained-release moxonidine in patients with heart failure (MOXCON). Eur J Heart Fail. 2003; 5:659–667. [PubMed: 14607206]

130. Sumners C, Raizada MK. Angiotensin II stimulates norepinephrine uptake in hypothalamus-brain stem neuronal cultures. Am J Physiol. 1986; 250:C236–C244. [PubMed: 3513605]

131. Weber MA, Purdy RE. Catecholamine-mediated constrictor effects of aldosterone on vascular smooth muscle. Life Sci. 1982; 30:2009–2017. [PubMed: 7109834]

132. Benedict CR, Francis GS, Shelton B, Johnstone DE, Kubo SH, Kirlin P, Nicklas J, Liang CS, Konstam MA, Greenberg B, et al. on behalf of the SOLVD Investigators. Effect of long-term enalapril therapy on neurohormones in patients with left ventricular dysfunction. Am J Cardiol. 1995; 75:1151–1157. [PubMed: 7762503]

133. Weber KT. Aldosterone in congestive heart failure. N Engl J Med. 2001; 345:1689–1697. [PubMed: 11759649]

134. Connell JM, Davies E. The new biology of aldosterone. J Endocrinol. 2005; 186:1–20. [PubMed: 16002531]

135. Marney AM, Brown NJ. Aldosterone and end-organ damage. Clin Sci. 2007; 113:267–278. [PubMed: 17683282]

136. Lymperopoulos A, Rengo G, Zincarelli C, Kim J, Soltys S, Koch WJ. An adrenal β-arrestin 1-mediated signaling pathway underlies angiotensin II-induced aldosterone production in vitro and in vivo. Proc Natl Acad Sci USA. 2009; 106:5825–5830. [PubMed: 19289825]

137. Lymperopoulos A, Rengo G, Zincarelli C, Kim J, Koch WJ. Adrenal beta-3arrestin 1 inhibition in vivo attenuates post-myocardial infarction progression to heart failure and adverse remodeling via reduction of circulating aldosterone levels. J Am Coll Cardiol. 2011; 57:356–365. [PubMed: 21232674]

138. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. N Engl J Med. 1999; 341:709–717. [PubMed: 10471456]

139. Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurley S, Kleiman J, Gatlin M. Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study Investigators. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med. 2003; 348:1309–1321. [PubMed: 12668699]

140. Petersen JW, Felker GM. Inotropes in the management of acute heart failure. Crit Care Med. 2008; 36:S106–S111. [PubMed: 18158469]

141. Grose R, Strain J, Greenberg M, LeJemtel TH. Systemic and coronary effects of intravenous milrinone and dobutamine in congestive heart failure. J Am Coll Cardiol. 1986; 7:1107–1113. [PubMed: 3958369]

142. Triposkiadis F, Dalampiras P, Kelepeshis G, Skoularigis J, Sitafidis G. Hemodynamic effects of dobutamine in patients with an exacerbation of chronic systolic heart failure treated with low doses of carvedilol. Int J Clin Pharmacol Ther. 2008; 46:136–139. [PubMed: 18397684]

143. Packer M. The development of positive inotropic agents for chronic heart failure: how have we gone astray? J Am Coll Cardiol. 1993; 22:119A–126A. [PubMed: 8509531]

144. Birks EJ, Tansley PD, Hardy J, George RS, Bowles CT, Burke M, Banner NR, Khaghani A, Yacoub MH. Left ventricular assist device and drug therapy for the reversal of heart failure. N Engl J Med. 2006; 355:1873–1884. [PubMed: 17079761]

145. Soppa GK, Lee J, Stagg MA, Felkin LE, Barton PJ, Siedlecka U, Youssef S, Yacoub MH, Terracciano CM. Role and possible mechanisms of clenbuterol in enhancing reverse remodelling during mechanical unloading in murine heart failure. Cardiovasc Res. 2008; 77:695–706. [PubMed: 18178572]

146. Kamalakkannan G, Petrilli CM, George I, LaManca J, McLaughlin BT, Shane E, Mancini DM, Maybaum S. Clenbuterol increases lean muscle mass but not endurance in patients with chronic heart failure. J Heart Lung Transplant. 2008; 27:457–461. [PubMed: 18374884]

