CHAPTER

ADRENERGIC RECEPTORS 11

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ADRENERGIC SIGNALING: SYSTEMATIC AND UPDATED OVERVIEW

Adrenergic receptors (also known as adrenoceptors, ARs) belong to the guanine nucleotide-binding G protein–coupled receptor (GPCR) superfamily, and are membrane receptors that activate heterotrimeric G proteins following the binding of a ligand. GPCRs consist of one extracellular N-terminal domain, seven membrane-spanning domains, three intra- and three extracellular loops, and one intracellular C-terminal tail (Fig. 11.1). These heptahelical trans-membrane sensors account for approximately 4% of the total protein-coding genome and are considered the most important drug targets in medicine and physiology. G proteins typically stimulate (via G_s protein) or inhibit (via G_i protein) the enzyme adenylyl-cyclase or activate (via G_q protein) phospholipase C (PLC). A detailed and updated overview of the main cardiovascular GPCRs was recently published.[1] GPCR signaling is terminated by phosphorylation of the intracellular domains of the receptor by the family of G protein–coupled receptor kinases (GRKs).[2,3] GRK-mediated phosphorylation increases the affinity of GPCRs for the arrestin class of proteins, which uncouples the phosphorylated receptor from G protein and successively targets the receptor for internalization. Downregulation of GPCRs reduces the functional activity of classical signaling paradigms up to 80%[4,5] (Fig. 11.1).

Two classes of ARs have been identified: αAR and βAR. Phenylephrine is a selective pharmacological agonist of αAR while isoproterenol is considered a nonselective agonist for βAR.[6] The subfamily of α_1AR (G_q coupled receptors) consists of three highly homologous subtypes, including α_1A-, α_1B-, and α_1D-AR.[7] The α_2AR subfamily (coupled to G_i) comprises three subtypes: α_2A-, α_2B-, and α_2C-AR.[8] Some species other than humans express a fourth α_2D-AR as well.[9] In the βAR family there are three receptor subtypes:β_1AR is found at its highest levels in the heart,[10] β_2AR is distributed extensively throughout the body,[11] and β_3AR is mainly expressed in the white and brown adipose tissue.[12] All three βARs couple primarily to $G\alpha_s$ and subsequent cAMP-related pathways, although under certain conditions can also couple to $G\alpha_i$.[13] β_2AR and β_3AR signaling can also occur via G protein independent mechanisms.[14] In particular, cardiac β_3AR causes negative inotropic effects mainly mediated by activation of nitric oxide (NO) synthase, serving thereby as a brake in sympathetic overstimulation. These paradigms of signaling can be observed in the same cell type based on the functional state of the cell. Henceforth, the response to GPCR stimulus can be modified by various conditions, including chronic stimulation, acidosis, cell hypoxia, and aging.[15–17]

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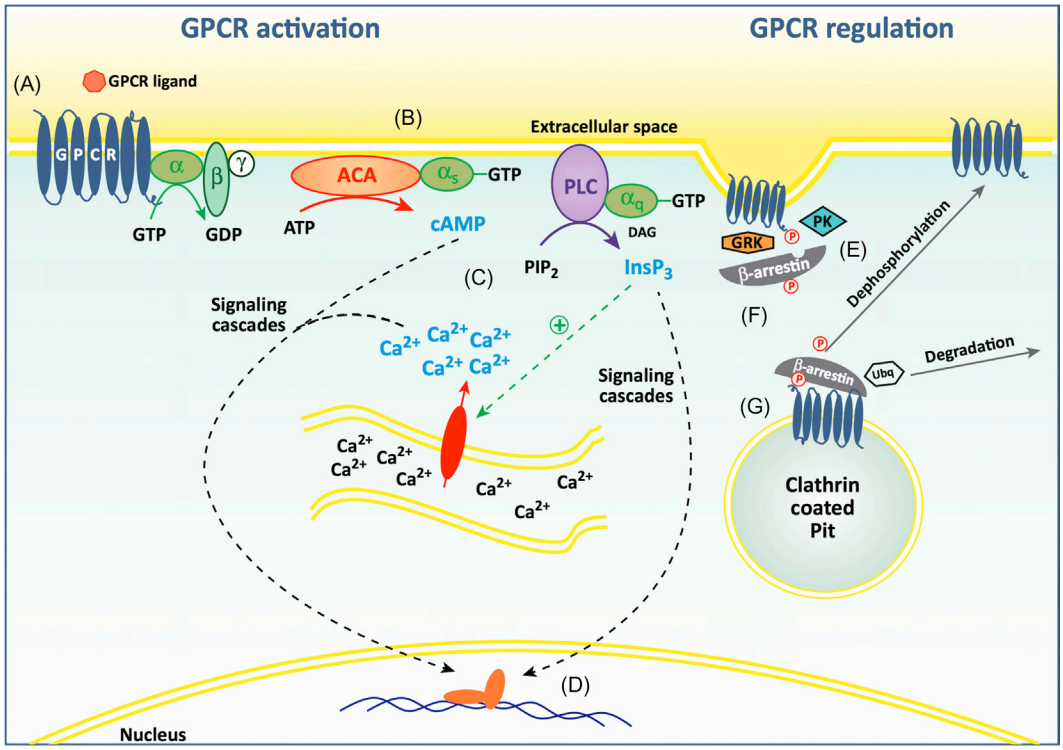

FIGURE 11.1

G protein–coupled receptor (GPCR) activation and regulation. (A) Binding of a GPCR ligand to the extracellular side of the receptor enables the exchange of GDP to GTP by the α subunit of the G protein. (B) The GTP-bound α subunit then acts on a second messenger-releasing enzyme such as adenylate cyclase (*ACA*) (Gαs) or phospholipase C (*PLC*) (Gαq), leading to their activation. (C) Second-messenger molecules such as cAMP and inositol-1,4,5-triphosphate ($InsP_3$) are direct products of enzymatic conversion of ATP and phosphatidylinositol-4,5-bisphosphate (PIP_2) respectively, whereas cytosolic Ca^{2+} is released upon activation of reticular calcium channels. (D) Second-messenger molecules can trigger cascade reactions that will lead to a downstream biological event (frequently gene expression regulation). (E) GPCR responsive elements such as protein kinases (*PKs*) or G protein–coupled receptor kinases (*GRKs*) phosphorylate the intracellular side of the receptor and decouple the G protein by steric exclusion. (F) β-Arrestins can recognize the phosphorylated GPCR and trigger the internalization process. (G) Modifications on the β-arrestin molecule such as dephosphorylation or ubiquitination define the fate of the internalized molecule either to recycling or degradation, respectively.

*Adapted from Martins SA, Trabuco JR, Monteiro GA, Chu V, Conde JP, Prazeres DM. Towards the miniaturization of GPCR-based live-cell screening assays. Trends Biotechnol 2012;***30***(11):566–74. (10.1016/j.tibtech.2012.07.004).*

SYMPATHETIC SYSTEM AND HYPERTENSION

ROLE OF ADRENERGIC SYSTEM IN REGULATION OF VASCULAR HOMEOSTASIS AND OXIDATIVE STRESS

The endothelium is central in the regulation of several vascular functions, including vasculature tone and permeability, thrombosis, hemostasis, and angiogenesis.[18–20] This integrates the overall information originating from the bloodstream and furnishing, in a time- and space-dependent manner, a fine tuning of vascular homeostasis by releasing specific factors including catecholamines, NO, vasoactive peptides, arachidonic acid metabolites, and reactive oxygen species (ROS).[21] The adrenergic system is the major regulator of cardiac and vascular function, and this is accomplished also through the activation of specific receptors localized on endothelial surface by local and systemic release of catecholamines.[22–25]

These receptors actively participate in the release of NO to regulate endothelial function.[26,27] Following its release, NO diffuses to the subjacent vascular smooth muscle where it elicits vasorelaxation through activation of soluble guanylyl-cyclase enzyme, which then catalyzes the formation of cGMP and hence activation of cGMP-dependent protein kinase.[28,29] Classically, NO is released from endothelial cells following activation of the endothelial or type 3 isoform of NO synthase (NOS-3 or eNOS), which is a Ca^{2+} and calmodulin-dependent enzyme; hence, many endothelium-dependent vasodilators cause NO release via an increase in intracellular Ca^{2+}. However, other pathways have also identified to act in Ca^{2+} independent manner and involve phosphorylation of various eNOS serine residues by a number of protein kinases.[30] Such a mechanism is particularly evident for β_2AR signaling where activation of eNOS involves specific kinases such as protein kinase A (PKA) and AKT.[31] The impaired ability of endothelium to appropriately vasodilate is defined as "endothelial dysfunction" and the major cause is decreased NO bioavailability (Fig. 11.2). Endothelial dysfunction has been associated with development of several cardiovascular disorders including hypertension, type 2 diabetes mellitus, and heart failure.[21,32]

However, altered NO production is not the only feature of the endothelial dysfunction. Indeed, increased ROS bioavailability and dysregulated redox signaling (oxidative stress) together with decreased NO production and increased NO consumption by ROS contribute to many of the molecular events underlying endothelial injury. [33,34] These findings have modified the molecular definition of endothelial dysfunction, leading to the concept of "eNOS uncoupling," characterized by the discrepancy between eNOS protein levels and NO production, with a switch in the enzymatic activity of eNOS to generate superoxide (O_2) rather than NO.[35]

ADRENERGIC SIGNALING AND ROS

ROS are products of normal cellular metabolism and derived from many sources in different cellular compartments. Enzymatic sources of ROS in endothelial cells include uncoupled NOS, xanthine oxidoreductase, mitochondrial respiratory enzymes, and NADPH oxidase.[36–38] However, the perceived role of ROS in regulation of cellular physiology has changed in the recent years. Indeed, on the one hand they can be considered detrimental for cell survival; however, they also have important physiological roles and act as part of the intracellular signaling, promoting beneficial cellular process such as mitohormesis (e.g., replacement and organization of the mitochondrial network), induction of host

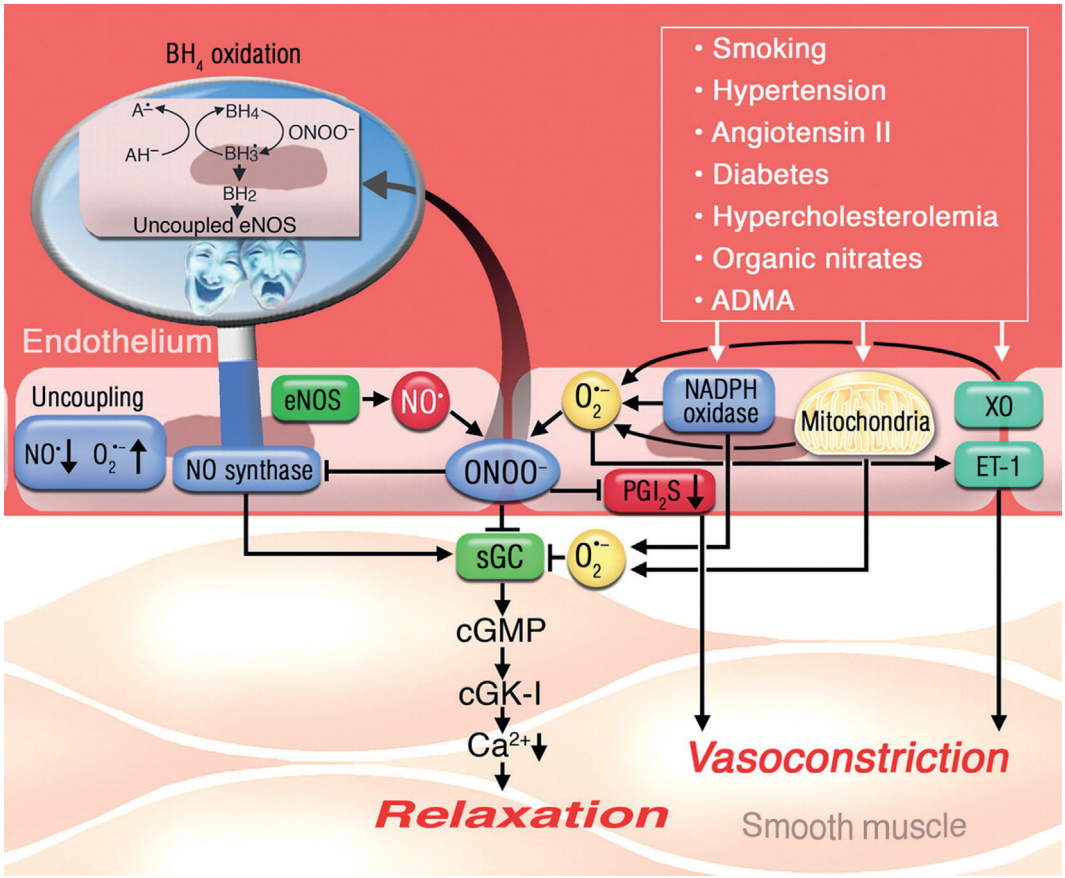

FIGURE 11.2

Mechanisms underlying endothelial (vascular) dysfunction in vascular disease. An unbalanced production of nitric oxide (NO) and superoxide (O_2^-) leads to inappropriate formation of peroxynitrite ($ONOO^-$). Peroxynitrite and superoxide cause vascular dysfunction through several mechanisms (reviewed in Forstermann and Munzel [1]). Peroxynitrite is a strong inhibitor of NO and prostacyclin (PGI_2) signaling, and it may cause eNOS uncoupling, causing this enzyme to produce superoxide instead of NO. *ADMA* = asymmetrical dimethylarginine; *cGMP* = guanosine 3′,5′-cyclic monophosphate; *cGK* = cGMP-dependent kinase; *eNOS* = endothelial nitric oxide synthase; *ET* = endothelin; *sGC* = soluble guanylate cyclase; *TXA* = thromboxane.

*From Münzel T, Gori T. Nebivolol. J Am Coll Cardiol 2009;***54(***16):1491–9. (10.1016/j.jacc.2009.05.066).*

defense genes, activation of transcription factors and stimulation of ion transport systems.[39] In the vascular system, ROS play a physiological role in controlling endothelial function, vascular tone, and vascular integrity but also a pathophysiological role in inflammation, hypertrophy, proliferation, apoptosis, constriction, migration, fibrosis, angiogenesis, and rarefaction, important factors contributing to endothelial dysfunction, vascular contraction, and arterial remodeling in cardiovascular diseases.

