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REVIEW

Current and future G protein-coupled receptor signaling targets for heart failure therapy

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Abstract: Although there have been significant advances in the therapy of heart failure in recent decades, such as the introduction of β-blockers and antagonists of the renin–angiotensin–aldosterone system, this devastating disease still carries tremendous morbidity and mortality in the western world. G protein-coupled receptors, such as β-adrenergic and angiotensin II receptors, located in the membranes of all three major cardiac cell types, ie, myocytes, fibroblasts, and endothelial cells, play crucial roles in regulation of cardiac function in health and disease. Their importance is reflected by the fact that, collectively, they represent the direct targets of over one-third of the currently approved cardiovascular drugs used in clinical practice. Over the past few decades, advances in elucidation of the signaling pathways they elicit, specifically in the heart, have led to identification of an increasing number of new molecular targets for heart failure therapy. Here, we review these possible targets for heart failure therapy that have emerged from studies of cardiac G protein-coupled receptor signaling in health and disease, with a particular focus on the main cardiac G protein-coupled receptor types, ie, the β-adrenergic and the angiotensin II type 1 receptors. We also highlight key issues that need to be addressed to improve the chances of success of novel therapies directed against these targets.

Keywords: heart failure, G protein-coupled receptor, signaling, cardiac, therapeutic target

Introduction

Heart failure (HF) is a complex pathophysiological syndrome that arises from a primary defect in the ability of the heart to fill and/or eject blood sufficiently. The clinical manifestations of HF result from the primary myocardial insult (most commonly coronary artery disease, hypertension, or genetic factors) and the attendant sequelae. In general, the primary insult brings about an increase in myocardial wall stress that induces an orchestrated cascade of remodeling stimuli within the heart, as well as neurohormonal, vascular, renal, and skeletal muscle alterations. Within this conceptual framework, chronic HF is generally a progressive disorder that results from continued left ventricular (LV) remodeling and a progressive loss of function. It should be noted that abnormalities of systolic and/or diastolic function can result in similar symptoms and they might share some common underlying mechanisms. It is estimated that symptomatic HF currently affects 0.4%–2% of the general population in the western world.[1–3] Importantly, however, the incidence of symptomatic HF rises substantially with increasing age; estimates of symptomatic HF prevalence for individuals over 65 years of age range between 6% and 10%.[1,4,5] Up to 50% of patients diagnosed with HF will die within 4 years, and for patients with end-stage HF, the 1 year survival rate is 50% – worse than most advanced malignancies.[6,7]

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The most important neurohormonal receptors that regulate cardiac function and physiology belong to the superfamily of G protein-coupled receptors (GPCRs) (or seven transmembrane-spanning receptors [7TMRs]).[8] For instance, cardiac function (contractility) is tightly controlled by the activity of β-adrenergic receptors (β_1- and β_2ARs) located in the membranes of cardiac myocytes.[9] Cardiac structure and morphology are regulated by angiotensin II (AngII) type 1 receptors (AT_1Rs) present (mainly) in cardiac fibroblast and endothelial cell membranes, but also, to a lesser extent, in cardiomyocyte membranes.[8] Heart rate (HR) is modulated by the balance between the activities of β-adrenergic and muscarinic cholinergic (mAChR) receptors located in various anatomical segments of the cardiac electrical conduction system.[8,9] Furthermore, even the neurohormonal control of the circulatory system, whether it be catecholamine and corticosteroid release by the adrenal glands or activation of the renin–angiotensin–aldosterone system (RAAS) by the juxtaglomerular apparatus of the kidneys or release of neurotransmitters by central and peripheral neurons innervating cardiovascular organs, is under tight regulation by various GPCRs (eg, α_2ARs) as well.[8,9] Thus, given that signaling from all these cardiac GPCRs constitutes an integral part of regulation of cardiac function, it comes as no surprise that drugs directly targeting (ie, binding) these receptors represent over one-third of currently used cardiovascular drugs in clinical practice, and the vast majority of currently approved HF drugs target GPCR function and signaling in one way or another.[8,9] However, there is still an enormous potential for development of novel HF therapies targeting these receptors, either directly (ie, the GPCR per se) or some other signaling molecule down the pathway the receptor activates. The cloning and molecular and structural characterizations of GPCRs, highlighted by last year's Nobel prize in chemistry award to the two pioneers of the field, Bob Lefkowitz and Brian Kobilka,[10] has spurred many significant advances in delineation and understanding of cardiac GPCR signaling in health and disease over the past couple of decades. The present review will discuss, receptor and signaling molecule type-by-type, all the important findings in the field of cardiac GPCR signaling that can be harnessed for development of novel HF therapeutics, and will also highlight the salient issues that complicate exploitation of these GPCR signaling targets for future HF clinical therapies.

