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Review

Cardiac β-Adrenoceptor Signaling: The New Insight on An Old Target in the Therapy of Cardiovascular Disease

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Abstract: A variety of G protein-coupled receptors (GPCRs) are involved in the regulation of cardiovascular function. The β-adrenoceptors (β-ARs) are the dominant receptor species in the heart, in which the β_1-AR and the β_2-AR are considered functional. Stimulation of the β-ARs produces myocardial inotropy via activation of the G_s-cAMP-PKA signaling cascade. Prolonged stimulation of the β_1-AR is cardiac harmful because the stimulated β_1-AR couples only to G_s proteins and it mediates a cardiotoxic signal. On the other hand, the β_2-AR couples dually to both G_s and G_i proteins and the β_2-AR-G_i pathway is antiapoptotic. The activated G_i signal also counteracts the β-AR-G_s-promoted positive inotropic effect. Other key players in cardiac β-AR signaling include Ca^{2+}/calmodulin-dependent protein kinases (CaMKs), GPCR kinases (GRKs), β-arrestins and phosphodiesterases. During heart failure, excessive sympathetic stimulation results in the activation of the cardiotoxic β_1-AR-CaMKIIδ pathway and the upregulation of GRK2 and G_i in the heart. GRK2 promotes the desensitization of β-ARs and enhances a β_2-AR-mediated G_i signaling. These signal transduction processes accompanying the downregulation of the β_1-AR are involved in cardiac dysfunction, maladaptive cardiac remodeling, and the progression of chronic heart failure. β-blockers are widely used in the treatment of cardiovascular disease. They have established their position as one of the "four pillars of heart failure" more than twenty years ago. In the present review, we provide an overview of the recent progress in the basic research of GPCRs focusing on cardiac β-AR signal transduction.

Keywords: heart; receptors, adrenergic, beta; signal transduction; heart failure.

1. GPCRs and Cardiovascular Disease

1.1. Brief Introduction of GPCRs

G protein-coupled receptors (GPCRs), or seven-fold transmembrane receptors, are the largest class of membrane proteins in the human genome. GPCRs share a common structure consisting of peptides with an extracellular N-terminus, an intracellular C-terminus (CT), and seven hydrophobic transmembrane helices linked by three extracellular and three intracellular loops (ICLs). The extracellular part of the receptor is usually glycosylated, and the highly conserved cysteine residues in the extracellular loops can form disulfide bonds to stabilize the spatial structure of the receptor. The CT and the ICLs are binding sites for G proteins, G protein-coupled receptor kinases (GRKs), arrestins and other downstream signaling molecules. GRKs and arrestins are key molecules participating in the desensitization of GPCRs. In addition, adenylyl cyclase (AC), phospholipase C, phosphodiesterases (PDEs), cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), inositol triphosphate, Ca^{2+}, etc. are also involved in GPCR signal transduction. Classical theory suggests that upon binding to a ligand, a GPCR undergoes a conformational change that promotes interaction of its intracellular part with a G protein. The exchange of GDP for GTP in the G protein results in the dissociation of the heterotrimeric G protein into the G_{α} subunit and the $G_{\beta\gamma}$ subunits. Both of which interact with various effectors to regulate second messenger production or ion channel opening. Over the past two decades, numerous studies have shown that receptor-bound arrestins trigger a second round of signaling that is quite different from that mediated by G proteins [1]. Some GPCR ligands are 'balanced' while some others are 'biased' in terms of their preferences on activating one type of G protein or arrestin signal over another [1]. Adding to this complexity is the capability of GPCRs to form heterodimers, and the new receptor species thus formed can have signaling properties different from those of their parent receptors. Thus, GPCR heterodimers constitute very interesting targets for future drugs [2,3].

The human GPCR superfamily have more than 800 members. Some GPCRs have sensory function mediating smell, taste, vision or pheromone signals. GPCRs without sensory function participate in growth, development, reproduction and other physiological processes. They are also involved in the development of diseases including cardiovascular diseases, diabetes, cancer, immune disorders, infectious diseases, neurological and mental diseases. GPCRs can be divided into five categories according to their structures, as shown in Table 1. Such taxonomy is known as the GRAFS nomenclature system [4].

Table 1. GPCR Classes.

Categories	Representative Receptors	Number of Receptors with Known Ligands	Number of Orphan Receptors	Number of Olfactory Receptors	Number of Visual Receptors	Number of Taste Receptors
Glutamate or class C	metabolic glutamate receptor, calcium sensing receptor, GABAB receptor, taste 1 receptor, pheromone V2 receptor	12	8	-	-	3
Rhodopsin or class A	Various small molecule receptors, neurotransmitter receptor, peptide and hormone receptors, olfactory receptor, visual receptor, pheromone V1 receptor	197	87	390	10	-
Adhesion	CD97, GPR133, GPR144	0	26	-	-	-
Frizzled/ Taste 2	Frizzled receptor, taste 2 receptor	11	0	-	-	30
Secretin or class B	Glucagon receptor, vasoactive intestinal peptide receptor, secretin	15	-	-	-	-

The orphan receptors in Table 1 refer to GPCRs lacking endogenous ligands. Although molecular biology and bioinformatic techniques have enabled the identification of orphan receptors, it has been a great challenge to find their endogenous ligands. Over 300 GPCRs have been de-orphaned in the last few decades. These receptors are potential cardiovascular disease targets. For example, the G_i protein-coupled receptor APJ, once considered an orphan receptor, is found to be a receptor with its endogenous ligand apelin, which exerts peripheral and central cardiovascular effects by promoting vasodilation, angiogenesis and contractility of the heart [5]. For receptors that are not de-orphaned, endogenous ligands can be bypassed to develop alternative ligand drugs. Modulators of GPR119, GPR35, GPR55, MAS and GPR84 have already entered clinical trials [6].

GPCRs are among the most well-established drug targets. With the development of structural biology and other technologies, drugs targeting GPCRs are rapidly developed and utilized. New GPCR targets continue to emerge, bringing new opportunities for drug development.

1.2. GPCR and Cardiovascular Disease

Cardiovascular diseases are the leading cause of mortality and morbidity worldwide, accounting for a quarter of global deaths every year [7]. Myocardial infarction, cardiomyopathy, valvular heart disease, viral myocarditis, chronic hypertension, coronary artery disease, heart injuries, etc. can eventually lead to heart failure. Heart failure is a complex clinical syndrome characterized by a reduced pumping and/or refilling capacity of the heart. Physiologically, heart failure can be defined as insufficiency of the cardiac output to meet metabolic demands. Globally, about 26 million adults suffer from heart failure, and therefore heart failure is defined as a global pandemic. The five-year mortality rate of heart failure is as high as 42.3% [8]. Patients with heart failure usually require long and repeated hospitalization. A rising patient population in heart failure brings serious economic, social and personal burdens.