These various lines of evidence suggest ROS as a specific cellular messenger able to promote either cellular survival and adaptation or apoptosis, according to the specific characteristics of the stressors. According to this view, the physiological or pathological ROS level is more likely to be associated to the impaired redox signaling and equilibrium rather than the imbalance between pro-oxidants and antioxidants.[40]

The adrenergic system is also implicated in ROS production by the endothelium. Mounting evidence suggest an important roles for catecholamines in vascular cell growth and tissue remodeling following atherosclerosis, hypertension, and vascular injury.[41] Meanwhile, noradrenaline (norepinephrine) is also known to be a potential pro-inflammatory factor, since noradrenaline induces TNF-α, matrix metalloproteinase (MMP)-2, MMP-9, ROS, and toll-like receptor (TLR4) release from cells.[42] Furthermore, data suggest that noradrenaline stimulates the phosphorylation of mitogen-activated protein kinases (MAPK) and ROS synthesis leading to cell proliferation in vitro.[43]

THE ROLE OF G PROTEIN–COUPLED RECEPTOR KINASE 2 IN VASCULAR HOMEOSTASIS

GRKs have a significant role in adrenergic regulation of endothelial function (Figs. 11.1 and 11.2). Of the seven mammalian isoforms of GRKs, G protein-coupled receptor kinase 2 (GRK2) appears to be the most important isoform related to cardiac physiology. GRK2 is found in the striated (heart and skeletal) and smooth muscle in addition to WBCs (bone marrow, lymph nodes, and thymus) and many other organs. Indeed, homozygous GRK2-deficient mice exhibited embryonic lethality whereas gene ablation for the other GRKs resulted in relatively mild phenotypes.[44–46] The physiological relevance of GRK2 was further confirmed by its participation in diverse fundamental cellular processes, including cell cycle progression, migration, and differentiation.[47,48] Notably, GRK-mediated desensitization does not always rely on its catalytic activity but also on protein–protein interactions[49,50] that occur in different cellular compartments.[51] It is likely that both up- and downregulation of GRK2 affect cellular function and survival. Alterations in GRK2 expression and activity were observed in several diseases, such as heart failure,[2] Alzheimer's disease,[52] multiple sclerosis,[53] thyroid gland disorders,[54] opioid addiction,[55] rheumatoid arthritis,[56] ovarian cancer,[57] and cystic fibrosis.[58]

GRK2 participates in the development of experimental portal hypertension, which appears dependent upon the physical interaction between GRK2 and AKT.[59] Since AKT is able to activate eNOS, the GRK2-mediated inhibition of AKT shifts the vascular tone toward constriction in the setting of endothelial dysfunction due to decreased eNOS activity.[59] Given its close relationship to the adrenergic system, GRK2 may represent the specific link between adrenergic system and endothelial ROS production.

The functional role of GRK2 in vascular smooth muscle cells was explored in a transgenic animal model (targeted overexpression of GRK2),[60] where mice exhibited an increase in resting mean arterial pressure accompanied by an attenuated response to β-AR signaling compared with nontransgenic littermates. The increased blood pressure was also accompanied by cardiac hypertrophy and vascular thickening, two hallmarks of hypertensive phenotype.[60]

The endothelium-mediated modulation of the contractile state of vascular smooth muscle is impaired in atherosclerosis and in several conditions associated with the premature development of atherosclerosis.[61] The correlation between GRK2 abundance and hypertension is also present in other conditions characterized by increased blood pressure, such as portal hypertension[59] and preeclampsia.[62] In gestational hypertension, the increase in GRK2 in the placental vasculature seems to be compensatory

rather than causative of increased blood pressure. This compensation helps balance the excessive vascular tension as the lack of protective effect of elevated GRK2 expression levels negatively affect the outcome of the hypertensive state.[62] In this case, a potential explanation could rely on the metabolic effect of GRK2, which is able to place the cell in a low energy state that might favor survival in stress conditions.[51,63–65]

GRK2 levels in peripheral blood lymphocytes was reported to mirror changes in kinase expression in other organs under several pathophysiological settings. In particular, GRK2 levels and activity were increased in lymphocytes from hypertensive patients. Impairment of β-adrenergic-mediated vasodilation was reported in both human hypertensive subjects[22,66] and animal models of hypertension[67]; such alterations have been related to the increased GRK2 abundance and activity.[66] Decreased β-adrenergic signaling due to increased GRK2 activity would reduce the vasodilatative response, leading to high blood pressure. This view is supported by the inverse correlation of GRK2 expression with blood pressure.[68] Other data from spontaneously hypertensive rats and Dahl salt-sensitive rats confirmed increased levels of GRK2 in vascular smooth muscle cells, consistent with the observations in peripheral lymphocytes.[69] An subsequent study observed higher GRK2 protein levels in circulating lymphocytes from patients with myocardial infarction; additionally, increased GRK2 levels associated with worse systolic and diastolic function.[70] Importantly, at 2-year follow-up patients with higher GRK2 levels at admission had worse systolic function and cardiac remodeling,[70] suggesting that GRK2 levels may reflect hemodynamic impairment and might have a meaningful prognostic value after myocardial infarction (Fig. 11.3).

Elevated GRK2 levels might imply metabolic alterations and lead to insulin resistance, a common feature of hypertensive state.[14,71,72] In myoblasts, increased GRK2 expression mediated insulin resistance via a mechanism that involves sequestration of G_q and the insulin receptor substrate-1 (IRS-1).[73] In addition, GRK2 was shown to bind and phosphorylate IRS1 and the inhibition of GRK2 action ameliorated insulin sensitivity.[74,75] Also, GRK2 negatively affected cardiac glucose uptake and lowering GRK2 after ischemic injury contributed to restoring cardiac metabolism and prevented the development of subsequent heart failure.[72,74] GRK2 appears to regulate cardiomyocyte function in part by controlling β_1-AR in the regulation of cardiac contractility and chronotropy; interestingly, GRK3 was implicated in the regulation of cardiac growth and hypertrophy by selectively controlling endothelin and α_1-AR (PMID 17573483). Taken together, the control of endothelial homeostasis relies on a complex interaction between adrenergic system and nitroxidative stress; specific molecules such as GRKs may interplay with and modulate the crosstalk across multiple cell types involved in vascular function.

ADRENERGIC SIGNALING IN HEART FAILURE

The sympathetic nervous system (SNS) has pronounced effects on cardiac physiology, including increases in atrioventricular conduction (positive dromotropy), heart rate (positive chronotropy), cardiac contractility (positive inotropy), and cardiac relaxation (positive lusitropy). Likewise, the SNS plays a crucial role in the regulation of vascular tone due by controlling peripheral resistance and cardiac output.[76]

Heart failure is a chronic clinical syndrome in which the heart is incapable of pumping a sufficient supply of blood to meet the metabolic requirements of the body or generating the required elevated ventricular filling pressures to maintain output.[72] Heart failure leads to a debilitating illness characterized by

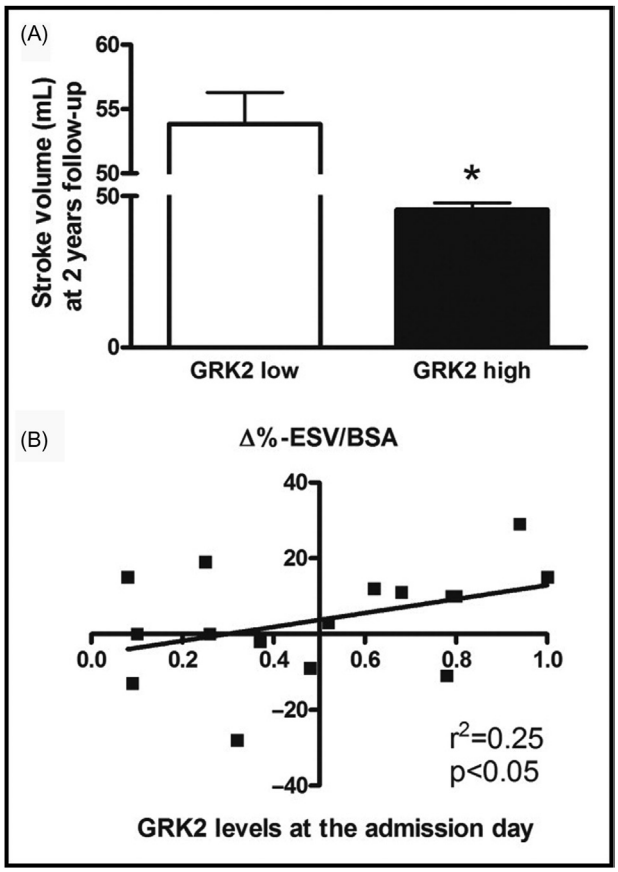

FIGURE 11.3

GRK2 levels and outcomes after myocardial infarction. (A) Two years after myocardial infarction, patients with higher GRK2 levels at admission had worse systolic function, with lower stroke volumes, than those with low GRK2 levels (independent-samples Student's *t*-test). (B) Also, cardiac remodeling, assessed by the change in end-systolic volume (ESV) corrected by body surface area (BSA) after 2 years of follow-up (Δ%-ESV/BSA) was correlated with GRK2 level at admission ($r^2 = 0.25$, $p < 0.05$).

From Santulli G, Campanile A, Spinelli L, et al. G protein-coupled receptor kinase 2 in patients with acute myocardial infarction. Am J Cardiol 2011;107(8):1125–30.

poor exercise tolerance and chronic fatigue, representing one of the most important causes of morbidity and mortality worldwide. Notwithstanding considerable advances in its treatment, heart failure still represents a severe social and clinical burden.[77,78] A complex neurohormonal regulatory system exists between the heart and multiple organ systems, including feedback loops mediated through a variety of vasoactive substances secreted by the adrenals, kidneys, lungs, and endothelium.[79] Perturbations of function in any of these organs affect the others. Accordingly, the cardiovascular system is best viewed as a

complex dynamic system, continually adapting to optimize organ perfusion. During heart failure, diverse neurohormonal mechanisms are triggered to maintain cardiac output.[4] Heart failure is indeed a progressive disease that begins long before symptoms or signs become evident. It is initially characterized by a complex adaptive neurohormonal activation, which includes the nervous system (see chapter: Neuronal Hormones and the Sympathetic/Parasympathetic Regulation of the Heart), the renin-angiotensin-aldosterone system (see chapter: Renin Angiotensin Aldosterone System and Heart Function), natriuretic peptides (see chapter: Cardiac Natriuretic Peptides), endothelin (see chapter: Endothelin-1 as a Cardiac-Derived Autocrine, Paracrine and Intracrine Factor in Heart Health and Disease), and vasopressin (see chapter: Renin Angiotensin Aldosterone System and Heart Function). These and other regulatory mechanisms are required to compensate for cardiac dysfunction[80]; however, the process progressively becomes maladaptive when the left ventricular (LV) dysfunction is persistent. This eventually leads to increased mechanical stress on the failing heart, causing detrimental electrical and structural events, further impairment of systolic and diastolic function, and progressive cardiac fibrosis and apoptosis.[81] Thus, β-blockers, angiotensin-converting enzyme inhibitors, Angiotensin II AT_1 receptor blockers and mineralocorticoid receptor antagonists represent cornerstones for the treatment of patients with a failing heart.[82]

The central part of the adrenal gland, called adrenal medulla, is the main source of catecholamines and comprises groups of adrenergic and noradrenergic chromaffin cells and, to a lesser extent, ganglionic neurons.[83] Chromaffin cells secrete roughly 80% adrenaline and 20% noradrenaline whereas this proportion is reversed in the sympathetic nerves, which contain and secrete predominantly noradrenaline.[79] The adrenergic and noradrenergic secretion in different groups of chromaffin cells relies on the different α_2AR subtypes expression.[84] The adrenal gland can be compared to a specialized sympathetic ganglion, receiving inputs from the SNS via preganglionic fibers.[85] However, the adrenal gland directly secretes neurohormones into the bloodstream. Indeed, chromaffin cells are postganglionic sympathetic neurons that have lost part of their characteristics as axons and dendrites and are able to secrete their hormones into the blood by exocytosis. The suggestive link between the adrenal gland and the heart has become quite interesting and stimulating in the last few years, with several studies investigating the molecular mechanisms underlying such a complex relationship, especially in the pathophysiology of heart failure.[79]

Adrenaline and noradrenaline generally have similar effects, although they differ from each other in certain of their actions. In particular, noradrenaline constricts almost all blood vessels, while adrenaline constricts many networks of minute blood vessels but dilates the vessels in the skeletal muscles and the liver.[86] Both sympathomimetic agents increase heart rate and myocardial contractility, thereby augmenting cardiac output and blood pressure.[87] Sympathetic overdrive observed in heart failure correlates with a higher risk of arrhythmias and LV dysfunction.[88] Plasma concentrations of noradrenaline are negatively associated with survival in heart failure patients.[89] Augmented levels of circulating catecholamines can cause myocardial damage via enhanced cardiac oxygen demand and by increasing peroxidative (and lipoperoxidative) metabolism and the ensuing production of free radicals.[90] These reactive species lead to structural alterations in the myocardium, including focal necrosis and inflammation, increased collagen deposition and subsequent interstitial fibrosis.[91] Noradrenaline can also increase cardiac oxygen consumption and cause apoptosis, ultimately leading to dilated cardiomyopathy.[92,93]

At the vascular level, systemically circulating or locally released catecholamines[94] trigger two main classes of ARs: α_1AR and β_2AR, causing vasoconstriction and vasodilatation, respectively.[23,95] With aging, such a fine equilibrium is progressively shifted toward increased vasoconstriction, most likely due to a defective vasodilatation in response to βAR stimulation. Supporting this hypothesis, βAR

agonist administration in the human brachial artery induces vasodilatation and this response is attenuated in hypertensive patients.[66] The mechanistic role of β_2AR in the vasculature is also corroborated by the fact that genetic variants of β_2AR cause excessive desensitization and lead to reduced vasodilation, promoting the development of atherosclerosis.[96]

Increased basal levels of circulating catecholamines were observed both in heart failure and with advancing age, mirrored by a decrease in the number of high-affinity βARs, suggesting that these alterations might be due to βAR desensitization rather than actual reduction in βAR density.[11] As mentioned above, βAR affinity for the ligand is mainly dependent upon GPCR phosphorylation, which in turn is in the domain of GRKs.[70] Modulation of sympathetic nervous signaling via GRKs mediated downregulation of βARs in the heart plays a key role in heart failure. In particular, heterozygous GRK2 knockout mice display augmented cardiac contractility and function, whereas transgenic mice overexpressing cardiac GRK2 exhibit decreased myocardial function due to βAR dysfunction.[97]

GRK2 expression and activity increase in vascular tissue with aging.[1] Equally important, an impairment in βAR-mediated vasorelaxation was observed in hypertensive patients[66] and in animal models of hypertension[21,67]: these alterations related to increased GRK2 abundance and activity. Transgenic overexpression of GRK2 in the vasculature impaired βAR signaling and the vasodilatative response, eliciting a hypertensive phenotype in rodents. This aspect was confirmed in humans: GRK2 expression correlated with blood pressure and impaired βAR-mediated adenylyl-cyclase activity.[1] Additionally, genetic variants of the β_2AR that affect its translational efficiency associated with longevity.[11] The decrease in βAR-mediated responses were attributed to different mechanisms, including attenuation of PKA activation, impaired generation of cyclic AMP, decreased receptor density, and less efficient coupling to adenylyl-cyclase.[11] Variations in cyclooxygenase expression and vasoactive prostanoids levels are suggested as potential mechanisms. However, currently there is no single molecular or cellular factor that fully explains the decline in βAR function. Nevertheless, the etiology seems to be most likely associated with alterations in the ability of βAR to respond to agonists at the cellular level.