βAR signaling targets for HF therapy

The sympathetic nervous system (SNS) neurotransmitters norepinephrine (NE) and epinephrine (Epi) mediate their effects in cells and tissues by binding to specific cell surface adrenoceptors (ARs), three α_1ARs, three α_2ARs, and three βARs (β_1, β_2, β_3).[9] All ARs primarily signal through heterotrimeric G proteins. The human heart contains all three βAR subtypes.[9] β_1AR is the predominant subtype in the (normal, healthy) myocardium, representing 75%–80% of total βAR density; followed by β_2AR, which accounts for about 15%–18% of total cardiomyocyte βARs; and the remaining 2%–3% is β_3ARs (under normal conditions).[9,11] The principal role of βARs in the heart is the regulation of cardiac rate and contractility in response to NE and Epi. Stimulation of β_1ARs (mainly) and of β_2ARs (to a lesser extent) increases cardiac contractility (positive inotropic effect), frequency (positive chronotropic effect), and rate of relaxation (lusitropic effect), and accelerates impulse conduction through the atrioventricular node (positive dromotropic effect) and pacemaker activity from the sinoatrial node.[9] β_3ARs are predominantly inactive during normal physiologic conditions;[12] however, their stimulation seems to produce a negative inotropic effect opposite to that induced by β_1ARs and β_2ARs, involving the nitric oxide synthase (NOS) pathway,[13] thus acting as a "fuse" against cardiac adrenergic overstimulation.[14] The most powerful physiologic mechanism to increase cardiac performance is activation of cardiomyocyte β_1ARs and β_2ARs, which, in turn, activates G_s proteins (stimulatory G proteins). G_s protein signaling stimulates the effector adenylate cyclase (AC), which converts adenosine triphosphate (ATP) to the second messenger adenosine 3′,5′-monophosphate or cyclic AMP (cAMP), which in turn binds to and activates the cAMP-dependent protein kinase (protein kinase A [PKA]).[9] PKA is the major effector of cAMP and, by phosphorylating a variety of substrates, it ultimately results in a significant raise in free intracellular Ca^{2+} concentration, which is the master regulator of cardiac muscle contraction (Figure 1).[15] Of note, PKA can phosphorylate the βARs themselves (and other GPCRs) in the heart, causing G protein uncoupling and functional desensitization of the receptor (heterologous or agonist-independent desensitization).[16] Given that cAMP and PKA augment cardiac contractility, drugs that enhance signaling through these molecules (such as inhibitors of cAMP-specific phosphodiesterase, an enzyme that degrades cAMP and reduces PKA activation) have been developed for HF (Figure 1).[17] Although they might be useful for acute decompensated HF, when acute increases in contractility are needed to sustain life, these drugs increase mortality in human HF in the long run (possibly because they increase cardiac workload and oxygen demand) and are nowadays contraindicated in chronic HF.[17] This is entirely consistent