GPCRs are widely expressed in the cardiovascular system and the corresponding cell types, such as cardiomyocytes, fibroblasts, endothelial cells and vascular smooth muscle cells. Over 200 GPCRs participate in the regulation of cardiovascular function. GPCRs present in the cardiovascular system are involved in the sensing and responses to various stimuli (such as mechanical stress, hormones, cytokines and growth factors). They also participate in the development of cardiovascular diseases, comprising 24% of the drug targets in the cardiovascular system [9]. The most well-studied ones include adrenoceptors (ARs), angiotensin receptors (ATRs), muscarinic receptors, endothelin receptors, and adenosine receptors.

ATRs, especially the AT1R, play a key role in the pathophysiology of the cardiovascular system. AT1Rs are expressed in the heart as well as in blood vessels. Angiotensin II acts on vascular AT1Rs to produce vasoconstriction causing blood pressure to rise. Overexpression of the AT1R promotes myocardial fibrosis and hypertrophy while its knockdown enhances cardiac function after myocardial infarction, suggesting that AT1R mediates a harmful effect on the heart. Myocardial AT1R is upregulated during ischemia and is associated with adverse cardiac remodeling in heart failure [10]. Therefore, AT1R antagonists (or angiotensin II receptor blockers, ARBs) are widely used in the treatment of cardiovascular diseases such as hypertension, coronary artery disease and heart failure. The AT1R couples to $G_{\alpha q}$ proteins and it may also mediate signals via $G_{\beta\gamma}$ subunits and β-arrestins [11]. Recent studies have suggested that activation of β-arrestin signaling in addition to the blockade of G protein signaling downstream of the AT1R may provide a beneficial cardiotonic effect in heart failure. This may lead to the development of a superior class of ARBs [12,13]. Thus, similar basic studies on GPCR signal transduction may open up new opportunities for the development of future cardiovascular drugs.

2. β-AR Signaling in the Cardiovascular System

2.1. β-ARs in the Cardiovascular System

Vital body functions such as heart beat and blood pressure maintenance are under the control of the autonomic nervous system composed of the sympathetic nervous system (SNS) and the parasympathetic nervous system. Target organs of the cardiovascular system such as the heart and peripheral arteries respond to sympathetic stimulation by means of ARs expressed on the plasma membranes of target cells. Norepinephrine secreted from sympathetic nerve terminals and circulatory catecholamines (mainly epinephrine with a small amount of norepinephrine) are the endogenous agonists of these receptors. In vascular smooth muscles, catecholamines stimulate α-ARs to cause vasoconstriction and β_2-ARs to cause vasodilation. In the heart, stimulation of β-ARs increases heart rate and myocardial contractility. Adaptive activation of the SNS increases blood pressure and cardiac output to meet the metabolic requirements of the body.

β-ARs are the most abundant GPCR members expressed in mammalian hearts. All the three subtypes of the β-ARs, β_1, β_2 and β_3, exist in the human heart. The β_1-AR has the highest abundance followed by the β_2-AR. Under normal conditions, the expression level of the β_3-AR is much lower [14]. The expression patterns of β-ARs in specific cells is worth exploring and refining. The most abundant cell types in the mammalian heart are cardiomyocytes, cardiac fibroblasts, endothelial cells and vascular smooth muscle cells. Among them, cardiomyocytes account for about 30% in quantity and 70% in mass and volume, constituting the myocardium and providing contractile force for the heart [15]. Recent data show that β_1-ARs are expressed in every cardiomyocyte in mice [16]. Cardiac fibroblasts, endothelial cells and vascular smooth muscle cells generally express β_2-ARs. With the increase of age, the level of β_1-ARs in human ventricular muscles decreases, especially in women [17]. But sex and age have no impact on β-AR-mediated inotropy in isolated human atrial muscles [18].

2.2. Cardiac β-AR Signaling

2.2.1. Classical β-AR Signaling in Cardiomyocytes

β-ARs are G_s protein-coupled receptors. Signal transduction begins with the binding of an adrenergic agonist to a β-AR causing it to couple to intracellular G_s proteins. The activated G_s protein enhances cAMP synthesis by AC, thereby activating cAMP-dependent protein kinase (PKA) which subsequently phosphorylates various PKA substrates to produce a cellular response. In cardiomyocytes, these PKA substrates include proteins responsible for intracellular Ca^{2+} regulation (e. g. phospholamban (PLB), L-type calcium channel (LTCC) and ryanodine receptor (RyR)), other cation channels involved in the generation of an action potential (e.g. the delayed rectifier potassium channel I_{Ks}) and regulatory proteins in the contractile machinery (e.g. cardiac troponin I and cardiac myosin-binding protein C).

Excitation-contraction coupling is the mechanism to relay electrical activity in a muscle cell to the process of cellular contraction. The transport of Ca^{2+} across different membranes play a central role in this process. LTCC on the plasma membrane (sarcolemma) is responsible for the voltage-dependent influx of extracellular Ca^{2+} , which is spatially coupled with a cluster of RyRs on subsarcolemmal cisternae of sarcoplasmic reticulum (SR) responsible for the release of stored Ca^{2+} into the cytosol. An action potential in a cardiomyocyte triggers a small influx of Ca^{2+} ($I_{Ca,L}$) through LTCC. The increase in local Ca^{2+} levels induces a large efflux of stored Ca^{2+} via RyRs in a process known as calcium-induced calcium release [19], generating Ca^{2+} sparks. Spatial synchronization of these Ca^{2+} sparks results in a large surge of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$). Binding of Ca^{2+} to cardiac troponin C activates myofilaments and initiates cell contraction. In the repolarization phase of the action potential, actions of the sarcolemmal sodium-calcium exchanger to transport intracellular Ca^{2+} out of the cell and that of the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) to refill the Ca^{2+} pool restore the resting $[Ca^{2+}]_i$ level, allowing the cardiomyocyte to relax.

Acute β-adrenergic stimulation increases myocardial contractility through enhancement of Ca^{2+} transport, myofilament Ca^{2+} sensitivity and membrane repolarization. These effects are the result of increased PKA-dependent phosphorylation of proteins. Studies on isolated cardiomyocytes have shown that β -adrenergic stimulation produces a positive inotropic effect and increases I_{Ks} and both of these effects depends on PKA activation [20,21] (Figure 1).