ADRENERGIC SYSTEM IN THE HEART: BEYOND THE REGULATION OF CONTRACTILITY

The activation of the sympathetic system leads to noteworthy metabolic responses, including increased gluconeogenesis and lipolysis with subsequent elevated plasma levels of free fatty acids (FFAs) and glucose.[98,99] Essentially, the increased availability of glucose and FFAs can be used by the organism as fuel in times of stress or danger, when increased exertion or alertness is required.[100] Different therapeutic approaches targeting myocardial metabolism have been suggested to regulate metabolic pathways in the failing heart, in an attempt to improve cardiac function and metabolic elasticity.[101]

During the flight or fight response, sympathetic activation causes α_1-AR–mediated vasoconstriction in less vital vascular beds, including splanchnic and skin, diverting blood to skeletal muscle. AR activation also mobilizes blood from the capacitance veins, involving α_1 and α_2ARs.[102] These acute physiological responses, typical of the stress conditions, are disadvantageous when they become chronic. Actually, a common feature of many pathological conditions involving sympathetic system hyperactivity is the development of metabolic alterations, including insulin resistance, impaired glucose and lipid metabolism, and mitochondrial dysfunction.[103,104] The myocardium has high metabolic demands, among the highest in the body: with minimal ATP reserves and complete ATP turnover approximately every ten seconds, the heart heavily depends on a continuous energy supply,[105] though the heart

possesses a strategic metabolic flexibility that supports its function during stressful conditions. Cardiac muscle generates ATP almost exclusively via oxidative phosphorylation by using different metabolic substrates: in the healthy state cardiac ATP production mainly relies on FFA oxidation, whereas the relative contribution of glucose increases during stress or injury.[106]

Imbalance in adrenergic activation and cardiac energy metabolism represents a risk factor for the development of cardiac disease. Therefore, heart failure represents a classical endpoint in the study of metabolic alterations related to the sympathetic system. Indeed, there are multiple disturbances in various metabolic pathways, including the tricarboxylic acid cycle and β-oxidation in heart under pathological conditions. Metabolic remodeling observed in failing hearts is characterized by a lower oxidative capacity, contractile dysfunction, and insulin resistance.[107,108] Circulating insulin levels are chronically augmented in both type 2 diabetes mellitus and heart failure, leading to persistent stimulation of insulin receptors[109,110] (see chapter: Insulin Signaling in Cardiac Health and Disease). Such an increase in insulin signaling in the heart promotes FFAs uptake and enhances lipotoxicity.[111] Moreover, hyperactive insulin signaling also accelerates adverse LV remodeling.[112,113] Insulin itself can directly impair adrenergic signaling pathways required for contractile function via an insulin receptor/β_2AR signaling complex,[107] providing a potential novel mechanism underlying cardiac dysfunction in heart failure. Of note, insulin resistance highly correlates with neuroadrenergic function,[21] and the onset of type 2 diabetes is associated with increased central sympathetic outflow.[114] In addition, both nutritional sympathetic responsiveness and baseline sympathetic drive are important prognostic biological markers for dietary weight loss outcome in obese subjects with metabolic syndrome.[115,116]

The prevalence of sympathetic over parasympathetic activity might be initially responsible, at least in part, for an increased metabolic state. However, as in different hormone-regulated pathways, such a state is subsequently followed by a decrease in βAR metabolic responsiveness. This compensatory response results in a reduced basal metabolic rate and an increased tendency toward anabolic processes, leading to insulin resistance and reduced ability to dissipate energy, with an overall weight gain, particularly at the visceral level.[117] This complex metabolic network can eventually cause a vicious circle, where insulin resistance further stimulates sympathetic activity, worsening insulin resistance itself. A sustained βAR stimulation is widely known to induce insulin resistance.[118] β_2ARs and β_3ARs seem to play a pivotal, although not exclusive, role in regulating glucose and lipid homeostasis, respectively: whereas β_2AR regulates both pancreatic β-cell hormone secretion and peripheral glucose metabolism,[14] β_3AR is more involved in the modulation of FFAs metabolism.[119] GRKs actively participate in this complex scenario. In fact, GRKs have been proposed as pleiotropic proteins involved in the regulation of countless cellular functions, not exclusively via the classic phosphorylation pathway. Mounting evidence indicates that GRKs exert different effects depending on cell type, localization, stimuli, and pathophysiological context.[120–123] For instance, Iaccarino and colleagues were the first to demonstrate the mitochondrial localization of GRK2,[51] later confirmed by other investigators[124] with imperative functional implications (Fig. 11.4).

Insulin also up-regulates GRK2, which in turn inhibits insulin signaling and glucose uptake.[1,125] Various conditions associated with insulin resistance, including hypertension and diabetes, are characterized by elevated GRK2 levels.[1] In murine failing hearts, GRK2 inhibition was demonstrated to be beneficial, preventing the derangement of insulin signaling and delaying the reduction of glucose uptake, thereby preserving myocardial function.[74] In the clinical setting, lymphocyte GRK2 levels were augmented in patients with end-stage heart failure[126] and in patients with myocardial infarction, correlating with a worse systolic and diastolic function.[70]

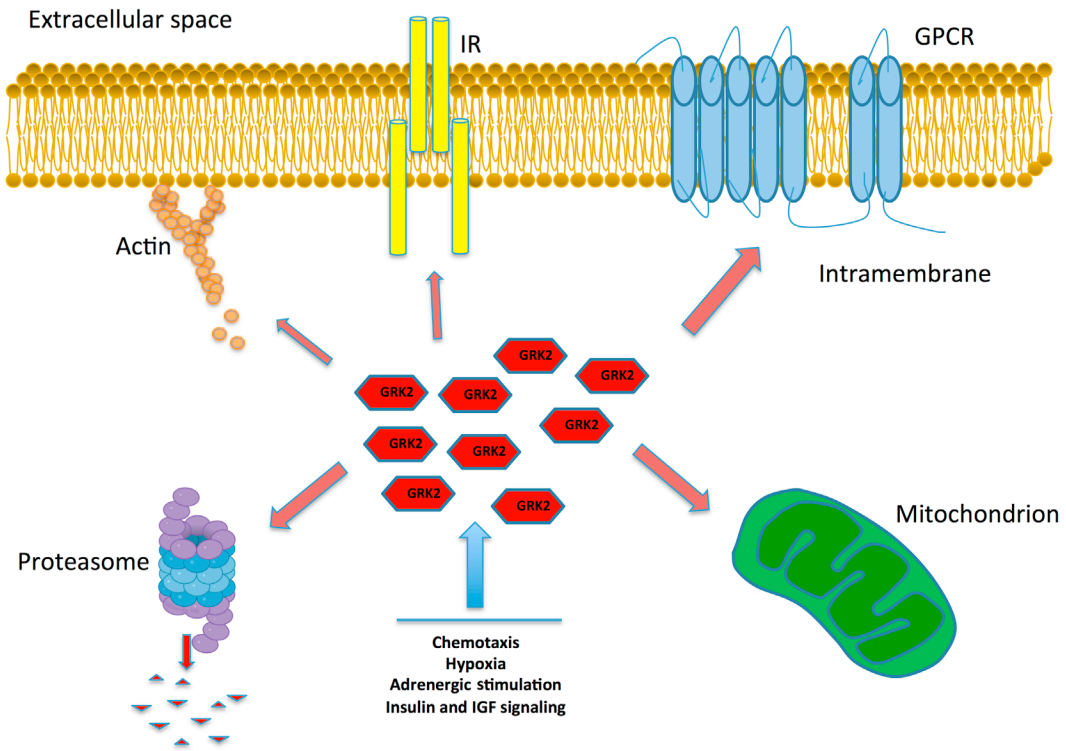

FIGURE 11.4

Intracellular localization of GRK2. GRK2 is localized to the cytosol in resting conditions, but GRK2 translocates in response to a variety of stimuli to different subcellular compartments where GRK2 regulates several cellular functions, including GPCR and IR desensitization at the plasma membrane level, actin polymerization in the cytoskeleton, metabolism, and ROS production in mitochondria. *GPCR*: G protein–coupled receptor; *IR*: insulin receptor.

ADRENERGIC RECEPTORS AND CARDIAC METABOLISM

Cardiac function relies to a great extent on oxidative metabolism. Given its the high mitochondrial content cardiac muscle generates ATP almost exclusively through oxidative phosphorylation.[127] Accordingly, cardiac muscle possesses a metabolic flexibility or plasticity, allowing it to maintain its function during stressful conditions. In the adult heart the major pathway for ATP production is fatty acid oxidation, while the relative contribution of glucose increases during stress or injury, such as exercise or ischemia.[128,129] Thus, it is not surprising that an impairment of cardiac muscle energy metabolism represents an important risk factor for the development of cardiac diseases.[130] The heart exhibits a severe malfunction of different pathways with a metabolic remodeling characterized by a lower oxidative capacity, contractile dysfunction, and cardiac muscle insulin resistance under pathological conditions.[127,130] Different therapeutic strategies have been undertaken to modulate metabolic

pathways in the failing heart, though it remains controversial whether targeting glucose versus fatty acid metabolism individually or in combination represents an optimal approach to improve metabolic flexibility and cardiac function.[131]

Activation of the adrenergic system is deeply involved in regulating diverse metabolic pathways. Increased circulating catecholamines and activation of the different adrenergic receptors present in the various organs produce important metabolic responses which include: (1) increased lipolysis and elevated levels of fatty acids in plasma, (2) increased gluconeogenesis by the liver to provide substrate for the brain, and (3) moderate inhibition of insulin release by the pancreas to conserve glucose and to shift fuel metabolism of muscle in the direction of fatty acid oxidation (Fig. 11.5). This physiological

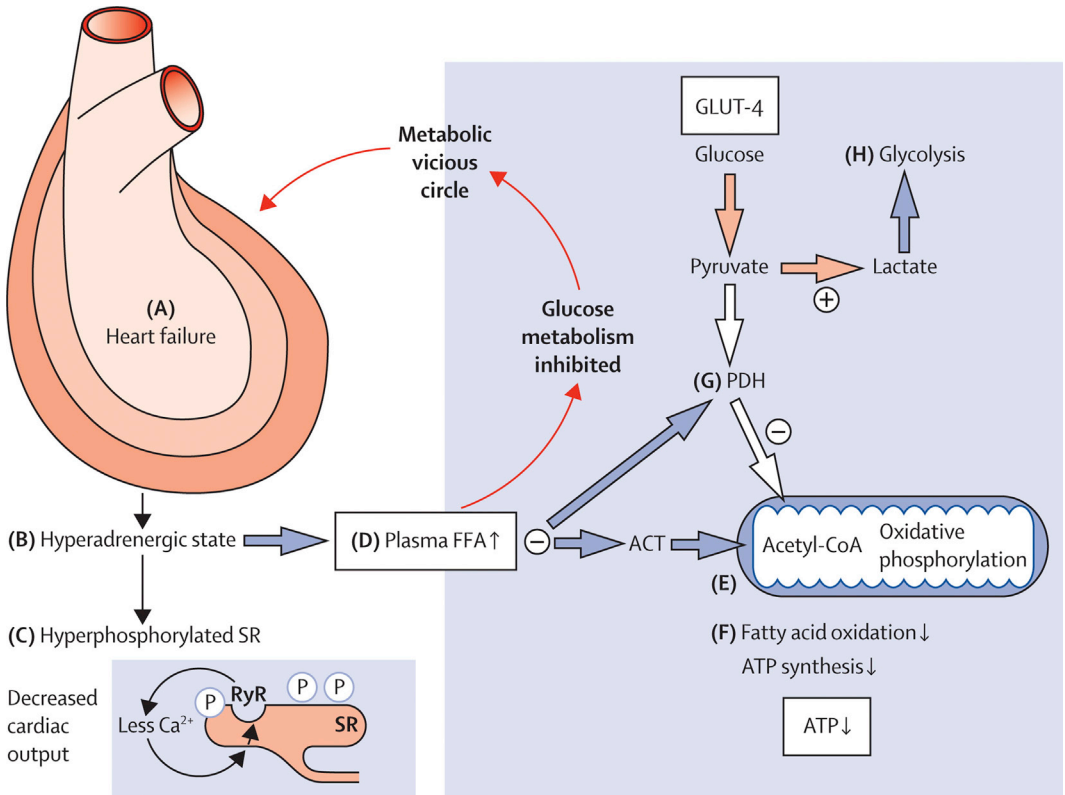

FIGURE 11.5

The metabolic vicious circle in heart failure. Dilation of the myocardium in heart failure (A) leads to adrenergic activation (B) that in turn hyperphosphorylates the SR (C) and increases concentrations of circulating FFA (D). FFA inhibit mitochondrial function at the level of ACT (E), thus inhibiting fatty acid oxidation and synthesis of ATP (F). Plasma FFA also inhibit PDH (G) to promote anaerobic glycolysis (H) rather than oxidative metabolism. SR = sarcoplasmic reticulum. RyR = ryanodine receptor. *FFA* = free fatty acids. *ACT* = acyl carnitine transferase. *PDH* = pyruvate dehydrogenase. *GLUT-4* = glucose uptake transporter 4.

*Adapted from Heusch G, Libby P, Gersh B, Yellon D, Böhm M, Lopaschuk G, Opie L. Cardiovascular remodelling in coronary artery disease and heart failure. Lancet 2014;***383***(9932):1933–43. (10.1016/S0140-6736(14)60107-0).*

response, typical of the stress condition, was demonstrated to be detrimental for the functioning of different organs like the cardiac muscle when it becomes chronic. Indeed, a common feature of many pathological conditions involving over-activation of the adrenergic system is the development of metabolic alterations which can include insulin resistance, altered glucose and lipid metabolism, and mitochondrial dysfunction.[104] These alterations are seen in a number of different pathological conditions; however, they are, in general, highly correlated to the level of activation of the adrenergic system.