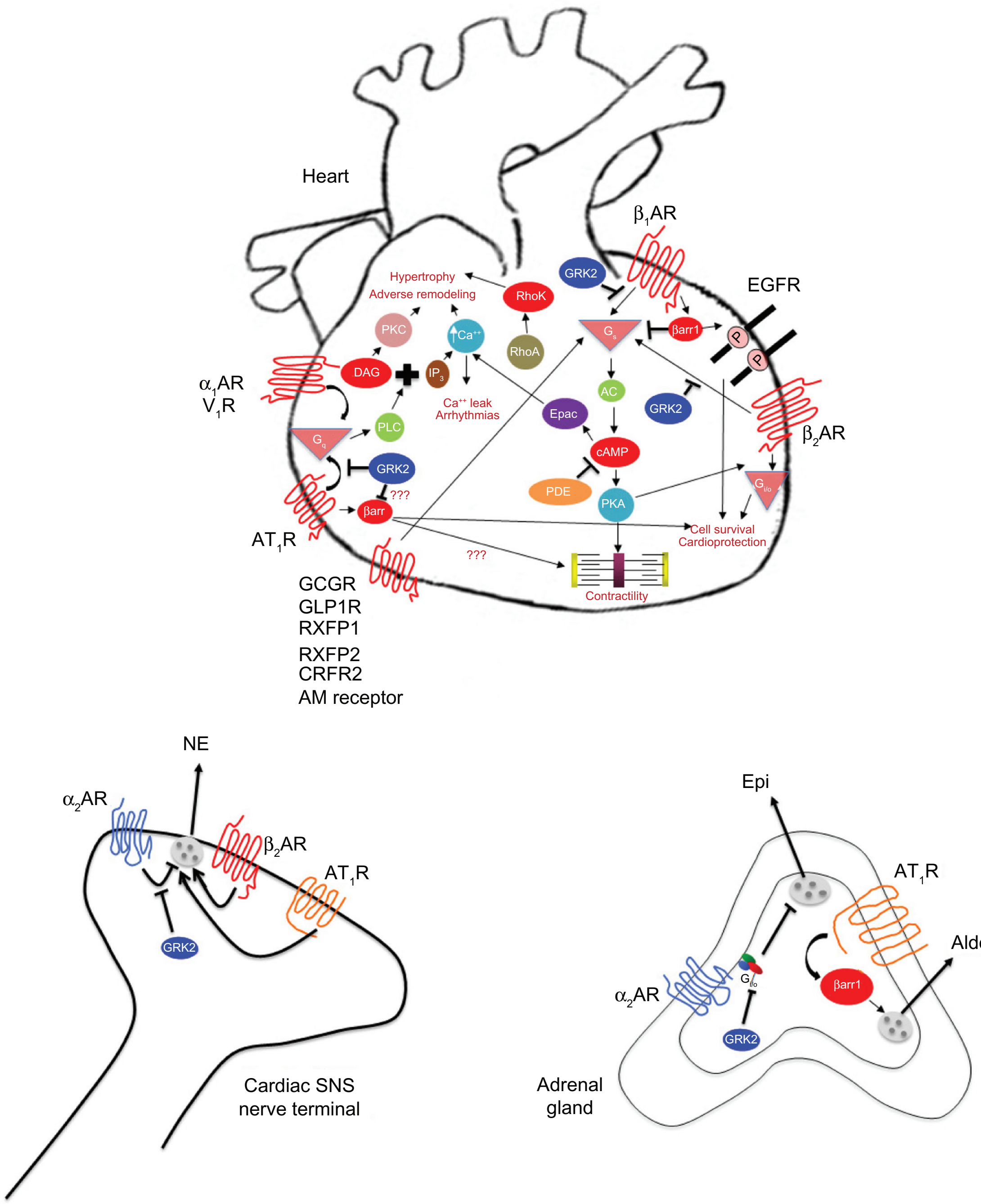

Figure 1 G protein-coupled receptors and their signaling pathways involved in heart failure pathophysiology.