2.2.2. Differential Effects of Cardiac β-AR Subtype Signaling in Regulating a Contractile Response

The β_1-AR couples to G_s protein while the β_2-AR and the β_3-AR couple to both G_s and G_i proteins [22]. The β_2-AR can 'switch' its G protein-coupling preference from G_s to G_i and this is dependent on the phosphorylation of the β_2-AR by PKA [23]. G_i inhibits the activity of the G_s-activated AC in synthesizing cAMP. In cardiomyocytes, β_2-AR-G_i signaling restricts the inotropic response mediated by the G_s-AC-cAMP-PKA signaling cascade [24] (Figure 1). Studies on rabbit, eel, frog, rat, dog, human and other species have found that β_3-ARs generally mediate a negative inotropic effect or are nonfunctional in the heart [14,25]. The G_i and the nitric oxide (NO) pathways have been implicated in the negative effect of the β_3-AR but details of the signal transduction mechanism (e.g. the link between G_i protein-coupling and the production of NO and the number of cell types involved) are still unclear [25].

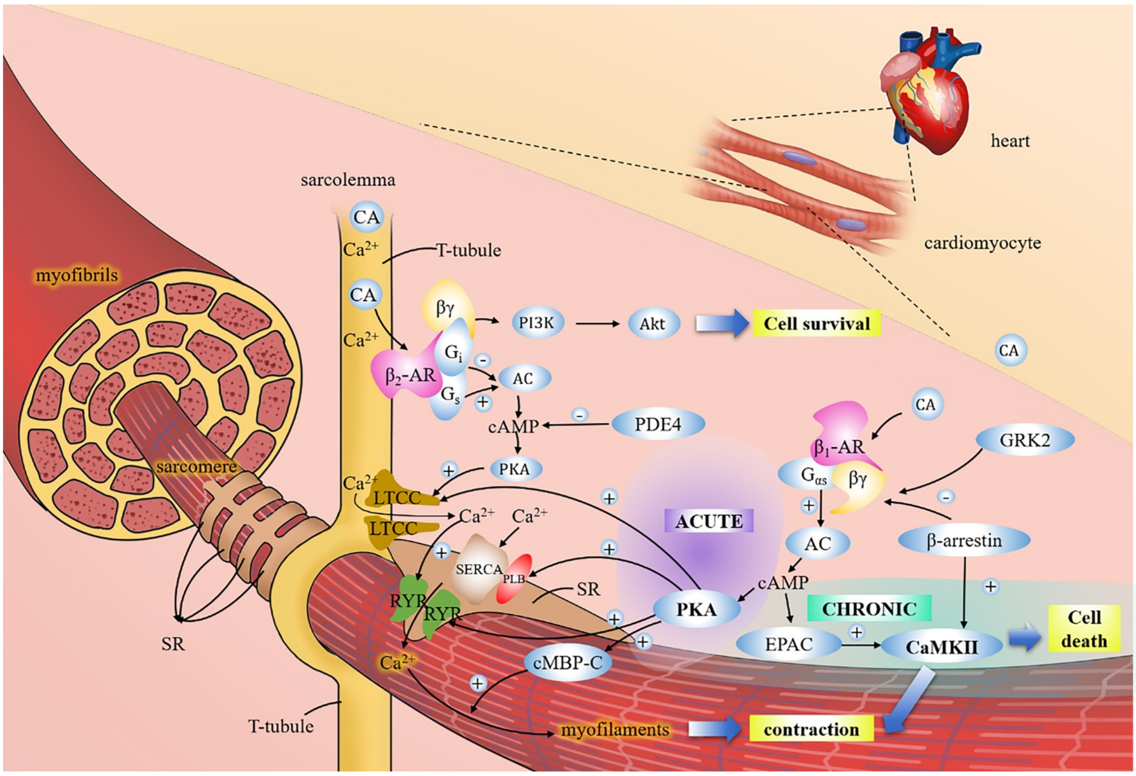

Figure 1. Schematic representation of β-AR signaling in cardiomyocytes. The $β_1$-AR and the $β_2$-AR mediate the biological effects of catecholamines (CA) on cardiomyocytes. These effects include one on contractility enhancement (i. e. a positive inotropic effect) and another one on cell survival and death regulation. Though both of these receptors by means of coupling to G_s mediate a positive inotropic effect, the promiscuously G_i-coupled $β_2$-AR also mediates a negative effect on contractility and a pro-survival effect. The positive inotropic effect of acute $β_1$ or $β_2$ stimulation depends on the activation of PKA and the resultant phosphorylation of proteins (LTCC, PLB, RyR, cMBP-C, etc.) that promote the mobilization and efficiency of cellular Ca^{2+}. The $β_2$-AR-G_s-cAMP signal is compartmentalized near t-tubules and caveolae due to subcellular localization of the $β_2$-ARs and PDE4 (which hydrolyzes cAMP) at these membrane compartments. On the other hand, the $β_1$-AR-G_s-cAMP signal is a global one thanks to the even distribution of $β_1$-ARs on the sarcolemma. 'Chronic' (long-term) stimulation of the $β_1$-AR turns on a $β_1$-AR-CaMKII signal, which mediates both a positive inotropic effect and a pro-apoptotic effect. GRKs and β-arrestins are important regulators of cardiac β-AR-signaling. They take part in homologous desensitization that terminates CA-triggered β-AR-G_s signaling. GRK2 is the most abundant GRK in the heart and has been proven to be a strong negative regulator of β-AR-G_s signaling. The role of β-arrestin is more diverse because β-arrestin also mediates signaling of its own and it has been implicated in the activation of the $β_1$-AR-CaMKII signal as well as in heterologous desensitization of the $β_2$-AR and cardioprotection (not shown). Heart diseases, particularly heart failure, is manifested by excessive CA stimulation and changes of the expression pattern of proteins in this signaling network, causing disturbances on the normal balance of cellular function and well-being. Thus, targeting on the cardiac β-AR signaling provides an important means to treat heart diseases. See the text for details. AC, adenylyl cyclase; Akt, protein kinase B; $β_1$-AR, $β_1$-adrenoceptor; $β_2$-AR, $β_2$-adrenoceptor; βγ, $G_{βγ}$ subunits; CA, catecholamine; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; cAMP, cyclic adenosine monophosphate; cMBP-C, cardiac myosin-binding protein C; EPAC, exchange protein activated by cAMP; GRK2, G protein-coupled receptor kinase 2; $G_{αs}$, α subunit of the stimulatory G protein; G_s, stimulatory G protein; G_i, inhibitory G protein; LTCC, L-type calcium channel; PDE, phosphodiesterase; PI3K, phosphatidylinositol 3 kinase; PKA, cAMP-dependent protein kinase; PLB, phospholamban; RyR, ryanodine receptor; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; SR, sarcoplasmic reticulum.