The SNS is maladaptively activated in response to a chronic reduction in cardiac output and this response is characterized by an increased adrenal secretion and reduced cardiac reuptake of catecholamines.[132] The effects of the catecholamine secretion on cardiac metabolism are mediated by both central and peripheral mechanisms. For example, increased catecholamines have directly detrimental effects on the heart, which cause marked enzyme loss as an index of diffuse myocardial damage, and substantial oxygen-wastage even in the absence of FFAs in the perfusate.[133,134] Furthermore, noradrenaline promotes both coronary vasoconstriction and increased plasma FFA levels, which further promote oxygen-wastage.[134] In turn, FFAs reciprocally augment sympathetic activity. In human skeletal muscle, a dose-response relationship exists between plasma FFAs[135] and defects in insulin signaling. This may in part be attributable to FFA-mediated activation of PKC, which phosphorylates insulin receptors and results in reduced capillary opening and reduced myocyte glucose uptake.[136]

Locally activated SNS appears to be relevant in altering cardiac metabolism. Using positron emission tomography in conjunction with a noradrenaline analog and 18F-fluorodeoxyglucose, myocardial segments with contractile dysfunction have reduced presynaptic noradrenaline reuptake and myocardial glucose uptake, compared to less impaired myocardial segments in the same patients.[118] Thus, after control for confounding variables, altered metabolism and insulin resistance directly relate to local sympathetic activity. The adverse effects of the SNS on the heart are mediated by ARs; however, extensive research indicates that ARs are differently involved in pathophysiology of heart failure, and so it is likely to be the same for modifications of cardiac metabolism observed during disease. Indeed, β_1- and β_2-adrenoceptors regulate different signal pathways, producing different outcomes on cardiac function. Stimulation of both β_1AR and β_2AR can activate the stimulatory G protein ($G\alpha_s$)/adenylyl-cyclase/cAMP/PKA signaling pathway, which subsequently leads to the phosphorylation of several target proteins within the cardiac myocyte, including intracellular calcium release channels (ryanodine receptors), L-type calcium channels, and phospholamban[16,127,137] (Fig. 11.1). Nonetheless, this signaling pathway is the main mechanism by which β_1AR rather than β_2AR regulates cardiac contractility/relaxation and rate.[138] In contrast, β_2ARregulates an alternative signaling pathway via activation of the inhibitory G protein ($G\alpha_i$) and the heterodimer formed by the β and γ subunits of the G protein ($G\beta\gamma$).[13] Besides the inhibition of adenylyl-cyclase, the main signal pathway regulated by β_2AR through $G\alpha_i$/$G\beta\gamma$ appears to be the phosphatidylinositol-3 kinase (PI3K)–signaling cascade, although other proteins such as the AMP-dependent protein kinase (AMPK), mammalian target of rapamycin, and extracellular signal-regulated kinase 1 and 2 (ERK1/2) have been proposed as novel targets of β_2AR.[139,140]

Regarding the effects of adrenergic system on metabolism, it is known that sustained beta adrenergic stimulation induces insulin resistance and in this context the β_2AR appears to have a major role in overall glucose homeostasis by modulating pancreatic islet hormone secretion as well as liver and muscle glucose homeostasis. For the heart, several studies have raised the possibility of using selective β_2AR agonists as potential modulators of cardiac muscle energy metabolism. Short- and long-term stimulation of the β_2AR has been associated with the modulation of fatty acid and glucose metabolism.[141] Indeed, acute treatment of myocytes in vitro or skeletal muscle ex vivo with β_2AR agonists increases glucose uptake to levels comparable to those seen after insulin stimulation.[142]

A putative mechanism for β_2AR function in insulin resistance involves the activation of PI3K and its downstream signal pathway[143,144] and in particular the phosphorylation and inactivation of TBC1D4 (also known as AKT substrate of 160 kDa, AS160) by AKT.[145] TBC1D4 inhibits the translocation of the glucose transporter type4 (GLUT4) from intracellular vesicles to the plasma membrane, hence an increase in TBC1D4 phosphorylation enhances glucose uptake.[145] Moreover, TBC1D4 is also targeted by AMPK, which represents a key mechanism in the regulation of insulin-independent glucose uptake.[145,146] Consistent with the potential role of β_2AR in glucose metabolism, higher levels of AMPK phosphorylation, and activity was seen in response to βAR stimulation[109,147] as a result of changes in the AMP/ATP ratio or activation of upstream AMPK kinases.[148] Therefore, it is tempting to speculate that β_2AR-agonists would induce GLUT4 translocation in this situation in an insulin-independent manner. Moreover in vivo studies show a greater efficiency of carvedilol, a nonselective beta AR antagonist, in ameliorating myocardial insulin sensitivity and glucose extraction in an animal model of heart failure, compared to the selective β1AR antagonist metoprolol.[149] These conflicting results may be due to differences in preclinical models but are more likely due to differences in response to acute and chronic stimulation of the β_2AR. While acute activation of the receptor can favor glucose uptake by increasing GLUT4 translocation to the plasma membrane, chronic adrenergic stimulation, as seen during heart failure, would be detrimental by mechanisms involving other molecular mechanisms, such as JNK, β-arrestins, and GRKs.[4,150]

ROLE OF ADRENERGIC RECEPTORS IN THE PATHOPHYSIOLOGY OF CARDIAC HYPERTROPHY

Cardiac hypertrophy can be observed in both physiological and pathological conditions where the increased hemodynamic or metabolic stress produces a remodeling of cardiac geometry.[151,152] However, under pathological conditions the hypertrophy is not compensatory, rather it reflects activation of maladaptive cellular processes that promote disease progression. In this sense, myocardial hypertrophy might serve as diagnostic and prognostic marker of cardiac remodeling (Fig. 11.6), and underlies several biochemical and molecular changes involved in metabolic and contractile regulatory pathways.[153,154]

The pathways attributed to pathological hypertrophy promoted the identification of specific pharmacological targets able to counteract adverse remodeling and foster prognosis improvement. Common triggers of cardiac remodeling include hypertension, myocardial infarction, chronic ischemia, inflammation, valvular disease, prolonged tachycardia or bradycardia, and genetic predisposition.[113] Cardiac remodeling represents the result of increased cell death (apoptosis and/or necrosis) and hypertrophy of the surviving cardiomyocytes. A resulting increase in LV mass is defined by LV wall thickness and LV diameter as concentric or eccentric hypertrophy.[155] In LV concentric hypertrophy cardiomyocytes grow thicker but retain normal length, while dilation of the LV in eccentric hypertrophy is associated with cardiomyocyte elongation through sequential addition of sarcomeres.[156] The type of hypertrophic remodeling depends on the trigger and concomitant progression of contractile dysfunction, such that concentric hypertrophy is a common response to increased afterload (valve stenosis, hypertensive heart disease), whereas eccentric hypertrophy is often observed in conditions of LV volume overload (e.g., valve regurgitation or shunt) or after myocardial infarction (as a late response).[157] Overall, cardiac hypertrophy can be considered a general term that indicates both concentric hypertrophy, with prevalent diastolic dysfunction, and eccentric hypertrophy with prevalent systolic dysfunction. These two patterns of cardiac remodeling represent the two extremes of a continuum.[158] Clinical echocardiography defines four distinct geometric

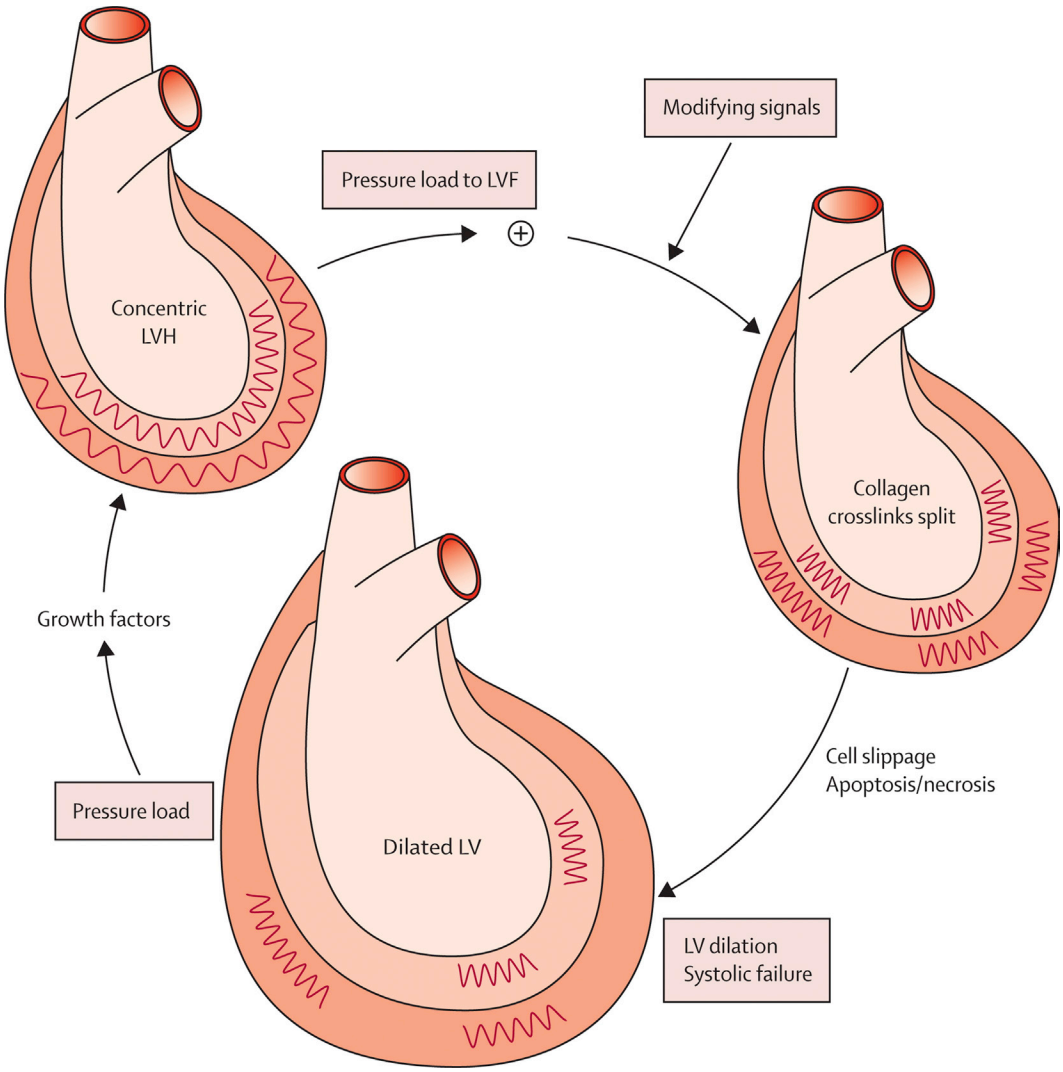

FIGURE 11.6

Myocardial remodeling in response to pressure load. Proposed transition from pressure load to concentric hypertrophy to dilated failing left ventricle (LV). Concentric remodeled myocardium undergoes splitting of the collagen crosslinks in response to modifying molecular signals such as metalloproteinases and other signals that disrupt collagen crosslinks to promote LV dilation and systolic heart failure. *LVF* = left ventricular failure. *LVH* = left ventricular hypertrophy.

*Adapted from Heusch G, Libby P, Gersh B, Yellon D, Böhm M, Lopaschuk G, Opie L. Cardiovascular remodelling in coronary artery disease and heart failure. Lancet 2014;***383***(9932):1933–43. (10.1016/S0140-6736(14)60107-0).*

patterns: (1) normal geometry, (2) concentric remodeling, (3) concentric hypertrophy, and (4) eccentric hypertrophy.[159] Additionally, nine intermediate phenotypes have been recently identified.[160]

Activation of the SNS in response to normal or disease-related stimuli is essential to maintain homeostasis and for the body to adapt in a constantly changing environment. The physiological and metabolic responses to sympathetic activation are mediated through the action of the endogenous catecholamines on adrenergic receptors. Regulation of cardiac function in response to catecholamine stimulation is controlled primarily through activation of βARs. As mentioned above, both β_1 and β_2AR subtypes couple to G_s and activate adenylyl-cyclase, thereby increasing cAMP levels and activating PKA.[161] In addition to G_s, β_2ARs also possess a nonclassic pathway through which are coupled to G_i (pertussis-toxin sensitive pathway).[162] Studies in neonatal cardiomyocytes of β_2AR knockout mice showed that stimulation of β_2AR is characterized by a biphasic effect on contraction rate with an initial PKA-independent increase in rate, followed by a PTX-sensitive decrease in rate of contraction.[163] Switching of β_2AR from G_s to G_i-coupling was found not only to inhibit adenylyl-cyclase activity but also to initiate signaling of MAPK by the Gβγ subunits of G_i in a process that is regulated by PKA-mediated phosphorylation of the receptor. βAR/G_i coupling can also activate the cytosolic effector molecule phospholipase A2 (cPLA2) in the heart in a cascade that triggers positive enhancement of calcium signaling and contraction, and which is independent of cAMP production.[164] In contrast with β_1-β_2ARs, β_3ARs are expressed at very low levels in the unstressed heart and are upregulated in various conditions with adrenergic overstimulation. Moreover, β_3ARs are typically activated by high concentrations of catecholamines (e.g., noradrenaline), are resistant to homologous desensitization, and their activation has potential negative inotropic effects since genetic deletion of β_3ARs results in enhancement of cardiac myocyte contractility.[165]

Transgenic overexpression of β_3ARs in the mouse heart was described as a neutral phenotype, with no deterioration of LV function at baseline.[166] However, systemic deletion of β_3ARs in mice subjected to transverse aortic constriction produced an adverse cardiac phenotype, thus arguing in favor of protection conferred by β_3ARs.[167] Nonetheless, mouse models with cardiac-specific overexpression of the human β_3ARs subjected to various neurohormonal stresses appear protected from hypertrophic and fibrotic remodeling. Dissection of signaling in isolated cardiomyocytes identifies β_3AR coupling to NOS/cGMP and downstream protein kinase G (PKG) as key components for this protection.[12]

Cardiac hypertrophy and heart failure are typically characterized by derangement of βARs signaling and a reduction of the adrenergic reserve of the heart.[168] This is primarily due to the selective reduction (downregulation) of β_1AR density at the plasma membrane and by the uncoupling of the remaining β_1ARs and β_2ARs from G proteins (functional desensitization). Moreover β_2AR signaling in the failing heart is different from that seen in the normal heart, switching from a compartmentalized to a diffuse pro-apoptotic cAMP signaling pattern, similar to that seen for the β_1AR.[169] These modifications are strictly connected to myocardial levels and activities of the most important, versatile, and ubiquitous GRKs, GRK2, and GRK5, which were elevated both in humans and in animal models of heart failure.[2] Excessive catecholamines stimulation of cardiac βARs triggered the GRK2 upregulation in cardiomyocytes, thus leading to a reduction in cardiac βAR density and responsiveness, ultimately resulting in cardiac inotropic reserve depletion.[4,170] Such GRK2 elevation is a homeostatic protective mechanism aimed at defending the heart against excessive catecholaminergic toxicity.[151] Thus, elevated sympathetic activity in chronic heart failure cause enhanced GRK2-mediated cardiac β_1AR and β_2AR desensitization and β_1ARs downregulation, eventually leading to the progressive loss of the adrenergic and inotropic reserves of the heart.

GRK2 SUBCELLULAR LOCALIZATION: A MOLECULAR LINK BETWEEN MYOCARDIAL CONTRACTILITY AND CARDIAC METABOLISM

Activation of the adrenergic system has a profound effect on cell function and metabolism regulating several metabolic responses. The over-activation in stress conditions becomes detrimental for the correct functioning of organs leading to the development of metabolic alterations (insulin resistance, altered glucose, and lipid metabolism and mitochondrial dysfunction). Among adrenergic receptors, it was demonstrated that the activation of βARs subtypes induces insulin resistance[75] and, in particular the βARs regulates overall glucose homeostasis.[14,73] At the cardiac level, β_2AR stimulation associated with the modulation of fatty acid and glucose metabolism.[141] In particular, acute treatment of myocytes in vitro or skeletal muscle ex vivo with β_2AR agonists increased glucose uptake, comparable to the increase produced by insulin stimulation.[171] Recent discoveries have suggested that GRK2 is a potential molecular link between chronic adrenergic stimulation and development of altered myocardial metabolism observed during heart failure.[74,127] The failing heart is characterized by an upregulation of GRK2 levels,[2] which is involved in the inhibition of βARs signaling and cardiac inotropism[5,172] (Fig. 11.7).