147. Mancini DM, Walter G, Reichek N, Lenkinski R, McCully KK, Mullen JL, Wilson JR. Contribution of skeletal muscle atrophy to exercise intolerance and altered muscle metabolism in heart failure. Circulation. 1992; 85:1364–1373. [PubMed: 1555280]

148. Khan MH, Sinoway LI. Muscle reflex control of sympathetic nerve activity in heart failure: the role of exercise conditioning. Heart Fail Rev. 2000; 5:87–100. [PubMed: 16228918]

149. Negrao CE, Middlekauff HR. Adaptations in autonomic function during exercise training in heart failure. Heart Fail Rev. 2008; 13:51–60. [PubMed: 17932745]

150. Fraga R, Franco FG, Roveda F, de Matos LN, Braga AM, Rondon MU, Rotta DR, Brum PC, Barretto AC, Middlekauff HR, Negrão CE. Exercise training reduces sympathetic nerve activity in heart failure patients treated with carvedilol. Eur J Heart Fail. 2007; 9:630–636. [PubMed: 17475552]

151. O'Connor CM, Whellan DJ, Lee KL, Keteyian SJ, Cooper LS, Ellis SJ, Leifer ES, Kraus WE, Kitzman DW, Blumenthal JA, Rendall DS, Miller NH, Fleg JL, Schulman KA, McKelvie RS, Zannad F, Piña IL. HF-ACTION Investigators. Efficacy and safety of exercise training in patients with chronic heart failure: HF-ACTION randomized controlled trial. J Am Med Assoc. 2009; 301:1439–1450.

152. Adams V, Doring C, Schuler G. Impact of physical exercise on alterations in the skeletal muscle in patients with chronic heart failure. Front Biosci. 2008; 13:302–311. [PubMed: 17981548]

153. Leosco D, Rengo G, Iaccarino G, Golino L, Marchese M, Fortunato F, Zincarelli C, Sanzari E, Ciccarelli M, Galasso G, Altobelli GG, Conti V, Matrone G, Cimini V, Ferrara N, Filippelli A, Koch WJ, Rengo F. Exercise promotes angiogenesis and improves beta-adrenergic receptor signalling in the post-ischaemic failing rat heart. Cardiovasc Res. 2008; 78:385–394. [PubMed: 18093988]

154. Leosco D, Rengo G, Iaccarino G, Filippelli A, Lymperopoulos A, Zincarelli C, Fortunato F, Golino L, Marchese M, Esposito G, Rapacciuolo A, Rinaldi B, Ferrara N, Koch WJ, Rengo F. Exercise training and beta-blocker treatment ameliorate age-dependent impairment of beta-adrenergic receptor signaling and enhance cardiac responsiveness to adrenergic stimulation. Am J Physiol Heart Circ Physiol. 2007; 293:H1596–H1603. [PubMed: 17557919]

155. Olshansky B, Sabbah HN, Hauptman PJ, Colucci WS. Parasympathetic nervous system and heart failure: pathophysiology and potential implications for therapy. Circulation. 2008; 118:863–871. [PubMed: 18711023]

156. Azevedo ER, Parker JD. Parasympathetic control of cardiac sympathetic activity: normal ventricular function versus congestive heart failure. Circulation. 1999; 100:274–279. [PubMed: 10411852]

157. Kubo T, Parker JD, Azevedo ER, Atchison DJ, Newton GE, Picton P, Floras JS. Vagal heart rate responses to chronic beta-blockade in human heart failure relate to cardiac norepinephrine spillover. Eur J Heart Fail. 2005; 7:878–881. [PubMed: 16087140]

158. Xu XL, Zang WJ, Lu J, Kang XQ, Li M, Yu XJ. Effects of carvedilol on M2 receptors and cholinesterase-positive nerves in adriamycininduced rat failing heart. Auton Neurosci. 2006; 130:6–16. [PubMed: 16798104]