2.2.3. Pathophysiological Role of Cardiac β-AR Signaling

β-Adrenergic stimulation is a powerful means to increase cardiac output in the fight-or-flight state but if the stimulation is continued, it will cause harm to the heart by promoting detrimental β-AR signaling. Dysregulation of the cardiac β-AR signaling is central to the pathogenesis of heart failure. In compensated heart failure, cardiac output and blood pressure is maintained, despite with a lower cardiac reserve, by activation of neuroendocrine axes, for instance the SNS and the renin-angiotensin-aldosterone system. A sustained increase of catecholamine levels is a leading feature of this mechanism [18]. In the failing hearts, chronic sympathetic stimulation ultimately leads to cardiomyocyte death and maladaptive cardiac remodeling, precipitating decompensation [26].

β_1-AR signaling and β_2-AR signaling are diverged in their effects on cardiomyocyte survival and death. Studies have implicated the central role of the β_1-AR in heart failure. Of particular importance is that chronic stimulation of the β_1-AR activates a cardiotoxic (pro-apoptotic) Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)-dependent signal transduction pathway [27]. In contrast, β_2-AR signaling is largely cardioprotective. In particular, β_2-AR signaling involves a G_i-$G_{\beta\gamma}$-phosphatidylinositol 3 kinase (PI3K) - protein kinase B (Akt) antiapoptotic pathway [28] (Figure 1). This is, however, a greatly simplified picture. As will be discussed in the following section, regulatory modification of β-AR signaling can have profound impacts on the biological effects produced.

Persistent stimulation of the cardiac β-AR induces cardiac hypertrophy and fibrosis and cardiomyocyte apoptosis [29], and these pathological changes are the basis of cardiac remodeling in heart failure. Evidence suggests that paracrine interactions between cardiomyocytes, cardiac fibroblasts and other cell types are key to this process. Cardiac fibroblasts play an important role in cardiac remodeling, fibrosis and hypertrophy due to their involvement in cell proliferation, extracellular matrix production and autocrine/paracrine signaling. A study on a transgenic mouse model has shown that sustained activation of the β_2-AR-G_s-PKA pathway in myofibroblasts promotes cardiac hypertrophy possibly via a paracrine effect [30]. In addition, activation of the β_2-AR in cardiac fibroblasts upregulates the pleotropic pro-inflammatory cytokine interleukin-6, also a hypertrophic factor in the heart, via different signaling mechanisms [31, 32]. Conversely, paracrine factors released from cardiomyocytes in response to sustained β-adrenergic stimulation may promote the growth of cardiac fibroblasts and consequently induce myocardial fibrosis [33]. A recent study has identified the adhesive cell surface protein galectin-3 as a key regulatory component in promoting inflammatory cell mobilization, fibroblast activation and myocardial fibrosis [34]. Galectin-3 is upregulated in cardiac-restricted β_2-AR transgenic mice [34], and its serum levels are also increased in human heart failure [35] and post-myocardial infarction [36]. Another study has found that β-adrenergic stimulation enhances cardiac expression of galectin-3 through the Hippo signaling pathway [37]. Interestingly, β_2-adrenergic stimulation in cardiac fibroblasts alone promotes autophagy and this is correlated with an enhanced degradation of collagen. Thus, β_2-AR signaling in cardiac fibroblasts may actually reduce cardiac fibrosis [38].

2.3. Regulation of Cardiac β-AR Signaling and Its Implications

2.3.1. Upregulation and Downregulation

In heart failure, chronic elevation of catecholamines leads to downregulation of the cardiac β_1-AR while the expression level of the cardiac β_2-AR remains unchanged [39]. Reduced transcription of the β_1-AR is the primary cause of the downregulation [40]. Hence, the ratio of β_1-AR to β_2-AR drops from about 4: 1 in the normal human heart to about 3:2 in the failing heart. This is accompanied by subsensitivity of the β_1-AR and β_2-AR signal transduction pathways, a leading cause of cardiac insufficiency in heart failure [39].

Downregulation of the cardiac β_1-AR is viewed as a protective mechanism to counteract excessive sympathetic stimulation but it also aggravates contractile dysfunction in heart failure. Despite of the downregulation, residual β_1-ARs still mediate a significant cardiotoxic effect of catecholamines. The result is a progressive loss of myocytes and myocardial contractile function in the failing heart [41, 42]. Given the important role of this process in the pathogenesis of heart failure, the use of β_1-AR antagonists becomes logical. Indeed, clinical data have suggested that β_1-AR antagonists not only blocked the cardiotoxic β_1-AR pathway and improved survival in heart failure [43], but also restored the number of downregulated cardiac β_1-ARs and improved cardiac function in the long run [44,45].

Heart failure is also associated with upregulation of other proteins involved in β-AR signaling, for instance G_i proteins and GRK2 [44, 46]. G_i proteins and GRK2 are negative regulators of β-AR-G_s-cAMP signaling in cardiomyocytes (Figure 1). Their upregulation in heart failure contributes to contractile dysfunction as detailed below.

Manipulation of β-AR levels in genetic animal models has provided important insights into the role of β

-ARs in heart failure. In cardiac-specific overexpression mouse models, β_2-ARs at moderate levels (30-fold over normal) reduced the incidence of heart failure while β_2-ARs at high levels (300-fold or higher) led to heart failure [47,48]. Similarly, overexpression of the β_1-AR in mouse hearts for 15-fold led to spontaneous heart failure and lower levels of the β_1-AR (5-fold) led to cardiac hypertrophy, consistent with the detrimental effect of persistent β_1 stimulation [49].