In addition to the effects on heart function, GRK2 upregulation also affects cardiac metabolism, and in particular, myocardial glucose uptake, at the early stages of the disease, when cardiac dilation and reduced function are not yet evident, indicating that metabolic modifications are involved in the progression of heart failure. These findings suggest that GRK2 is the molecular link between the over-activation of the adrenergic system and the altered glucose uptake during heart failure.[5,74] The connection between GRK2 and insulin signaling derives from the proof of concept that insulin increases the cellular content of GRK2.[75] Several reports suggest that GRK2 is a crucial modulator of insulin resistance, both systemically and in the heart.[173] GRK2 can directly induce insulin resistance and reduce glucose metabolism in cardiomyocytes through its catalytic activity. When GRK2 is overexpressed in myocytes, there was a decrease in myocardial glucose uptake and impaired insulin signaling and fatty acid metabolism.[74,174] GRK2 directly phosphorylates IRS1 at the inhibitory Ser^{307} residue, inducing the dissociation of the insulin receptor signaling complex and attenuating signaling to downstream effectors such as AKT and GLUT4.[74,150] Moreover, elevated myocardial GRK2 levels exacerbated defects in cardiac glucose metabolism after ischemic injury, before inducing ventricular contractile dysfunction,[173] demonstrating a proposed link between GRK2 and adrenergic control of contractility and metabolism. To further support this concept, myocardial glucose uptake is elevated in cardiac-specific GRK2 knockout mice, compared to wild-type mice, and glucose uptake is maintained even after ischemia. Moreover, insulin signaling is modified as evidenced by decreased phosphorylation of IRS1. Overall, glucose metabolism was improved, which prevents heart failure because cardiac contractility is not adversely affected.

The interaction between GRK2 and IRS1 is dependent on an intact C terminus of GRK2 as demonstrated in studies using the βARKct peptide, which reproduces the C-terminal sequence, inhibits insulin-mediated GRK2-dependent IRS1 phosphorylation, and improves AKT activation and GLUT4 translocation in response to insulin. Moreover, βARKct gene delivery to the hearts of rats through adeno-associated virus serotype 6 before ischemic injury prevented insulin resistance and myocardial glucose uptake remained high. These results could suggest that the direct interaction between GRK2 and IRS1 occurs within the C-terminal tail of GRK2 or that activation of the insulin receptor stimulates a pool of G proteins that recruit GRK2 to the membrane through Gβγ where it can interact with IRS1.

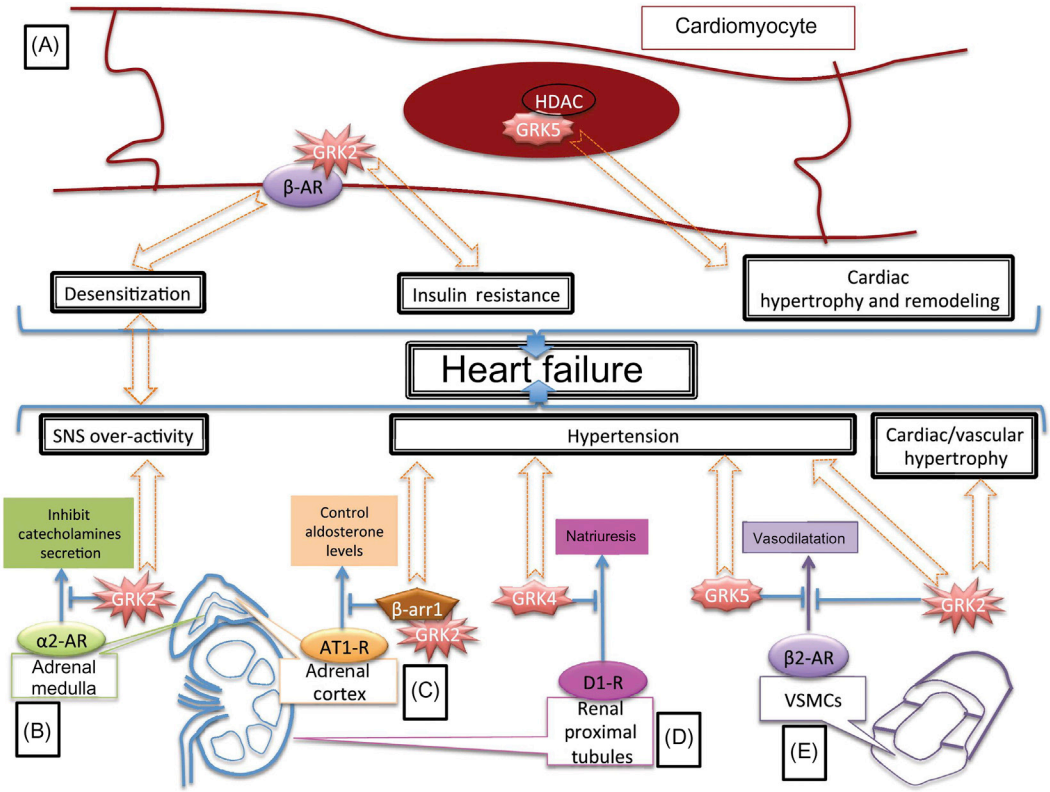

FIGURE 11.7

GRKs and cardiovascular disease. Persistent sympathetic stimulation of the cardiomyocyte triggers GRK2 upregulation that aggravates β-AR desensitization and impairs adrenergic signaling. GRK2 upregulation due to sympathetic nervous system overdrive has also been shown to be involved in the development of insulin resistance. (A) In the nucleus, GRK5 mediates cardiac hypertrophy by phosphorylating HDAC. (B) The adrenal gland was recently recognized as an attractive target for HF therapy because adrenal medullary chromaffin cells are the sites of catecholamine synthesis. GRK2 upregulation has been correlated with elevated catecholamine synthesis and secretion, primarily due to desensitization of the inhibitory α2-AR receptors. (C) In the adrenal cortex, angiotensin regulates aldosterone synthesis, where GRK2-β-arrestin 1–mediated signaling interferes with this regulatory pathway and elevated aldosterone secretion. (D) In the kidneys, dopamine 1-R–mediated natriuresis is dampened by GRK4; water and sodium retention are key factors of hypertension. (E) In the vasculature, β2-AR–mediated vasodilation is impaired by GRK2, leading to hypertension and cardiac and vascular hypertrophy. GRK5 hyperactivity in the vasculature precipitates hypertension.

*Adapted from Kamal FA, Travers JG, Blaxall BC. G protein–coupled receptor kinases in cardiovascular disease: why "where" matters. Trends Cardiovasc Med 2012;**22**(8):213–19.*

Overall, given the higher efficiency of glucose in ATP production and the lower effect in oxidative stress with respect to other substrates, these data argue that the role of GRK2 in the pathogenesis of heart failure is due, at least in part, to negative alterations in cardiac metabolism.[74,174] Since GRK2 upregulation causes insulin resistance, its inhibition has positive effects on cellular metabolism. Indeed, peptide inhibitors of GRK2 have been designed that prevent its binding to the substrate[175] and correct glucose levels in diabetic gerbils. In spontaneously hypertensive rats, chronic treatment with a similar inhibitor of GRK2 kinase activity, Ant-124, ameliorates the glucose dyshomeostasis and reduction of the blood pressure levels.[75] Moreover, the inhibition of GRK2 delays the reduction of glucose uptake and protects insulin signaling in the heart, preserving cardiac dimension and function.[74] This nurtures a novel scenario in which GRK2 inhibition might correct impaired metabolism in those conditions characterized by poor energy utilization by the cell, such as heart failure. In particular, it is known that GRK2 inhibition obtained through means of βARKct transgenic expression of the truncated mutant which prevents GRK2 localization on membranes or deletion of GRK2 gene is beneficial for the failing heart. Nevertheless, this benefit is thought to be dependent upon inhibition of βARs in the heart.

GRK2 inhibition may lead to an improved cardiac energy utilization. Indeed, as described above, during the development of heart failure impaired glucose metabolism precedes depressed cardiac contractility in mice with myocardial infarction.[13] These findings support the idea that the inhibition of GRK2 kinase activity could be a potential therapeutic target and that excessive elevation of GRK2 is deleterious for the cell. However, recent evidence challenges this view, since GRK2 exerts different effects within the cell depending on its localization, cell type, stimuli, and the pathophysiological context (Fig. 11.4). Beside the known localization of GRK2 in plasma membrane and cytosol, recently it has been demonstrated that GRK2 is also able to localize in mitochondria under specific experimental conditions.[51] Such mitochondrial localization suggests a potential role of GRK2 in the regulation of energy metabolism but to date there are only few and apparently contradictory reports on this topic. In the basal condition, GRK2 is in mitochondria and stressors can induce further accumulation. In macrophages, GRK2 levels in mitochondria increase during inflammation or endotoxin stimulation, facilitating biogenesis, and restoring mitochondrial function.[176] In the early pathogenesis of Alzheimer's disease and in models of ischemia/reperfusion brain injury, GRK2 accumulates in damaged mitochondria.[177] Furthermore, both in hearts in vivo and in cultured myocytes, GRK2 localizes into mitochondria after ischemia/reperfusion[124]. As to the role of GRK2 in mitochondria, in HEK-293 cells the kinase enhances mitochondrial biogenesis, leading to an increase of ATP cellular content.[51] The removal of GRK2 from the skeletal muscle in vivo leads to reduced ATP production and impaired tolerance to ischemia.[51] These findings support a positive regulatory role of GRK2 for mitochondrial biogenesis and ATP generation.[176]

In conclusion, GRK2 is involved in the regulation of cell metabolism, and its effects are strictly dependent on its subcellular localization. Collectively, the literature demonstrates this kinase is an important adaptive mechanism to stress, such as receptor dependent and independent stimuli. As for all adaptive mechanisms, the effect is beneficial in the beginning but then becomes detrimental. Indeed, increased levels of GRK2 have a deleterious effect in the development of heart failure and insulin resistance when it is increased on plasma membranes,[74,75] whereas it is advantageous for energy metabolism when it is localized in mitochondria.[51,176] Subcellular localization of GRK2 might in the future pose the strategy for selective inhibition of the kinase, and the possibility to modulate GRK2 accumulation within cellular compartments could be a useful approach to regulate its negative or positive effects on cell metabolism.

PHARMACOLOGY OF ADRENERGIC RECEPTOR BLOCKADE

α-ADRENERGIC RECEPTOR BLOCKADE

As powerful vasodilators, α_1AR blockers were initially considered as promising drugs to treat heart failure. Nevertheless, chronic administration of the α_1AR blocker prazosin led to increased catecholamine levels. Two clinical trials failed to support the use of α_1AR blockers to treat heart failure: in the Veterans Administration Cooperative Study (aka Vasodilator Heart Failure Trial or V-HeFT)) patients receiving prazosin experienced worse outcomes than those receiving the combined vasodilator therapy of isosorbide dinitrate and hydralazine;[178] and in the ALLHAT (Antihypertensive and Lipid-Lowering Treatment to prevent Heart Attack Trial) study, the doxazosin arm was terminated early because of higher incidence of heart failure.[179] Growing evidence indicates that the central nervous system plays a determinant role in the sympathetic excitation observed in heart failure.[83] Moreover, the association between the degree of sympathetic activation and mortality raised the possibility that a more complete adrenergic blockade might produce better outcome. Since the excitation of central α_2AR inhibits the activation of the SNS, such a receptor has been considered a possible target in the treatment of heart failure.[180] Clonidine is a centrally acting drug with α_2AR agonist action that at modest doses can markedly attenuates cardiac and renal sympathetic tone in patients with failing hearts enrolled in a small and short-term clinical study. However, a trial investigating the centrally acting sympatholytic agent moxonidine, had to be terminated early,[181] despite a significant dose-related reduction in plasma noradrenaline, because the drug was associated with increased mortality and hospitalizations for heart failure and myocardial infarction. These findings indicate that peripheral receptor inhibition may be better tolerated than central suppression of the SNS. A marked sympatholytic effect has also been associated with adverse outcomes in the Beta Blocker Evaluation of Survival Trial, where patients receiving bucindolol showed a decrease in noradrenaline levels and exhibited a 169% increase in mortality.[182] Notably, one of the oldest drugs used to treat heart failure, digoxin, which mainly acts by indirectly increasing intracellular Ca^{2+} available in the sarcoplasmic reticulum, has been also shown to modulate the adrenergic nervous system by improving baroreceptor function and decreasing sympathetic tone.[183]

β-ADRENERGIC RECEPTOR BLOCKADE

Based on receptor-level activity, β-blockers can be classified into three generations (Fig. 11.8): (1) first generation—nonselective drugs that block both β_1AR and β_2AR; (2) second generation—cardioselective agents, with higher affinity for β_1AR; and (3) third generation—β-blockers with vasodilative properties, mediated by α_1AR blockade, β_2AR agonism, or NO synthesis. Both selective and nonselective β-blockers have negative inotropic and chronotropic effects. The reduced inhibitory effect on β_2ARs makes the selective β-blockers less likely to cause peripheral vasoconstriction.[184] Hence, exercise performance may be impaired to a lesser extent by β_1AR selective drugs, at least in part because β_2AR blockade tends to blunt the exercise-induced increase in skeletal muscle blood flow.[103] Exercise training can improve βAR signaling and function, augmenting peak oxygen uptake, increasing cardiac inotropic reserves, and restoring normal sympathetic outflow and circulating catecholamine levels.[103]

β-blockers differ in their physicochemical properties. For instance, lipophilic compounds, including metoprolol, carvedilol, nebivolol, and bucindolol are rapidly adsorbed in the gastrointestinal tract and are extensively metabolized in the liver (first-pass metabolism), resulting in a shorter half-life when