159. Goldsmith RL, Bigger JT, Bloomfield DM, Krum H, Steinman RC, Sackner-Bernstein J, Packer M. Long-term carvedilol therapy increases parasympathetic nervous system activity in chronic congestive heart failure. Am J Cardiol. 1997; 80:1101–1104. [PubMed: 9352991]

160. De Ferrari GM, Schwartz PJ. Vagus nerve stimulation: from pre-clinical to clinical application: challenges and future directions. Heart Fail Rev. 2011; 16:195–203. [PubMed: 21165697]

161. Sabbah HN, Ilsar I, Zaretsky A, Rastogi S, Wang M, Gupta RC. Vagus nerve stimulation in experimental heart failure. Heart Fail Rev. 2011; 16:171–178. [PubMed: 21128115]

Circulation Research Compendium on Heart Failure

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Eugene Braunwald, Editor

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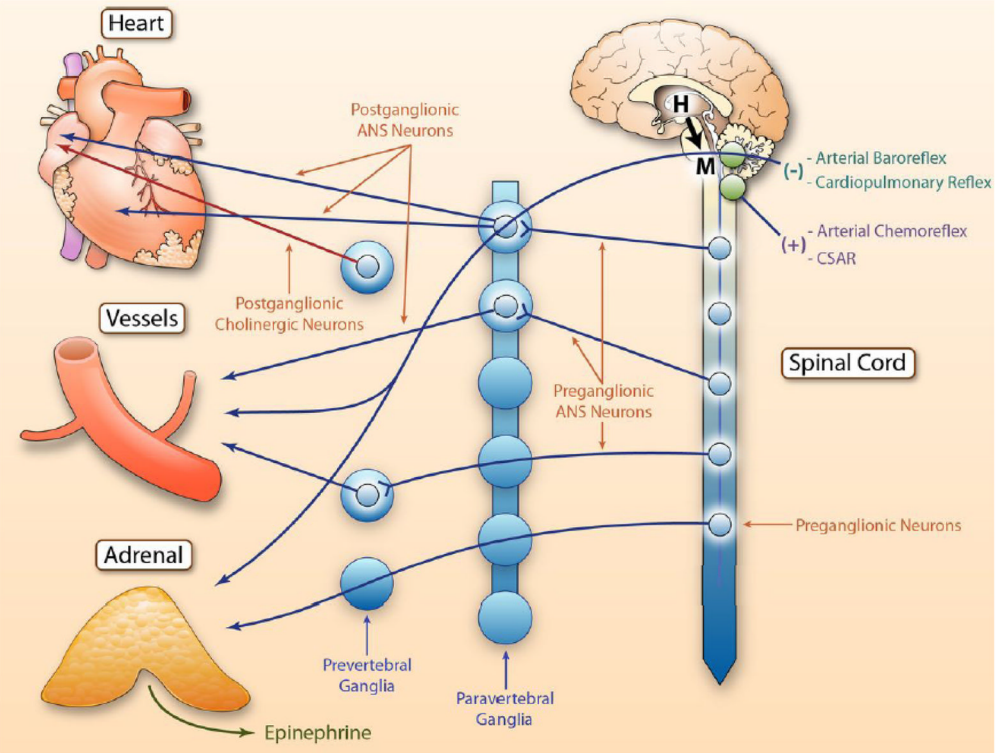

Figure 1. Overview of the ANS innervation of the cardiovascular system

Note that, in contrast to the ANS which innervates both atria and ventricles of the heart, the cholinergic (parasympathetic) nervous system mainly innervates cardiac atria only. See text for more details. CSAR: Cardiac Sympathetic Afferent Reflex. (Illustration Credit: Ben Smith).