2.3.2. Desensitization

Upon β-adrenergic stimulation, β-AR signaling is attenuated through cooperative actions of a series of regulatory molecules. This regulatory process, known as desensitization, reduces the responsiveness of a GPCR to continuous or repeated stimuli. In one of the most well-characterized processes called homologous desensitization, activation of β-ARs (β_1 and β_2) promotes their own phosphorylation by GRKs at specific sites on their CTs. β-Arrestins are then recruited to the phosphorylated receptor sites to form complexes with the β -ARs. β-Arrestins sterically inhibit the coupling of β-ARs with G proteins, thus weakening G protein signaling. They also interact with proteins in the endocytic machinery such as clathrin to initiate receptor internalization. Internalized receptors are degraded or recycled, and thus losing their abilities temporally or permanently to interact with agonists. Agonist-induced receptor internalization is more rapid and obvious in β_2-ARs, but less sensitive in β_1-ARs [50]. Overall, homologous desensitization reduces G protein-mediated signal transduction, and is believed to produce a beneficial effect on the failing heart by restricting the β_1-AR-mediated cardiotoxic pathway [22], but it may also, to a lesser extent, contribute to the functional decline of the failing heart by causing β_2-AR subsensitivity [39].

Apart from GRKs, phosphorylation of GPCRs by second messenger-dependent protein kinases (e. g. PKA and protein kinase C (PKC)) provides another means for GPCR signal regulation, that is, heterologous desensitization. The β_2-AR is among the most well-studied GPCRs in the process of heterologous desensitization. Stimulation of G_s protein- or G_q protein-coupled receptors promotes phosphorylation of the β_2-AR by PKA and PKC, respectively, leading to functional uncoupling of the β_2-AR from the G_s protein-mediated pathway without receptor internalization [51]. Heterologous desensitization of the β_2-AR is a common mechanism for the suppression of β-AR-stimulated myocyte contractile function by different hormones [51]. Importantly, it is believed to play a major role in causing β_2-AR subsensitivity in heart failure due to upregulation of G_i proteins and, hence, augmentation of the inhibitory effect on G_s-cAMP signaling [44]. Studies have shown that other proteins (e. g. PDE4D and β-arrestin2, see below) also participate in heterologous desensitization of the β_2-AR [51].

2.3.3. Spatial Distribution

Cardiomyocytes display a highly sophisticated membrane structure. Small pits (caveolae) and invaginations (transverse or T-tubules) exist on the surface of the plasma membrane of a cardiomyocyte. T-tubules extend from the cell surface deep into the cell and connected with longitudinal tubules to form the transverse-axial tubules system, which serves to conduct action potential and extracellular Ca^{2+} to the vicinity of each of the smallest unit of contraction – the sarcomere. Integrity of this membrane structure is critical for spatial synchronization of calcium-induced calcium release and, hence, coordinated contraction of sarcomeres throughout the cardiomyocyte upon induction by an action potential. Heart failure is manifested by an aberrant change of this membrane structure and relocation of proteins in it. This can cause important changes on the compartmentalized signals of cAMP and Ca^{2+} as discussed below. In late-stage heart failure, disordered Ca^{2+} waves occur more readily in cardiomyocytes, promoting arrhythmogenesis [52].

In a healthy cardiomyocyte, β_1-ARs are evenly distributed on the sarcolemma while β_2-ARs are located on T-tubules and caveolae [53]. In addition, β_2-ARs are generally located within signal complexes containing LTCC, PDEs, G_i, and other proteins involved in signal transduction [51, 54, 55]. In particular, PDE4 limits diffusion of cAMP, so that the β_2-AR-mediated cAMP signal is generally confined to the vicinities of T-tubules and caveolae as opposed to the β_1-AR-mediated cAMP signal which is global [52,54] (Figure 1). Our recent study has found that β_2-adrenergic stimulation transforms a β_1-AR-mediated cAMP signal from the global mode to the local mode at nanometer level [56]. We call this PDE4-dependent β_2-AR signal localization spillover effect 'offside' compartmentalization. It prevents the β_1-stimulated PKA activity from acting on RyRs adjacent to activated β_2-ARs, which may to some extent limit the β_1-AR-mediated cardiotoxicity and sharpen the transient β_1-AR response of sympathetic stimulation. Moreover, the β_2-AR is directly associated with an LTCC in a signal complex that also contains a G_s protein, AC, PKA, and the counterbalancing phosphatase PP2A (type 2A protein phosphatase) [57]. Thus, the compartmentalized (offside or otherwise) β_2-AR signaling is uncoupled from a global cAMP signal and phosphorylation of most PKA substrates in a cardiomyocyte but can still effectively enhance $I_{Ca,L}$ and a contractile response [58].

Heart failure is characterized by progressive loss of T-tubules in cardiomyocytes. β_2-ARs originally located on T-tubules are relocated to cell crests of cardiomyocytes. This leads to the loss of cAMP compartmentation with the production of a cAMP signal similar to the one elicited by β_1 stimulation [52]. LTCCs also exhibit similar relocation from T-tubules to cell crests in heart failure and this change has been implicated as a pathological basis for arrhythmogenesis at single cell level [59]. Moreover, β_2-ARs and LTCCs have been modelled to locate in cell crests and T-tubules of a cardiomyocyte in silico, which reveals enhanced phosphorylation of LTCC by PKA and, hence, increased $I_{Ca,L}$ when they are present in cell crests [60]. Thus, spatial redistribution of cardiac β_2-ARs in heart failure may enhance the pro-arrhythmogenic effect of β -AR-cAMP signaling.

2.3.4. Other Regulatory Effects of GRKs and β-Arrestins

GRKs and β-arrestins can regulate GPCR signaling by mechanisms other than homologous desensitization. The GRK family regulates various GPCRs. Among them, GRK2, GRK3 and GRK5 are highly expressed in the human heart. GRK2 and GRK5 are expressed in almost all cardiac cells and upregulated in heart failure, whereas GRK3 is detected only in cardiomyocytes [61]. GRK2 and GRK5 phosphorylate different sites on the CT of the β_2-AR and this has biological implications [62]. GRK3 phosphorylates α_1-ARs in the heart and plays no role on β-AR signaling [63].