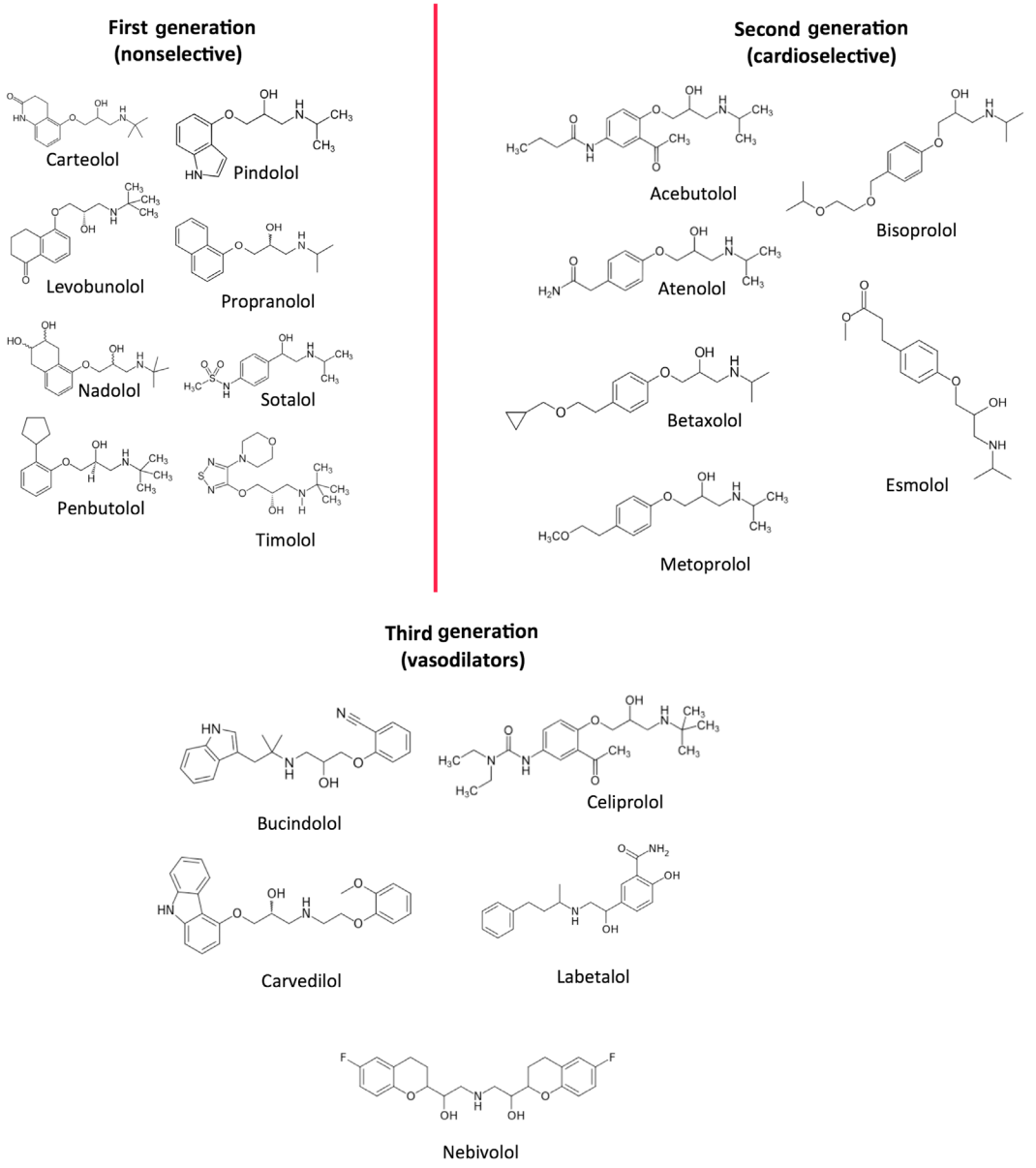

FIGURE 11.8

Graphic representation of the molecular structures of the three generations of β-blockers.

compared to other β-blockers. Additionally, the high lipophilicity leads to an enhanced penetration across the blood-brain barrier, which may justify the increased number of central effects as well as the membrane-stabilizing properties of antiarrhythmic molecules.[185]

The most common adverse events of β-blockers are attributable to the blockade of sympathetic stimulation, resulting in acute or chronic consequences at cardiovascular, metabolic, and respiratory levels.[186] In the heart, acute blockade of catecholamine effects worsens myocardial contractility and induces bradycardia. Starting with low doses and slowly titrating up is a commonly used approach to reduce these risks, patients may experience worsening of their symptoms during β-blocker titration, often requiring increased diuretic doses. Since a prolonged β-blocker treatment can enhance the sensitivity to catecholamines, an abrupt withdrawal should be avoided. As a result of antagonizing β_2ARs, β-blockers can cause bronchoconstriction, therefore β-blockers, especially nonselective agents, are contraindicated in patients suffering from asthma or chronic obstructive pulmonary disease. A risk/benefit assessment should be performed in each patient to avoid under treatment of heart failure.

The variability in the response to β-blockers has been at least in part ascribed to polymorphisms in the cytochrome P450 (CYP) gene CYP2D6, which is highly polymorphic in humans.[187] Indeed, many β-blockers are partially or totally metabolized by CYP2D6, and opportune dose modifications should be accurately considered in patients treated with drugs processed by the same cytochrome P450 isoforms, including antipsychotics and antidepressants.[188] Equally important, half-life and peak plasma concentration are influenced by the formulation of the molecule.[189] For instance, metoprolol is available in two different formulations: metoprolol-succinate, with a long-lasting action (Metoprolol CR/XL Randomized Intervention Trial in Congestive Heart Failure—MERIT-HF), and metoprolol-tartrate, which has a short half-life and demonstrated a reduced efficacy when compared to carvedilol (Carvedilol or Metoprolol European Trial—COMET study). The FDA has approved metoprolol-succinate for the treatment of patients with heart failure.[189]

Counteracting adrenergic overdrive via βAR antagonists reduces cardiac workload and increases O_2 sparing in patients with failing heart.[189] However, β-blockers have also noteworthy metabolic implications, including alterations in the lipoprotein profile, namely, a reduction in high-density lipoprotein cholesterol and an increase in triglycerides, and a deranged glucose homeostasis, which can be partially attributed to the blockade of β_2AR-dependent insulin release from the pancreatic islets of Langerhans.[14,190] Thus, β_1AR selective antagonists are generally preferred in patients with diabetes and heart failure.[110,186]

REFERENCES

1. Santulli G, Trimarco B, Iaccarino G. G-protein-coupled receptor kinase 2 and hypertension: molecular insights and pathophysiological mechanisms. *High Blood Press Cardiovasc Prev Off J Italian Soc Hypertens* 2013;**20**(1):5–12.
2. Iaccarino G, Barbato E, Cipolletta E, et al. Elevated myocardial and lymphocyte GRK2 expression and activity in human heart failure. *Eur Heart J* 2005;**26**(17):1752–8.
3. Penela P, Ribas C, Mayor Jr. F. Mechanisms of regulation of the expression and function of G protein-coupled receptor kinases. *Cell Signal* 2003;**15**(11):973–81.
4. Hatton R, Cvjeticanin A, Lymperopoulos A. The adrenergic system of the adrenal glands as a remote control of cardiac function. *JCvD* 2015;**5**(3):394–7.

5. Kamal FA, Travers JG, Blaxall BC. G protein-coupled receptor kinases in cardiovascular disease: why "where" matters. *Trends Cardiovasc Med* 2012;**22**(8):213–9.
6. Mei Y, Yin N, Jin X, He J, Yin Z. The regulatory role of the adrenergic agonists phenylephrine and isoproterenol on fetal hemoglobin expression and erythroid differentiation. *Endocrinology* 2013;**154**(12):4640–9.
7. Lampri E, Ioachim E.. In: Santulli G, editor. *Angiogenesis: something old, something new. Angiogenesis: insight from a systematic overview*. New York, NY: Nova Science Publishers; 2013. p. 1–30.
8. O'Connell TD, Jensen BC, Baker AJ, Simpson PC. Cardiac alpha1-adrenergic receptors: novel aspects of expression, signaling mechanisms, physiologic function, and clinical importance. *Pharmacol Rev* 2014;**66**(1):308–33.
9. Ruuskanen JO, Laurila J, Xhaard H, et al. Conserved structural, pharmacological and functional properties among the three human and five zebrafish alpha 2-adrenoceptors. *Br J Pharmacol* 2005;**144**(2):165–77.
10. Vicco MH, Pujato N, Bontempi I, Rodeles L, Marcipar I, Bottasso OA. beta1-Selective adrenoceptor antagonists increase plasma levels of Anti-p2beta antibodies and decrease cardiac involvement in chronic progressive chagas heart disease. *Can J Cardiol* 2014;**30**(3):332–7.
11. Santulli G, Iaccarino G. Pinpointing beta adrenergic receptor in ageing pathophysiology: victim or executioner? Evidence from crime scenes. *Immun Ageing* 2013;**10**(1):10.
12. Belge C, Hammond J, Dubois-Deruy E, et al. Enhanced expression of beta3-adrenoceptors in cardiac myocytes attenuates neurohormone-induced hypertrophic remodeling through nitric oxide synthase. *Circulation* 2014;**129**(4):451–62.
13. Xiao RP. Beta-adrenergic signaling in the heart: dual coupling of the beta2-adrenergic receptor to G(s) and G(i) proteins. *Sci STKE* 2001;**2001**(104):re15.
14. Santulli G, Lombardi A, Sorriento D, et al. Age-related impairment in insulin release: the essential role of beta(2)-adrenergic receptor. *Diabetes* 2012;**61**(3):692–701.
15. Kaya AI, Onaran HO, Ozcan G, et al. Cell contact-dependent functional selectivity of beta2-adrenergic receptor ligands in stimulating cAMP accumulation and extracellular signal-regulated kinase phosphorylation. *J Biol Chem* 2012;**287**(9):6362–74.
16. Santulli G, Marks AR. Essential roles of intracellular calcium release channels in muscle, brain, metabolism, and aging. *Curr Mol Pharmacol* 2015;**8**(2):206–22.
17. Yuan Q, Chen Z, Santulli G, et al. Functional role of Calstabin2 in age-related cardiac alterations. *Sci Rep* 2014;**4**:7425.
18. Santulli G, Wronska A, Uryu K, et al. A selective microRNA-based strategy inhibits restenosis while preserving endothelial function. *J Clin Invest* 2014;**124**(9):4102–14.
19. Santulli G, Basilicata MF, De Simone M, et al. Evaluation of the anti-angiogenic properties of the new selective alphaVbeta3 integrin antagonist RGDechiHCit. *J Transl Med* 2011;**9**:7.
20. Santulli G, Ciccarelli M, Palumbo G, et al. In vivo properties of the proangiogenic peptide QK. *J Transl Med* 2009;**7**:41.
21. Iaccarino G, Ciccarelli M, Sorriento D, et al. AKT participates in endothelial dysfunction in hypertension. *Circulation* 2004;**109**(21):2587–93.
22. Iaccarino G, Cipolletta E, Fiorillo A, et al. Beta(2)-adrenergic receptor gene delivery to the endothelium corrects impaired adrenergic vasorelaxation in hypertension. *Circulation* 2002;**106**(3):349–55.
23. Iaccarino G, Ciccarelli M, Sorriento D, et al. Ischemic neoangiogenesis enhanced by beta2-adrenergic receptor overexpression: a novel role for the endothelial adrenergic system. *Circul Res* 2005;**97**(11):1182–9.
24. Ciccarelli M, Sorriento D, Cipolletta E, et al. Impaired neoangiogenesis in beta-adrenoceptor gene-deficient mice: restoration by intravascular human beta-adrenoceptor gene transfer and role of NFkappaB and CREB transcription factors. *Br J Pharmacol* 2011;**162**(3):712–21.
25. Santulli G. Angiopoietin-like proteins: a comprehensive look. *Front Endocrinol* 2014;**5**:4.

26. Boer C, Scheffer GJ, de Lange JJ, Westerhof N, Sipkema P. Alpha-1-adrenoceptor stimulation induces nitric oxide release in rat pulmonary arteries. *J Vasc Res* 1999;**36**(1):79–81.
27. Guimaraes S, Moura D. Vascular adrenoceptors: an update. *Pharmacol Rev* 2001;**53**(2):319–56.
28. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* 1987;**84**(24):9265–9.
29. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987;**327**(6122):524–6.
30. Bockman CS, Gonzalez-Cabrera I, Abel PW. Alpha-2 adrenoceptor subtype causing nitric oxide-mediated vascular relaxation in rats. *J Pharmacol Exp Ther* 1996;**278**(3):1235–43.
31. Queen LR, Ji Y, Xu B, et al. Mechanisms underlying beta2-adrenoceptor-mediated nitric oxide generation by human umbilical vein endothelial cells. *J Physiol* 2006;**576**(Pt 2):585–94.
32. Lembo G, Iaccarino G, Vecchione C, et al. Insulin modulation of an endothelial nitric oxide component present in the alpha2- and beta-adrenergic responses in human forearm. *J Clin Invest* 1997;**100**(8):2007–14.
33. Ozaki M, Kawashima S, Yamashita T, et al. Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. *J Clin Invest* 2002;**110**(3):331–40.
34. Endemann DH, Schiffrin EL. Endothelial dysfunction. *J Am Soc Nephrol* 2004;**15**(8):1983–92.
35. Bouloumie A, Bauersachs J, Linz W, et al. Endothelial dysfunction coincides with an enhanced nitric oxide synthase expression and superoxide anion production. *Hypertension* 1997;**30**(4):934–41.
36. Forstermann U. Oxidative stress in vascular disease: causes, defense mechanisms and potential therapies. *Nat Clin Pract* 2008;**5**(6):338–49.
37. Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 2004;**4**(3):181–9.
38. Theccanat T, Philip JL, Razzaque AM, et al. Regulation of cellular oxidative stress and apoptosis by G protein-coupled receptor kinase-2; The role of NADPH oxidase 4. *Cell Signal* 2016;**28**(3):190–203.
39. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002;**82**(1):47–95.
40. Gutierrez J, Ballinger SW, Darley-Usmar VM, Landar A. Free radicals, mitochondria, and oxidized lipids: the emerging role in signal transduction in vascular cells. *Circul Res* 2006;**99**(9):924–32.
41. Fu YC, Yin SC, Chi CS, Hwang B, Hsu SL. Norepinephrine induces apoptosis in neonatal rat endothelial cells via a ROS-dependent JNK activation pathway. *Apoptosis* 2006;**11**(11):2053–63.
42. Lopez Farre A, Casado S. Heart failure, redox alterations, and endothelial dysfunction. *Hypertension* 2001;**38**(6):1400–5.
43. Finkel T. Reactive oxygen species and signal transduction. *IUBMB Life* 2001;**52**(1–2):3–6.
44. Gainetdinov RR, Bohn LM, Sotnikova TD, et al. Dopaminergic supersensitivity in G protein-coupled receptor kinase 6-deficient mice. *Neuron* 2003;**38**(2):291–303.
45. Lyubarsky AL, Chen C, Simon MI, Pugh Jr. EN. Mice lacking G-protein receptor kinase 1 have profoundly slowed recovery of cone-driven retinal responses. *J Neurosci* 2000;**20**(6):2209–17.
46. Walker JK, Gainetdinov RR, Feldman DS, et al. G protein-coupled receptor kinase 5 regulates airway responses induced by muscarinic receptor activation. *Am J Physiol Lung Cell Mol Physiol* 2004;**286**(2):L312–9.
47. Kahsai AW, Zhu S, Fenteany G. G protein-coupled receptor kinase 2 activates radixin, regulating membrane protrusion and motility in epithelial cells. *Biochim Biophys Acta* 2010;**1803**(2):300–10.
48. Chen Y, Sasai N, Ma G, et al. Sonic Hedgehog dependent phosphorylation by CK1alpha and GRK2 is required for ciliary accumulation and activation of smoothened. *PLoS Biol* 2011;**9**(6):e1001083.
49. Lodowski DT, Pitcher JA, Capel WD, Lefkowitz RJ, Tesmer JJ. Keeping G proteins at bay: a complex between G protein-coupled receptor kinase 2 and Gbetagamma. *Science* 2003;**300**(5623):1256–62.
50. Tesmer VM, Kawano T, Shankaranarayanan A, Kozasa T, Tesmer JJ. Snapshot of activated G proteins at the membrane: the Galphaq-GRK2-Gbetagamma complex. *Science* 2005;**310**(5754):1686–90.
51. Fusco A, Santulli G, Sorriento D, et al. Mitochondrial localization unveils a novel role for GRK2 in organelle biogenesis. *Cell Signal* 2011;**24**(2):468–75.