Abbreviations: Aldo, aldosterone; AM, adrenomedullin; AR, adrenoceptor; AT_1R, angiotensin II type 1 receptor; CRFR, corticotrophin-releasing factor receptor; DAG, 2-diacylglycerol; EGFR, epidermal growth factor receptor; Epi, epinephrine; GCGR, glucagon receptor; GLP1R, glucagon-like peptide-1 receptor; GPCRs, G protein-coupled receptors; GRK, GPCR kinase; NE, norepinephrine; PDE, phosphodiesterase; RXFP, relaxin family peptide; SNS, sympathetic nervous system; ???, indicates effect currently under investigation; βarr, beta arrestin; G_s, stimulatory G protein; G_i/G_o, inhibitory/other G protein; Rho, Ras homolog gene family member A; Epac, exchange protein directly activated by cAMP; AC, adenylate cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; V1R, vasopressin receptor type 1; RhoK, Rho-dependent kinase.

with the notion that chronic cardiac β_1AR activation is detrimental and pro-apoptotic in the heart (see below).

Importantly, the β_2AR also mediates the effects of catecholamines in the heart, but in a qualitatively different manner from β_1AR, as it can also couple to the AC inhibitory G protein (G_i). In fact, this switching of β_2AR signaling from G_s to G_i proteins is postulated to be induced by the phosphorylation of the β_2AR by PKA (Figure 1).[16] It is now generally accepted that in the heart, β_2AR signals and functions in a substantially different way than β_1AR:[18–20] whereas β_1AR activation enhances cardiomyocyte apoptosis, β_2AR exerts anti-apoptotic effects in the heart.[18–21] This essential difference between the two receptor subtypes is ascribed to the signal of β_2AR through $G_{i/o}$ proteins.[19] This is the rationale behind the notion that promotion of $G_{i/o}$ protein signaling might serve as a therapeutic strategy in HF in an effort to augment cardiac survival (anti-apoptotic, cardioprotective) (Figure 1 and Table 1). Nevertheless, studies using transgenic mice, β_2AR-selective stimulation, and adenoviral-mediated β_2AR overexpression have clearly demonstrated the protective effects of β_2AR signaling in the myocardium, including improved cardiac function and decreased apoptosis. Conversely, hyperstimulation or overexpression of β_1AR has detrimental effects in the heart.[21,22]

Of note, the differences between the two predominant cardiac βARs, ie, β_1AR and β_2AR, in terms of their signaling properties, might take quite a different shape and have a much bigger bearing on pathophysiologic implications in the setting of human HF: for instance, and as discussed in more detail in subsequent sections, β_1AR is selectively downregulated (ie, functional receptor number reduced) in human HF, thus shifting the above-mentioned stoichiometry of β_1AR:β_2AR toward 50:50 in the failing heart from ~75%:~20% in the normal, healthy heart.[23,24] However, β_2AR is also nonfunctional and does not signal properly in the failing heart.[23,24] In addition, emerging evidence suggests that β_2AR signaling in the failing heart is quite different from that in the normal heart, ie, is more diffuse and noncompartmentalized and resembles more the pro-apoptotic "diffuse" cAMP signaling pattern of the β_1AR.[25] Therefore, this stoichiometric shift in favor of the supposedly "good" β_2AR in HF appears unable to help the heart improve its structure and function.

Chronic β-blocker (ie, βAR antagonist) therapy reverses LV remodeling in HF, reduces risk of hospitalization, improves survival, reduces risk of arrhythmias (sudden cardiac death), improves coronary blood flow to the heart (relieves angina), and protects the heart against cardiotoxic overstimulation by the catecholamines (NE and Epi).[9] All of these effects result in a decrease in the oxygen/energy and metabolic demands of the heart (cardiac workload is decreased) and in an increase in its oxygen/energy supply, thereby improving, in the long run, LV function and performance. Various molecular mechanisms underlying these effects have been postulated: 1) direct antagonism of catecholaminergic cardiotoxic effects; 2) cardiac βAR upregulation and restoration of their signaling and function (ie, increase in adrenergic and inotropic reserves of the heart), which is needed in situations where the heart needs to work and sustain systemic circulation (eg, in acute stress or in acute HF episodes);[26] 3) suppression of the elevated cardiotoxic, adverse remodeling–promoting, and pro-apoptotic neurohormonal systems (RAAS, endothelin); 4) coronary blood flow enhancement (as a result of diastolic prolongation); and 5) restoration of the reflex controls on the heart and the circulation.[27] Given that β_2AR signaling, unlike β_1AR signaling, might be cardioprotective in HF, novel β_1AR-selective β-blockers (ie, like metoprolol) or combinations of a β-blocker with a β_2AR-selective agonist (eg, clenbuterol) might be preferable for HF therapy (Table 1),[28] although carvedilol, a very successful and efficacious β-blocker in HF therapy, lacks βAR subtype selectivity.[29]