Numerous studies have pinpointed GRK2 as a main culprit for cardiodepression, cardiac hypertrophy and maladaptive cardiac remodeling in the pathogenesis of heart failure [64–70], as upregulation of GRK2 occurs early in human heart diseases and is partly responsible for the diminished responses to adrenergic stimulation [71]. Particularly, in a murine model of pressure overload-induced heart failure, cardiac-specific overexpression of β_2-ARs with deletion of all PKA phosphorylation sites (β_2-AR PKA-) showed earlier disease-onset, more severe structural and functional cardiac damage and higher cardiac expression of GRK2 and G_i proteins as compared with mice overexpressing wild-type human β_2-ARs or β_2-ARs with deletion of all GRK phosphorylation sites [64]. Importantly, the blunted β-AR-mediated positive inotropic effect in the β_2-AR PKA- mice could be rescued by the G_i inhibitor pertussis toxin. These results indicate that GRK2-induced phosphorylation of β_2-ARs results in G_i-biased β_2-AR signaling, linking upregulation of GRK2 to cardiodepression, maladaptive cardiac remodeling and heart failure. In heart failure, the GRK2-promoted G_i signaling not only inhibits the β_2-AR-mediated positive inotropic effect but also cross-inhibits the β_1-AR-mediated positive inotropic effect. Increased cardiac expression of G_i plays a central role in the cross-inhibition. Recent structural biology data have revealed the structural basis of receptor-coupling to different G proteins by both the β_1- and β_2-AR [72, 73]. Under normal conditions, the coupling preference to G_i is much lower than that to G_s for both β-AR subtypes. Nevertheless, current evidence is in favor that the β_2-AR-G_i pathway plays an important role in the pathophysiology of human heart failure and Takotsubo syndrome [44,74, 75]. Activation of this pathway induces cardiodepression, but at the same time produces a cardioprotective effect [74, 75], corroborating the perception that β_2-AR-G_i signaling is equivalent to an endogenous β_1-AR antagonist in the heart [31]. Similar to the use of β-blockers in heart failure where low doses of them improve cardiac function in the long run whereas high doses of β-blockers induce acute cardiac decompensation, therefore, the GRK2-promoted β_2-AR-G_i signaling needs to be kept in check to avoid functional impairment while allowing delivery of a right amount of protective signals to the failing heart.

Increased levels of GRK5 in the failing hearts has been suggested to be a maladaptive mechanism due to the key role of GRK5 in cardiac hypertrophy [76,77]. Interestingly, carriers of the GRK5-Leu41 allele, which confers more β -AR desensitization and protection against catecholamine-induced cardiomyopathy in mice, have lower mortality than the wild-type GRK5-Gln4 allele carriers in African Americans with heart failure or cardiac ischemia [78]. Additionally, GRK5 has been implicated in β_1-AR-mediated β-arrestin2 signaling and the resultant cardiac fibrosis-inducing effect of the β-blocker metoprolol in wild-type mice and in neonatal rat cardiomyocytes [79]. In this study, the β-blocker acted as an antagonist for the G protein-dependent pathway and as an agonist for the β-arrestin-dependent pathway. Phosphorylation of β_1-ARs by GRK5 is the trigger for the recruitment of β-arrestins that set off signal transduction (presumably via activation of ERK) in a G protein-independent manner. This represents a classical example of β-arrestin-biased signaling, and metoprolol is defined as a β-arrestin-biased ligand in this paradigm. However, it should be note that β-blockers atenolol, metoprolol and propranolol also prevent cardiac fibrosis induced by β-adrenergic stimulation in cardiomyocytes [33]. The β-blocker carvedilol has also been suggested to be a β-arrestin-biased ligand at the β_1-AR and the GRK5/GRK6-dependent β-arrestin-biased signaling it induced is protective to cardiomyocytes via upregulation of multiple cardioprotective microRNAs [80]. GRK5 exhibits both harmful and protective effects on the heart, which needs to be further studied.

Interestingly, GRK2 and β-arrestin1 have been found to be involved in the offside compartmentalization effect of β_2-AR stimulation on β_1-AR signaling [56]. Activation of the β_2-AR leads to the translocation of GRK2 to the plasma membrane. GRK2 can also act on a β_1-AR in close proximity to the activated β_2-AR. Phosphorylation of the β_1-AR at the CT by GRK2 then triggers recruitment of β-arrestin1 to the β_1-AR, and the β-arrestin1 subsequently attracted PDEs to come close to the β_1-AR. The latter limits β_1-AR signaling by hydrolyzing cAMP. In contrast, heterologous desensitization of the β_2-AR by activation of other GPCRs involves β-arrestin2 but not GRKs. Phosphorylation of the β_2-AR by second messenger kinases likely triggers recruitment of β-arrestin2 that brings along PDE4D to limit β_2-AR-cAMP signaling [51].

2.3.5. CaMKII

CaMKII is an important molecule for the transmission of Ca^{2+} signals. Four isoforms of CaMKII exist, in which the δ isoform is the major one in mammalian hearts. CaMKII positively regulates Ca^{2+} signaling in cardiomyocytes through phosphorylation of LTCCs, RyRs and PLB [81,82]. Knockout of CaMKIIδ decreases β-AR-stimulated $I_{Ca,L}$ response in ventricular myocytes and reduces cardiac reserve and heart rate in response to β-adrenergic stimulation in mice, suggesting that CaMKIIδ is involved in the β-AR-mediated cardiac stimulatory effect [83].

We have found that β_1-adrenergic stimulation produces an early but rapidly desensitized cAMP-PKA signal, followed by a slow-onset but sustained CaMKII signal and both of these signals could facilitate biological processes such as SR Ca^{2+} refilling in cardiomyocytes [84]. Recently, a negative feedback mechanism on CaMKII activity regulation was identified in cardiomyocytes, in which CaMKII phosphorylates and activates PDE4D, causing a reduction in cAMP signaling which positively regulates CaMKII activity [85]. This may provide a mechanistic explanation for the rapid desensitization of the β_1-AR-cAMP signal in our observation. Our study suggests that the β_1-AR-mediated myocardial contractile response is sustained by switching from cAMP-PKA signaling to CaMKII signaling upon prolonged stimulation [84] (Figure 1).

Activation of CaMKII has been implicated in the detrimental effects of β-AR signaling on the heart. Particularly, sustained β_1-adrenergic stimulation has been shown to induce cardiomyocyte apoptosis through a CaMKII-dependent but PKA-independent pathway [86]. The involvement of CaMKII in β-AR-mediated cardiomyocyte apoptosis has also been demonstrated in vivo [87]. Moreover, CaMKIIδ also participates in β-AR-mediated SR Ca^{2+} leak in cardiomyocytes by enhancing RyR2 phosphorylation and this mechanism may be involved in the induction of cardiomyopathy in mice subjected to chronic catecholamine stimulation [82]. The β_1-AR-activated CaMKII signal is also implicated in catecholamine-induced cardiac hypertrophy in neonatal rat cardiomyocytes [88]. In the failing hearts, activated CaMKII phosphorylates RyRs and subsequently promotes SR Ca^{2+} leakage, delayed afterdepolarization and tachycardia [89]. Inhibition of CaMKII but not PKA decreases RyR2 activity and arrhythmogenic Ca^{2+} release in response to β-adrenergic stimulation [90,91].