52. Obrenovich ME, Morales LA, Cobb CJ, et al. Insights into cerebrovascular complications and Alzheimer disease through the selective loss of GRK2 regulation. *J Cell Mol Med* 2009;**13**(5):853–65.
53. Vroon A, Kavelaars A, Limmroth V, et al. G protein-coupled receptor kinase 2 in multiple sclerosis and experimental autoimmune encephalomyelitis. *J Immunol* 2005;**174**(7):4400–6.
54. Iacovelli L, Franchetti R, Masini M, De Blasi A. GRK2 and beta-arrestin 1 as negative regulators of thyrotropin receptor-stimulated response. *Mol Endocrinol* 1996;**10**(9):1138–46.
55. Ferrer-Alcon M, La Harpe R, Garcia-Sevilla JA. Decreased immunodensities of micro-opioid receptors, receptor kinases GRK 2/6 and beta-arrestin-2 in postmortem brains of opiate addicts. *Brain Res Mol Brain Res* 2004;**121**(1–2):114–22.
56. Lombardi MS, Kavelaars A, Cobelens PM, Schmidt RE, Schedlowski M, Heijnen CJ. Adjuvant arthritis induces down-regulation of G protein-coupled receptor kinases in the immune system. *J Immunol* 2001;**166**(3):1635–40.
57. King DW, Steinmetz R, Wagoner HA, et al. Differential expression of GRK isoforms in nonmalignant and malignant human granulosa cells. *Endocrine* 2003;**22**(2):135–42.
58. Mak JC, Chuang TT, Harris CA, Barnes PJ. Increased expression of G protein-coupled receptor kinases in cystic fibrosis lung. *Eur J Pharmacol* 2002;**436**(3):165–72.
59. Liu S, Premont RT, Kontos CD, Zhu S, Rockey DC. A crucial role for GRK2 in regulation of endothelial cell nitric oxide synthase function in portal hypertension. *Nat Med* 2005;**11**(9):952–8.
60. Eckhart AD, Ozaki T, Tevaearai H, Rockman HA, Koch WJ. Vascular-targeted overexpression of G protein-coupled receptor kinase-2 in transgenic mice attenuates beta-adrenergic receptor signaling and increases resting blood pressure. *Mol Pharmacol* 2002;**61**(4):749–58.
61. Santulli G. microRNAs distinctively regulate vascular smooth muscle and endothelial cells: functional implications in angiogenesis, atherosclerosis, and in-stent restenosis. *Adv Exp Med Biol* 2015;**887**: 53–77.
62. Napolitano R, Campanile A, Sarno L, et al. GRK2 levels in umbilical arteries of pregnancies complicated by gestational hypertension and preeclampsia. *Am J Hypertens* 2012;**25**(3):366–71.
63. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 2003;**111**(5):649–58.
64. Rana S, Powe CE, Salahuddin S, et al. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. *Circulation* 2012;**125**(7):911–9.
65. Abd Alla J, Graemer M, Fu X, Quitterer U. Inhibition of G-protein-coupled receptor kinase 2 prevents the dysfunctional cardiac substrate metabolism in fatty acid synthase-transgenic mice. *J Biol Chem* 2015;**291**(6):2583–600.
66. Izzo R, Cipolletta E, Ciccarelli M, et al. Enhanced GRK2 expression and desensitization of betaAR vasodilatation in hypertensive patients. *Clin Transl Sci* 2008;**1**(3):215–20.
67. Borkowski KR, Gros R, Schneider H. Vascular beta-adrenoceptor-mediated responses in hypertension and ageing in rats. *J Auton Pharmacol* 1992;**12**(6):389–401.
68. Gros R, Benovic JL, Tan CM, Feldman RD. G-protein-coupled receptor kinase activity is increased in hypertension. *J Clin Invest* 1997;**99**(9):2087–93.
69. Gros R, Chorazyczewski J, Meek MD, Benovic JL, Ferguson SS, Feldman RD. G-Protein-coupled receptor kinase activity in hypertension: increased vascular and lymphocyte G-protein receptor kinase-2 protein expression. *Hypertension* 2000;**35**(1 Pt 1):38–42.
70. Santulli G, Campanile A, Spinelli L, et al. G protein-coupled receptor kinase 2 in patients with acute myocardial infarction. *Am J Cardiol* 2011;**107**(8):1125–30.
71. De Boer MP, Meijer RI, Wijnstok NJ, et al. Microvascular dysfunction: a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. *Microcirculation* 2012;**19**(1):5–18.

72. Santulli G. The adrenergic system in cardiovascular metabolism and aging.. In: Lymperopoulos A, editor. *The cardiovascular adrenergic system*. New York, NY: Springer; 2015. p. 97–116.
73. Usui I, Imamura T, Babendure JL, et al. G protein-coupled receptor kinase 2 mediates endothelin-1-induced insulin resistance via the inhibition of both Galphaq/11 and insulin receptor substrate-1 pathways in 3T3-L1 adipocytes. *Mol Endocrinol* 2005;**19**(11):2760–8.
74. Ciccarelli M, Chuprun JK, Rengo G, et al. G protein-coupled receptor kinase 2 activity impairs cardiac glucose uptake and promotes insulin resistance after myocardial ischemia. *Circulation* 2011;**123**(18): 1953–62.
75. Cipolletta E, Campanile A, Santulli G, et al. The G protein coupled receptor kinase 2 plays an essential role in beta-adrenergic receptor-induced insulin resistance. *Cardiovasc Res* 2009;**84**(3):407–15.
76. Heinonen I, Wendelin-Saarenhovi M, Kaskinoro K, Knuuti J, Scheinin M, Kalliokoski KK. Inhibition of alpha-adrenergic tone disturbs the distribution of blood flow in the exercising human limb. *Am J Physiol Heart Circul Physiol* 2013;**305**(2):H163–72.
77. Wu L, Liu L. Systematic review and meta-analysis evaluating the impact of vitamin D on the risk of heart failure: new evidence from population-based studies. *J Cardiovasc Dis* 2014;**2**(3):159–73.
78. Al Maluli H, DeStephan C. Hemodynamic monitoring in the intensive care unit. *J Cardiovas Dis* 2014;**2**(2):101–15.
79. Santulli G. Adrenal signaling in heart failure: something more than a distant ship's smoke on the horizon. *Hypertension* 2014;**63**(2):215–6.
80. Latini R, Masson S, Staszewsky L, Barlera S. Neurohormonal modulation in heart failure of ischemic etiology: correlates with left ventricular remodeling. *Curr Heart Fail Rep* 2006;**3**(4):157–63.
81. Sardu C, Marfella R, Santulli G, Paolisso G. Functional role of miRNA in cardiac resynchronization therapy. *Pharmacogenomics* 2014;**15**(8):1159–68.
82. Senni M, Paulus WJ, Gavazzi A, et al. New strategies for heart failure with preserved ejection fraction: the importance of targeted therapies for heart failure phenotypes. *Eur Heart J* 2014;**35**(40):2797–815.
83. Lymperopoulos A, Chowdhary S, Sankar K, Simon I. Regulation of catecholamine production from the adrenal medulla. In: Santulli G, editor. *Adrenal glands: from pathophysiology to clinical evidence*. New York, NY: Nova Science Publisher; 2015.
84. Perez-Alvarez A, Hernandez-Vivanco A, Albillos A. Past, present and future of human chromaffin cells: role in physiology and therapeutics. *Cell Mol Neurobiol* 2010;**30**(8):1407–15.
85. Chung M, Song J. Imaging of adrenal gland. In: Santulli G, editor. *Adrenal glands: from pathophysiology to clinical evidence*. New York, NY: Nova Science Publisher; 2015.
86. Deo SH, Jenkins NT, Padilla J, Parrish AR, Fadel PJ. Norepinephrine increases NADPH oxidase-derived superoxide in human peripheral blood mononuclear cells via alpha-adrenergic receptors. *Am J Physiol Regul Integr Comp Physiol* 2013;**305**(10):R1124–32.
87. Ma Y, Krueger JJ, Redmon SN, et al. Extracellular norepinephrine clearance by the norepinephrine transporter is required for skeletal homeostasis. *J Biol Chem* 2013;**288**(42):30105–13.
88. Thireau J, Karam S, Roberge S, et al. Beta-adrenergic blockade combined with subcutaneous B-type natriuretic peptide: a promising approach to reduce ventricular arrhythmia in heart failure? *Heart* 2014;**100**(11):833–41.
89. Staroukine M, Devriendt J, Decoodt P, Verniory A. Relationships between plasma epinephrine, norepinephrine, dopamine and angiotensin II concentrations, renin activity, hemodynamic state and prognosis in acute heart failure. *Acta Cardiol* 1984;**39**(2):131–8.
90. Shiny KS, Kumar SH, Farvin KH, Anandan R, Devadasan K. Protective effect of taurine on myocardial antioxidant status in isoprenaline-induced myocardial infarction in rats. *J Pharm Pharmacol* 2005;**57**(10): 1313–7.
91. Huang CJ, Webb HE, Zourdos MC, Acevedo EO. Cardiovascular reactivity, stress, and physical activity. *Front Physiol* 2013;**4**:314.

92. Weng TP, Fu TC, Wang CH, Hsu CC, Wang JS. Activation of lymphocyte autophagy/apoptosis reflects haemodynamic inefficiency and functional aerobic impairment in patients with heart failure. *Clin Sci* 2014;**127**(10):589–602.
93. Berezin A, Kremzer A, Samura T, Martovitskaya Y. Apoptotic microparticles to progenitor mononuclear cells ratio in heart failure: relevance of clinical status and outcomes. *J Cardiovasc Dis* 2014;**2**(2):50–7.
94. Sorriento D, Santulli G, Del Giudice C, Anastasio A, Trimarco B, Iaccarino G. Endothelial cells are able to synthesize and release catecholamines both in vitro and in vivo. *Hypertension* 2012;**60**(1):129–36.
95. Ciccarelli M, Santulli G, Campanile A, et al. Endothelial alpha1-adrenoceptors regulate neo-angiogenesis. *Br J Pharmacol* 2008;**153**(5):936–46.
96. Gao W, Li J. Femoral artery occlusion increases muscle pressor reflex and expression of hypoxia-inducible factor-1α in sensory neurons. *J Cardiovascu Dis* 2014;**1**(2):34–40.
97. Buroker N. ADRBK1 (GRK2) rSNPs, transcriptional factor binding sites and cardiovascular disease in the black population. *J Cardiovasc Dis* 2014;**2**(2):62–7.
98. Santulli G. Coronary heart disease risk factors and mortality. *JAMA J Am Med Assoc* 2012;**307**(11):1137.
99. Szepietowska B, Zhu W, Czyzyk J, Eid T, Sherwin RS. EphA5-EphrinA5 interactions within the ventromedial hypothalamus influence counterregulatory hormone release and local glutamine/glutamate balance during hypoglycemia. *Diabetes* 2013;**62**(4):1282–8.
100. Neglia D, De Caterina A, Marraccini P, et al. Impaired myocardial metabolic reserve and substrate selection flexibility during stress in patients with idiopathic dilated cardiomyopathy. *Am J Physiol Heart Circul Physiol* 2007;**293**(6):H3270–8.
101. Dias P, Terracciano CM. Hyperpolarization-activated cyclic nucleotide-gated channels and ventricular arrhythmias in heart failure: a novel target for therapy? *J Am Heart Assoc* 2013;**2**(3):e000287.
102. Li YF, Shi ST. Age-dependent differential crosstalk between alpha(1)-adrenergic and angiotensin receptors. *Can J Cardiol* 2009;**25**(8):481–5.
103. Santulli G, Ciccarelli M, Trimarco B, Iaccarino G. Physical activity ameliorates cardiovascular health in elderly subjects: the functional role of the beta adrenergic system. *Front Physiol* 2013;**4**:209.
104. Ciccarelli M, Santulli G, Pascale V, Trimarco B, Iaccarino G. Adrenergic receptors and metabolism: role in development of cardiovascular disease. *Front Physiol* 2013;**4**:265.
105. Chakraborty M, Phillips A, Macdonald J, Windsor J, Hickey A. Mitochondrial respiration in mononuclear cells and heart fibers in spontaneously hypertensive rats. *J Cardiovascu Dis* 2014;**2**(1):7–14.
106. Leone TC, Kelly DP. Transcriptional control of cardiac fuel metabolism and mitochondrial function. *Cold Spring Harbor Symp Quant Biol* 2011;**76**:175–82.
107. Fu Q, Xu B, Liu Y, et al. Insulin inhibits cardiac contractility by inducing a Gi-biased beta2 adrenergic signaling in hearts. *Diabetes* 2014;**63**(8):2676–89.
108. Msolly A, Miled A, Kassab A. Hydrogen peroxide: an oxidant stress indicator in type 2 diabetes mellitus. *J Cardiovasc Dis* 2014;**1**(2):48–52.
109. Oriente F, Iovino S, Cassese A, et al. Overproduction of phosphoprotein enriched in diabetes (PED) induces mesangial expansion and upregulates protein kinase C-beta activity and TGF-beta1 expression. *Diabetologia* 2009;**52**(12):2642–52.
110. Sardu C, Marfella R, Santulli G. Impact of diabetes mellitus on the clinical response to cardiac resynchronization therapy in elderly people. *J Cardiovasc Transl Res* 2014;**7**(3):362–8.
111. Azevedo PS, Minicucci MF, Santos PP, Paiva SA, Zornoff LA. Energy metabolism in cardiac remodeling and heart failure. *Cardiol Rev* 2013;**21**(3):135–40.
112. Shimizu I, Minamino T, Toko H, et al. Excessive cardiac insulin signaling exacerbates systolic dysfunction induced by pressure overload in rodents. *J Clin Invest* 2010;**120**(5):1506–14.
113. Sorriento D, Santulli G, Fusco A, Anastasio A, Trimarco B, Iaccarino G. Intracardiac injection of AdGRK5-NT reduces left ventricular hypertrophy by inhibiting NF-kappaB-dependent hypertrophic gene expression. *Hypertension* 2010;**56**(4):696–704.