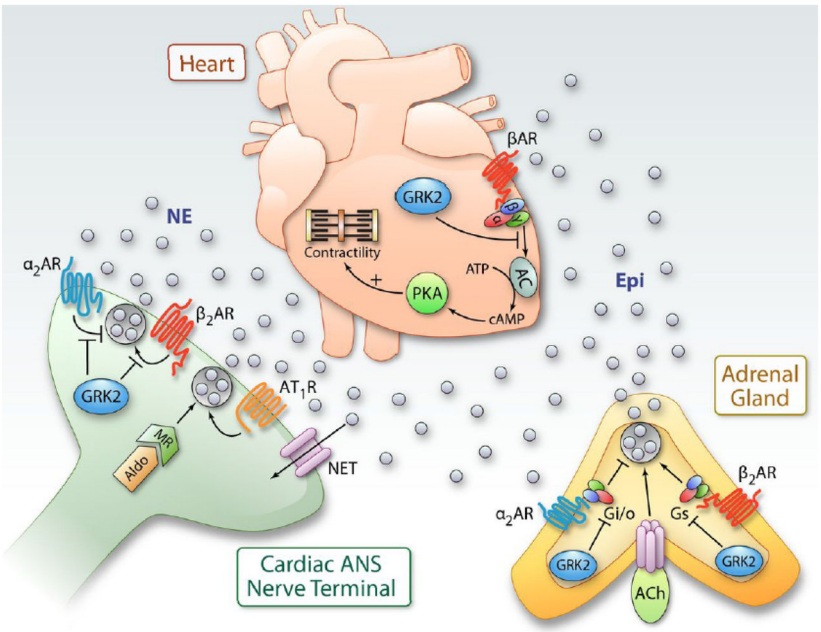

Figure 2. ANS input to the heart and its regulation

See text for details. $G_{i/o}$: inhibitory or other G protein; G_s: stimulatory G protein; ACh: Acetylcholine; NET: NE transporter; Aldo: Aldosterone; MR: Mineralocorticoid Receptor. (Illustration Credit: Ben Smith).

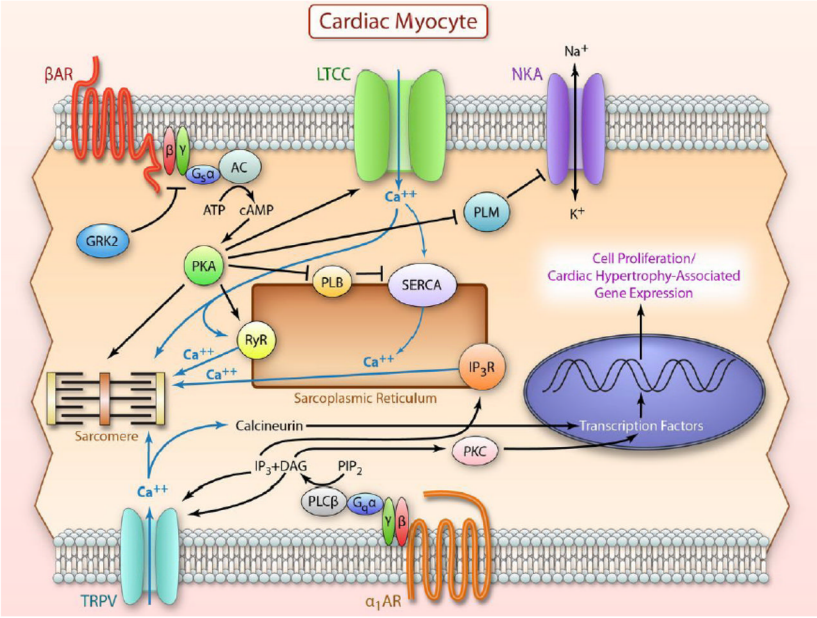

Figure 3. Signal transduction of cardiac myocyte contraction and its regulation by cardiac ARs

See text for details. LTCC: L-type Calcium Channel; NKA: Na^+, K^+-ATPase; PLM: Phospholemman; PLB: Phospholamban; SERCA: Sarcoplasmic/Endoplasmic Reticulum Ca^{2+}-ATPase; RyR: Ryanodine Receptor; IP_3R: IP_3 Receptor; PKC: Protein Kinase C; TRPV: Transient Receptor Potential Vanilloid. (Illustration Credit: Ben Smith).

Table 1

Overview of ANS-related therapeutics in HF.