Different signal transduction mechanisms have been proposed to relay sustained β-adrenergic stimulation to the activation of CaMKII. These mechanisms may be categorized according to the involvement of exchange protein activated by cAMP (Epac)-dependent and/or PKA. Epac is a signaling molecule responsive to intracellular cAMP elevation independent of PKA [92, 93]. In one of the most extensively studied Epac-dependent mechanism, stimulation of the β_1-AR induces the formation of a complex containing β-arrestin, Epac1 and CaMKII, leading to CaMKII activation [94]. It is proposed that the β-arrestin that binds to the CT of the β_1-AR assumes a specific conformation which keeps CaMKII and Epac in a stable complex with the receptor, whereas the conformation of the β_2-AR-bound β-arrestin is incapable of recruiting CaMKII and Epac. This explains why the β_1-AR, but not the β_2-AR, is the relevant receptor for activating CaMKII signaling [94]. Further studies in cardiomyocytes suggests that β-AR-CaMKII signaling involves NO, and a β-AR-cAMP-Epac-PI3K-Akt-NOS1-CaMKII signaling cascade has been proposed to link β-adrenergic stimulation to increased SR Ca^{2+} leaks [95,96]. In the PKA-dependent mechanism, increased $[Ca^{2+}]_i$ leads to CaMKII activation and the source of Ca^{2+} is cAMP-PKA-PLB-SR-mediated Ca^{2+} pool refilling [97]. This pathway has been shown to transmit a pro-cell death signal of β-adrenergic stimulation in cardiomyocytes. Interestingly, the same study has also discovered a cardioprotective β_1-AR-cAMP-Epac2-Rap1-Rac-ERK pathway. The regulatory effects of PKA on CaMKII expression and activity have also been demonstrated in vivo in transgenic mice with cardiac-specific overexpressed PKI (a peptide inhibitor of PKA). In particular, acute β -stimulation-induced CaMKII signaling that leads to heart rate and cardiomyocyte contractility increment was sensitive to PKI [20]. A recent study has proposed yet another mechanism of β_1-AR-stimulated CaMKII activation in cardiomyocytes that involves PKA, GRK5, SAP97, β-arrestin2 and Epac2 [27].

Recent experimental progress also holds promise for CaMKII inhibition in the treatment of heart failure and related myocardial abnormalities [98–100].

2.3.6. Other Regulatory Mechanisms of β-AR Signaling in the Heart

Acute β-adrenergic stimulation with dobutamine produced positive inotropic effects in mouse hearts while sustained β-adrenergic stimulation with isoproterenol resulted in cardiac hypertrophy in mice. Both effects could be attenuated in transgenic mice lacking B56α, a PP2A regulatory subunit highly expressed in the heart, indicating that PP2A is a potential regulator of cardiac function after β-AR agitation [101]. Another study has found that overexpression of the Raf kinase inhibitor protein produced a well-tolerated, persistent increase in cardiac contractility mediated by the β_1-AR via simultaneous activation of the β_2-AR subtype [102]. Signal transducers and activators of transcription 3 has been suggested to be a key transcriptional regulator of β-AR-mediated cardiac stress adaptation, pathological remodeling and heart failure [103]. Recently, we have found that 5-HT_{2B} receptors formed heterodimers with β_2-ARs, promoting cardioprotective G_i signaling with β_2-adrenergic stimulation [2]. We have also found that β_1-ARs and receptors of advanced glycation end products physically interacted with each other. Stimulation with either of their agonists generated a pro-cell death CaMKII signal with significant signal crosstalk, leading to cardiomyocyte injury and cardiac remodeling [104]. The G protein-coupled estrogen receptor 1, a G_i protein-coupled receptor, has been suggested to moderate myocardial Ca^{2+} dynamics and cardiomyocyte contraction by limiting β_1-AR-cAMP-PKA signaling [105]. This study may provide a mechanistic basis for the effects of estrogen on cardiac function regulation, and possibly cardioprotection.

Non-coding micro single-stranded RNA (microRNA, miRNA) is a class of small-molecular transcription regulator. Studies have shown that miR-133 regulates multiple components of β_1-AR signaling and has a cardioprotective effect by reducing apoptosis and fibrosis in pressure overload-induced heart failure [106]. miR-145 improves cardiac dilatation and fibrosis and alleviates heart failure-related cardiac remodeling by upregulating β_2-ARs and downregulating CaMKII [107]. Downregulation of non-coding RNA circ-HIPK3 attenuates fibrosis and maintains myocardial function after myocardial infarction in mice through miR-17-3p [108].

3. Interventions of β-AR Signaling and Cardiovascular Disease

3.1. β-Blockers

β-Blockers are widely used in tachyarrhythmias, coronary artery disease, hypertension and heart failure. Their use is considered to be a milestone in the treatment of heart failure [109]. Over the past six decades, three generations of β-blockers have been developed: the first generation nonselective β-blockers, the second generation cardioselective β-blockers (selectively antagonizing the β_1-AR), and the third generation vasodilating β-blockers (exhibiting vasodilating effects as a result of α_1-AR blockade or endothelial nitric oxide synthase (eNOS) activation via β_2-AR or β_3-AR agonism). Clinical studies have shown that the cardioselective β-blockers bisoprolol and metoprolol and the vasodilating β-blockers carvedilol and nebivolol significantly reduce morbidity and mortality from heart failure [110]. The reason causing the different efficacies of β-blockers in heart failure treatment is not totally clear, but it is quite certain that the side effect profiles of nonselective β-blockers are the key issue disfavoring their long-term use in heart failure and essential hypertension.

Propranolol, which has been widely used in clinical practice to treat arrhythmias, is a representative of the first generation β-blockers. They have similar affinities for the β_1-AR and the β_2-AR, so they are called nonselective β-blockers. Blocking of the β_1-AR in the heart is beneficial in both heart failure and myocardial ischemia [111], but blocking of the β_2- or β_3-AR is not beneficial because it antagonizes β_2-AR-mediated bronchodilation and the mechanisms of cardiac protection and vasodilation mediated by both the β_2- and β_3-AR [28, 112]. In blood vessels, β_2- and β_3-ARs are present in vascular smooth muscle cells and endothelial cells and promote vasodilation through the eNOS-NO-cGMP-protein kinase G pathway. The first generation β -blockers may cause fatal side effects such as bronchospasm due to the antagonism of the β_2-AR. Therefore, the use of these blockers is strictly prohibited in patients with asthma. A recent experimental study has suggested that propranolol may be useful in the treatment of congenital heart disease by promoting cardiomyocyte cytokinesis in neonates and could, therefore, increase the number of cardiomyocytes and confer benefit after myocardial infarction in adulthood [113].