114. Badimon L, Hernandez Vera R, Vilahur G. Determinants of cardiovascular risk in diabetes beyond hyperglycemia. *J Cardiovasc Dis* 2014;**1**(2):53–62.
115. Masuo K, Rakugi H, Ogihara T, Lambert GW. Different mechanisms in weight loss-induced blood pressure reduction between a calorie-restricted diet and exercise. *Hypertens Res Off J Jpn Soc Hypertens* 2012;**35**(1):41–7.
116. Santulli G. Effects of low-carbohydrate and low-fat diets. *Ann Int Med* 2015;**162**(5):392.
117. Quinones MJ, Nicholas SB, Lyon CJ. Insulin resistance and the endothelium. *Curr Diabetes Rep* 2005;**5**(4):246–53.
118. Mongillo M, John AS, Leccisotti L, Pennell DJ, Camici PG. Myocardial pre-synaptic sympathetic function correlates with glucose uptake in the failing human heart. *Eur J Nucl Med Mol Imaging* 2007;**34**(8):1172–7.
119. Larsen TM, Toubro S, van Baak MA, et al. Effect of a 28-d treatment with L-796568, a novel beta(3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men. *Am J Clin Nutr* 2002;**76**(4):780–8.
120. Sorriento D, Ciccarelli M, Santulli G, Illario M, Trimarco B, Iaccarino G. Trafficking GRK2: cellular and metabolic consequences of GRK2 subcellular localization. *Transl Med UniSa* 2014;**10**:3–7.
121. Horinouchi T, Hoshi A, Harada T, et al. Endothelin-1 suppresses insulin-stimulated Akt phosphorylation and glucose uptake via G protein-coupled receptor kinase 2 in skeletal muscle cells. *Br J Pharmacol* 2016;**173**(6):1018–32.
122. Lucas E, Cruces-Sande M, Briones AM, et al. Molecular physiopathology of obesity-related diseases: multiorgan integration by GRK2. *Arch Physiol Biochem* 2015;**121**(5):163–77.
123. Mehta N, Cheng AH, Chiang CK, et al. GRK2 fine-tunes circadian clock speed and entrainment via transcriptional and post-translational control of PERIOD proteins. *Cell Rep* 2015;**12**(8):1272–88.
124. Chen M, Sato PY, Chuprun JK, et al. Prodeath signaling of G protein-coupled receptor kinase 2 in cardiac myocytes after ischemic stress occurs via extracellular signal-regulated kinase-dependent heat shock protein 90-mediated mitochondrial targeting. *Circul Res* 2013;**112**(8):1121–34.
125. Taguchi K, Sakata K, Ohashi W, Imaizumi T, Imura J, Hattori Y. Tonic inhibition by G protein-coupled receptor kinase 2 of Akt/endothelial nitric-oxide synthase signaling in human vascular endothelial cells under conditions of hyperglycemia with high insulin levels. *J Pharmacol Exp Ther* 2014;**349**(2):199–208.
126. Hata JA, Williams ML, Koch WJ. Genetic manipulation of myocardial beta-adrenergic receptor activation and desensitization. *J Mol Cell Cardiol* 2004;**37**(1):11–21.
127. Santulli G, Xie W, Reiken SR, Marks AR. Mitochondrial calcium overload is a key determinant in heart failure. *Proc Natl Acad Sci USA* 2015;**112**(36):11389–94.
128. Wisneski JA, Gertz EW, Neese RA, Mayr M. Myocardial metabolism of free fatty acids. Studies with 14C-labeled substrates in humans. *J Clin Invest* 1987;**79**(2):359–66.
129. Bing RJ, Siegel A, Ungar I, Gilbert M. Metabolism of the human heart. II. Studies on fat, ketone and amino acid metabolism. *Am J Med* 1954;**16**(4):504–15.
130. Neubauer S. The failing heart—an engine out of fuel. *New Engl J Med* 2007;**356**(11):1140–51.
131. Kolwicz Jr. SC, Tian R. Metabolic therapy at the crossroad: how to optimize myocardial substrate utilization? *Trends Cardiovasc Med* 2009;**19**(6):201–7.
132. Eisenhofer G, Friberg P, Rundqvist B, et al. Cardiac sympathetic nerve function in congestive heart failure. *Circulation* 1996;**93**(9):1667–76.
133. Opie LH, Thandroyen FT, Muller C, Bricknell OL. Adrenaline-induced "oxygen-wastage" and enzyme release from working rat heart. Effects of calcium antagonism, beta-blockade, nicotinic acid and coronary artery ligation. *J Mol Cell Cardiol* 1979;**11**(10):1073–94.
134. Sasaoka T, Wada T, Tsuneki H. Lipid phosphatases as a possible therapeutic target in cases of type 2 diabetes and obesity. *Pharmacol Ther* 2006;**112**(3):799–809.
135. Banerjee S, Peterson LR. Myocardial metabolism and cardiac performance in obesity and insulin resistance. *Curr Cardiol Rep* 2007;**9**(2):143–9.

136. Wagenmakers AJ, van Riel NA, Frenneaux MP, Stewart PM. Integration of the metabolic and cardiovascular effects of exercise. *Essays Biochem* 2006;**42**:193–210.
137. Xiao RP, Cheng H, Zhou YY, Kuschel M, Lakatta EG. Recent advances in cardiac beta(2)-adrenergic signal transduction. *Circul Res* 1999;**85**(11):1092–100.
138. Baruscotti M, Barbuti A, Bucchi A. The cardiac pacemaker current. *J Mol Cell Cardiol* 2010;**48**(1):55–64.
139. Santulli G, Totary-Jain H. Tailoring mTOR-based therapy: molecular evidence and clinical challenges. *Pharmacogenomics* 2013;**14**(12):1517–26.
140. Perino A, Ghigo A, Ferrero E, et al. Integrating cardiac PIP(3) and cAMP signaling through a PKA anchoring function of p110gamma. *Mol Cell* 2011;**42**(1):84–95.
141. Philipson LH. beta-Agonists and metabolism. *J Allergy Clin Immunol* 2002;**110**(6 Suppl.):S313–7.
142. Ngala RA, O'Dowd J, Wang SJ, et al. Metabolic responses to BRL37344 and clenbuterol in soleus muscle and C2C12 cells via different atypical pharmacologies and beta2-adrenoceptor mechanisms. *Br J Pharmacol* 2008;**155**(3):395–406.
143. Jo SH, Leblais V, Wang PH, Crow MT, Xiao RP. Phosphatidylinositol 3-kinase functionally compartmentalizes the concurrent G(s) signaling during beta2-adrenergic stimulation. *Circul Res* 2002;**91**(1):46–53.
144. Zhu WZ, Zheng M, Koch WJ, Lefkowitz RJ, Kobilka BK, Xiao RP. Dual modulation of cell survival and cell death by beta(2)-adrenergic signaling in adult mouse cardiac myocytes. *Proc Natl Acad Sci USA* 2001;**98**(4):1607–12.
145. Sakamoto K, Holman GD. Emerging role for AS160/TBC1D4 and TBC1D1 in the regulation of GLUT4 traffic. *Am J Physiol* 2008;**295**(1):E29–37.
146. Maarbjerg SJ, Sylow L, Richter EA. Current understanding of increased insulin sensitivity after exercise - emerging candidates. *Acta Physiol (Oxford, England)* 2011;**202**(3):323–35.
147. Perez-Schindler J, Philp A, Baar K, Hernandez-Cascales J. Regulation of contractility and metabolic signaling by the beta2-adrenergic receptor in rat ventricular muscle. *Life Sci* 2011;**88**(19–20):892–7.
148. Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat Rev* 2012;**13**(4):251–62.
149. Nikolaidis LA, Poornima I, Parikh P, Magovern M, Shen YT, Shannon RP. The effects of combined versus selective adrenergic blockade on left ventricular and systemic hemodynamics, myocardial substrate preference, and regional perfusion in conscious dogs with dilated cardiomyopathy. *J Am College Cardiol* 2006;**47**(9):1871–81.
150. Shahid G, Hussain T. GRK2 negatively regulates glycogen synthesis in mouse liver FL83B cells. *J Biol Chem* 2007;**282**(28):20612–20.
151. Sorriento D, Santulli G, Franco A, et al. Integrating GRK2 and NFkappaB in the pathophysiology of cardiac hypertrophy. *J Cardiovasc Transl Res* 2015;**8**(8):493–502.
152. Santulli G, Cipolletta E, Sorriento D, et al. CaMK4 gene deletion induces hypertension. *J Am Heart Assoc* 2012;**1**(4):e001081.
153. Opie LH, Commerford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodelling. *Lancet (London, England)* 2006;**367**(9507):356–67.
154. Heusch G, Libby P, Gersh B, et al. Cardiovascular remodelling in coronary artery disease and heart failure. *Lancet* 2014;**383**(9932):1933–43.
155. Ganau A, Devereux RB, Roman MJ, et al. Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension. *J Am College Cardiol* 1992;**19**(7):1550–8.
156. Gerdes AM, Kellerman SE, Moore JA, et al. Structural remodeling of cardiac myocytes in patients with ischemic cardiomyopathy. *Circulation* 1992;**86**(2):426–30.
157. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. *Circulation* 1990;**81**(4):1161–72.
158. Lorell BH, Carabello BA. Left ventricular hypertrophy: pathogenesis, detection, and prognosis. *Circulation* 2000;**102**(4):470–9.

159. Foppa M, Duncan BB, Rohde LE. Echocardiography-based left ventricular mass estimation. How should we define hypertrophy? *Cardiovasc Ultrasound* 2005;**3**:17.
160. Tarone G, Balligand JL, Bauersachs J, et al. Targeting myocardial remodelling to develop novel therapies for heart failure: a position paper from the Working Group on Myocardial Function of the European Society of Cardiology. *Eur J Heart Fail* 2014;**16**(5):494–508.
161. Brodde OE, Michel MC, Zerkowski HR. Signal transduction mechanisms controlling cardiac contractility and their alterations in chronic heart failure. *Cardiovasc Res* 1995;**30**(4):570–84.
162. Xiao RP, Avdonin P, Zhou YY, et al. Coupling of beta2-adrenoceptor to Gi proteins and its physiological relevance in murine cardiac myocytes. *Circul Res* 1999;**84**(1):43–52.
163. Devic E, Xiang Y, Gould D, Kobilka B. Beta-adrenergic receptor subtype-specific signaling in cardiac myocytes from beta(1) and beta(2) adrenoceptor knockout mice. *Mol Pharmacol* 2001;**60**(3):577–83.
164. Pavoine C, Behforouz N, Gauthier C, et al. beta2-adrenergic signaling in human heart: shift from the cyclic AMP to the arachidonic acid pathway. *Mol Pharmacol* 2003;**64**(5):1117–25.
165. Moniotte S, Kobzik L, Feron O, Trochu JN, Gauthier C, Balligand JL. Upregulation of beta(3)-adrenoceptors and altered contractile response to inotropic amines in human failing myocardium. *Circulation* 2001;**103**(12):1649–55.
166. Kohout TA, Takaoka H, McDonald PH, et al. Augmentation of cardiac contractility mediated by the human beta(3)-adrenergic receptor overexpressed in the hearts of transgenic mice. *Circulation* 2001;**104**(20):2485–91.
167. Moens AL, Leyton-Mange JS, Niu X, et al. Adverse ventricular remodeling and exacerbated NOS uncoupling from pressure-overload in mice lacking the beta3-adrenoreceptor. *J Mol Cell Cardiol* 2009;**47**(5):576–85.
168. Port JD, Bristow MR. Altered beta-adrenergic receptor gene regulation and signaling in chronic heart failure. *J Mol Cell Cardiol* 2001;**33**(5):887–905.
169. Nikolaev VO, Moshkov A, Lyon AR, et al. Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation. *Science New York, NY* 2010;**327**(5973):1653–7.
170. Sorriento D, Ciccarelli M, Santulli G, et al. The G-protein-coupled receptor kinase 5 inhibits NFkappaB transcriptional activity by inducing nuclear accumulation of IkappaB alpha. *Proc Natl Acad Sci USA* 2008;**105**(46):17818–23.
171. Nevzorova J, Evans BA, Bengtsson T, Summers RJ. Multiple signalling pathways involved in beta2-adrenoceptor-mediated glucose uptake in rat skeletal muscle cells. *Br J Pharmacol* 2006;**147**(4):446–54.
172. Koch WJ, Rockman HA, Samama P, et al. Cardiac function in mice overexpressing the beta-adrenergic receptor kinase or a beta ARK inhibitor. *Science New York, NY* 1995;**268**(5215):1350–3.
173. Ciccarelli M, Cipolletta E, Iaccarino G. GRK2 at the control shaft of cellular metabolism. *Curr Pharm Des* 2012;**18**(2):121–7.
174. Sato PY, Chuprun JK, Ibetti J, et al. GRK2 compromises cardiomyocyte mitochondrial function by diminishing fatty acid-mediated oxygen consumption and increasing superoxide levels. *J Mol Cell Cardiol* 2015;**89**(Pt B):360–4.
175. Anis Y, Leshem O, Reuveni H, et al. Antidiabetic effect of novel modulating peptides of G-protein-coupled kinase in experimental models of diabetes. *Diabetologia* 2004;**47**(7):1232–44.
176. Sorriento D, Fusco A, Ciccarelli M, et al. Mitochondrial G protein coupled receptor kinase 2 regulates pro-inflammatory responses in macrophages. *FEBS Lett* 2013;**587**(21):3487–94.
177. Obrenovich ME, Smith MA, Siedlak SL, et al. Overexpression of GRK2 in Alzheimer disease and in a chronic hypoperfusion rat model is an early marker of brain mitochondrial lesions. *Neurotox Res* 2006;**10**(1):43–56.
178. Cheng JW. A review of isosorbide dinitrate and hydralazine in the management of heart failure in black patients, with a focus on a new fixed-dose combination. *Clin Ther* 2006;**28**(5):666–78.
179. Oparil S, Davis BR, Cushman WC, et al. Mortality and morbidity during and after Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial: results by sex. *Hypertension* 2013;**61**(5):977–86.

180. Gavras I, Manolis AJ, Gavras H. The alpha2 -adrenergic receptors in hypertension and heart failure: experimental and clinical studies. *J Hypertens* 2001;**19**(12):2115–24.
181. Pocock S, Wilhelmsen L, Dickstein K, Francis G, Wittes J. The data monitoring experience in the MOXCON trial. *Eur Heart J* 2004;**25**(22):1974–8.
182. Frantz RP, Lowes BD, Grayburn PA, et al. Baseline and serial neurohormones in patients with congestive heart failure treated with and without bucindolol: results of the neurohumoral substudy of the Beta-Blocker Evaluation of Survival Study (BEST). *J Card Fail* 2007;**13**(6):437–44.
183. Deneer VH, van Hemel NM. Is antiarrhythmic treatment in the elderly different? a review of the specific changes. *Drugs Aging* 2011;**28**(8):617–33.
184. Wong GW, Laugerotte A, Wright JM. Blood pressure lowering efficacy of dual alpha and beta blockers for primary hypertension. *Cochrane Database Syst Rev* 2015;**8**:CD007449.
185. Ellison KE, Gandhi G. Optimising the use of beta-adrenoceptor antagonists in coronary artery disease. *Drugs* 2005;**65**(6):787–97.
186. Santulli G. beta-Blockers in diabetic patients with heart failure. *JAMA Intern Med* 2015;**175**(4):657.
187. Lazalde-Ramos BP, Martinez-Fierro Mde L, Galaviz-Hernandez C, et al. CYP2D6 gene polymorphisms and predicted phenotypes in eight indigenous groups from northwestern Mexico. *Pharmacogenomics* 2014;**15**(3):339–48.
188. Rossello X, Pocock SJ, Julian DG. Long-term use of cardiovascular drugs: challenges for research and for patient care. *J Am Coll Cardiol* 2015;**66**(11):1273–85.
189. Santulli G. Sympathetic nervous system signaling in heart failure and cardiac aging. In: Jagadeesh G, editor. *Pathophysiology and pharmacotherapy of cardiovascular disease*. New York, NY: Springer; 2015. p. 83–105.
190. Santulli G, Pagano G, Sardu C, et al. Calcium release channel RyR2 regulates insulin release and glucose homeostasis. *J Clin Invest* 2015;**125**(5):1968–78.