Therapeutic modality	Mechanism(s) of action in HF	Effect on ANS function	Effect(s) on HF phenotype	Clinical outcome(s)-indication(s) in HF	Other Notes
β-blockers	Cardiac βAR antagonism-ANS neuronal β_2AR antagonism-↓ Cardiac & adrenal GRK2-↑ PNS outflow/activity	↓ Outflow/activity	Reversed adverse remodeling; ↓ arrhythmias; ↑ cardiac blood flow; protection against CA toxicity; ↓ cardiac oxygen, metabolic & energy demand/supply ratio	↓ all-cause & cardiac mortalities; ↑ adrenergic & inotropic reserves-Chronic HF, especially after MI	Contraindicated in acute HF; Certain polymorphisms in cardiac βAR & GRK5 genes affect response; individual agents not equal: carvedilol-metoprolol appear superior in HF
α-blockers	VSM α_1AR (and α_2AR) antagonism	↑ Outflow/ activity(reflex response to α_1AR blockade)	Worsening of HF; ↑ HF incidence; ↑ HF morbidity & mortality	↑ cardiac morbidity & mortality(prazosin-doxazosin)	Contraindicated in HF
Centrally acting sympatholytic agents	Central ANS & adrenalα_2AR (and putative "imidazoline receptor") agonism	↓ Outflow/activity	↓ Cardiac & renal ANS tones in HF; ↓ cardiac oxygen, metabolic & energy demand/supply ratio (?)	No clinical deterioration (clonidine) but ↑ mortality (due to excessive sympatholysis?) with moxonidine; benefit questionable	Contraindicated in acute HF; Central ANS & adrenalα_2ARs desensitized/dow nregulated in chronic HF (due to ↑ GRK2), so efficacy might be limited
RAAS-modulating agents	↓ AngII production-AngII & Aldo antagonism, leading to ↓ NE release & ↑ NE reuptake from ANS neurons	↓ Outflow/activity	Well-established benefits in reversed adverse remodeling & ↓ cardiac oxygen, metabolic & energy demand/supply ratio	↓ all-cause & cardiac mortalities-Chronic HF, especially after MI	Part of benefit in chronic HF due to ANS outflow/activity lowering
Sympathomimetics	Cardiac βAR agonism or PDE3 inhibition, leading to ↑ cardiac & VSM cAMP levels	Indirect ↓ outflow/ activity via improved cardiac hemodynamics but potential ↑ NE release from ANS neurons net effect unkown	Positive inotropy for acute HF; ↑ arrhythmias; ↑ cardiac oxygen, metabolic & energy demand/supply ratio	↑ all-cause & cardiac mortalities; ↓ adrenergic & inotropic reserves & cardiac βAR signaling/ function over time (due to ↑ cardiac GRK2)-Acute HF ONLY (cardiogenic shock)	Contraindicated in chronic HF; PDE3 inhibitors (milrinone) provide additional (direct) vasodilatory benefit
Exercise training	Improved hemodynamics-↑ Cardiac βAR signaling/ function-↓ Cardiac & adrenal GRK2-↑ ANS neuronal& adrenalα_2AR function	↓Outflow/activity	Improved arterial & chemoreflex controls; ↑ cardiac blood flow; ↓ CA toxicity; ↓ cardiac inflammation	↓ all-cause & cardiac mortalities; ↑ adrenergic & inotropic reserves; ↓ Cardiac ANS tone	Under investigation for chronic HF treatment
Cholinergic system stimulation	↓ HR & cardiac NE release-↑ cardiac function-↓ adverse remodeling	↓Outflow/activity	Improved arterial & chemoreflex controls; ↓ arrhythmias; ↓ CA toxicity	↓Cardiac ANS tone-Under investigation for chronic HF treatment, especially along with β-blockers	Cholinergic system function (bradycardia & vasodilatation) ↓ in chronic HF; benefit in HF significantly enhanced by β-blockers

See text for details. PNS: Parasympathetic Nervous System; CA: Catecholamine; MI: Myocardial Infarction; VSM: Vascular Smooth Muscle; AngII: Angiotensin II; Aldo: Aldosterone; PDE3: Phosphodiesterase type III; cAMP: cyclic adenosine monophosphate; HR: Heart Rate.