In order to find drugs that avoid the bronchial side effects of the first generation β-blockers, the second generation β-blockers have been developed. The most representative drugs are atenolol and metoprolol [114, 115]. Their affinities for the β_1 subtype are higher than those for the β_2 and β_3 subtypes. At low doses, they inhibit β_1-AR-mediated effects in the heart, but not β_2-AR-mediated vasodilation or bronchodilation. At higher doses, the selectivity to the β_1-AR is lost. Therefore, the use of the second generation β-blockers should still be considered with caution in patients with respiratory diseases.

In addition to its effects on cardiomyocytes, new research has shown that metoprolol also acts on the hematopoietic system. Metoprolol reduced neutrophil infiltration by blocking the β_1-ARs on neutrophils. In the absence of neutrophils, metoprolol lost its protective effect on acute myocardial infarction, indicating that neutrophils are one of the targets of metoprolol in the treatment of acute myocardial infarction [116]. Other studies have shown that the functional improvement of metoprolol after myocardial infarction is dependent on the β_3-AR [117]. Therefore, patients with β_3-AR dysfunction may be insensitive to metoprolol. In addition, the treatment benefit of metoprolol is not correlated with a change in CaMKII activity in experimental and human heart failure [118]. Therefore, direct inhibition of CaMKII on top of β_1-AR blockade might bring additional benefit to heart failure therapy.

The third generation β-blockers are drugs with vasodilating properties. This vasodilating activity is beneficial because it reduces peripheral vascular resistance and lowers blood pressure while maintaining or improving cardiac output and left ventricular function. Carvedilol and labetalol produce their vasodilating effects by antagonizing the α_1-AR in vascular smooth muscle cells. Nebivolol produces NO-mediated vasodilation by means of activating the β_2- and/or β_3-ARs in endothelial cells [119]. Vasodilating β-blockers also have neutral (labetalol and nebivolol) or beneficial (carvedilol) effects on glucose and lipid metabolism, whereas non-vasodilating β-blockers tend to have negative effects on glucose and lipid metabolism [120].

Carvedilol has the most evidence for reducing morbidity and mortality in patients with ischemic heart failure [110]. Its effectiveness might be due to its unique biological activity. Carvedilol possesses an antioxidant activity, and could prevent cardiomyocyte apoptosis induced by doxorubicin and the reduction of β_1-AR expression induced by hydrogen peroxide or doxorubicin [121]. One possible mechanism is that carvedilol contains a large aromatic amine substituent that is not present in other β-blockers. This large group forms a unique contact with the β_1-AR ligand binding pocket, causing a conformational rearrangement on the extracellular surface, thus burying the redox-sensitive disulfide bond in the receptor structure. Another possible explanation for its beneficial effect on heart failure is that carvedilol promotes the accumulation of N-terminal truncated β_1-ARs, which constitutively (without ligand induction) activate non-classical G_i protein-mediated Akt signaling in carvedilol-treated cardiomyocytes [121]. Unlike the classical β_1-AR-coupled G_s protein-mediated signal transduction pathway, this study and some others suggest that carvedilol promotes the recruitment of G_i proteins to the β_1-AR (but not β_2-AR) and initiates unique signal transduction

[80,122]. Carvedilol also reversed the deterioration of cardiac function in diabetes which seems to be related to β-arrestins [123].

Specific blocking of the β_1-AR in Golgi bodies of cardiomyocytes has recently been found to prevent cardiac hypertrophy [124], which has a potential to be developed into new-generation β-blockers.

β-Blockers differ a lot in their pharmacological properties, for instance receptor subtype specificity, intrinsic sympathomimetic activity, membrane-stabilizing activity, lipophilicity, side effects and effectiveness in treating heart failure. Thus, they need to be studied individually rather than by category [125].

3.2. Other Potential β-AR-related Therapies

Recent evidence has shown that β_2-AR agonists may be potentially useful in cardiovascular diseases [126,127]. Moreover, β_2-AR agonists with high specificity and activities have been designed and synthesized continuously [128,129]. Biased ligands and positive or negative allosteric modulators for β-ARs have also been designed and reported [130 – 136]. In addition, some researchers have activated β_2-AR-mediated β-arrestin-biased signaling by expressing allosteric regulatory peptides without the participation of agonists, and have confirmed the protective effect of this signal on cardiomyocytes [137]. It should be noted that long-term use of β_2-AR agonists has been shown to increase the risk of heart failure in some meta-analysis although the overall cardiovascular safety of β_2-AR agonists has been confirmed in many studies [138]. Whether β_2-AR ligands should be used alone or in combination with other drugs such as β_1-AR blockers in treating cardiovascular disease requires more clinical validation.

In addition, gold nanoparticles, a widely used drug transport carrier, have a property to accumulate in the heart through blood circulation, and have been found to play an anti-myocardial hypertrophy role by downregulating the β_1-AR and its downstream ERK pathway [139]. Thus, gold nanoparticles may be a potential multifunctional material for drug transport and anti-myocardial hypertrophy therapy. One possibility is to use gold nanoparticles to deliver small molecule inhibitors, microRNAs or gene therapy to combat the deleterious effect of GRKs in heart diseases [61]. Other new development includes light-controlled drugs with a high level of β_1-/β_2-AR selectivity [140]. Given the presence of multiple phosphorylation sites on GPCRs that are associated with distinct signal transduction pathways, mechanisms that generate different receptor phosphorylation patterns may be exploited for the development of pathway-selective drugs.

4. Future Prospects

Cardiovascular disease is a leading public health problem worldwide. In particular, heart failure is one of the most common complications of cardiovascular disease and an important cause of hospitalization and immature death. There is a large and growing demand of better and more personalized cardiovascular drugs. β-AR signal transduction plays an important role in cardiac physiology and the pathophysiology of heart failure, and it is also a therapeutic target with a long history. Technological advances, especially functional genomics and structure-based drug discovery, have enabled the continual discovery of existing and novel GPCR targets (i.e. orphan receptors) and GPCR-targeting drugs. Especially, emerging studies have suggested that biased GPCR signaling may be exploited as new therapeutic targets. Recently, the structural biology approach has begun to illuminate the structural basis of GPCR activation by different ligands or allosteric modulators, and hold promise for elucidating the structure-function relationships of GPCR signal complexes. It is anticipated that new drugs based on these structural understandings will be developed in the near future. Therefore, new disease models and transgenic systems need to be created along the way to promote basic research on cardiovascular science and drug development.

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