α_1AR signaling targets for HF therapy

The human heart also expresses α_1ARs, albeit at much lower levels than βARs (~20% of total βARs).[30] Their role in cardiac physiology is still a matter of debate, contrary to their well established effects in regulation of blood flow by inducing constriction in the smooth muscle wall of major arteries (eg, aorta, pulmonary arteries, mesenteric vessels, coronary arteries, etc).[31] The α_1ARs couple to the $G_{q/11}$ family of heterotrimeric G proteins, thereby activating phospholipase C (PLC)-β. PLCβ generates the second messengers, inositol [1,4,5]-trisphosphate (IP_3) and 2-diacylglycerol (DAG) from the cell membrane component phospholipid phosphatidylinositol (4,5)-bisphosphate (PIP_2). IP_3 binds specific receptors in the sarcoplasmic reticulum (SR) membrane, which cause release of Ca^{2+} from intracellular stores, whereas DAG activates protein kinase C (PKC) (Figure 1).[9] The end result is raised intracellular [Ca^{2+}], which leads to contraction in vascular smooth muscle (vasoconstriction) and to activation of hypertrophic programs in the heart (Figure 1).[15] α_1ARs in HF may function in a compensatory fashion to maintain cardiac inotropy, but their involvement in cardiac pathophysiology appears limited to situations of cardiac hypertrophy that ultimately lead to HF.[32] For instance, in the presence of pressure overload, cardiac α_{1A}ARs get activated and promote cardiomyocyte survival (ie, block apoptosis), protecting against adverse remodeling and decompensation to HF.[33,34] Thus, cardiac α_1AR antagonism, as well as inhibition of some cardiac α_1AR signaling components, eg, G_q proteins or PKC (PKCβ_2, for instance),[35,36] have been pursued as HF therapeutic modalities (Figure 1 and Table 1), but the jury is still out with regards to their effectiveness.

α_2AR signaling targets for HF therapy

Centrally located α_2ARs reduce SNS outflow (presynaptic inhibitory autoreceptors) and thus lower systemic blood

Table 1 Potential G protein-coupled receptor signaling targets for drug development in heart failure

Target/modality	Representative agents(s)	HF type to be treated	Potential desirable effect(s) in HF	Notes
β_2AR stimulation (with concurrent β_1AR inhibition)	Clenbuterol	Chronic HF	Cardioprotection ↑ Survival	Compatible with asthma, T2DM, and therapies of other diseases
α_2AR agonism/ sympatholysis	Clonidine, moxonidine, bucindolol	Chronic HF	Cardioprotection ↑ Survival ↓ Adverse remodeling	α_2AR desensitization in chronic HF and incompatibility of excessive sympatholysis with life are potential problems
AT_1R-dependent G_q protein signaling antagonism	ARBs (eg, losartan)	Chronic HF	Cardioprotection ↑ Survival ↓ Adverse remodeling ↓ Hypertrophy	↓ SNS tone additional advantage in HF treatment
$G_{i/o}$ protein stimulation	None known as yet	Chronic HF	Cardioprotection ↑ Survival	↓ contractility, CO (acutely) and bradycardia are disadvantages
G_q protein/PKCβ2 inhibition	G_{qI}, ruboxistaurin, LY379196	Chronic HF	↓ Adverse remodeling ↓ Hypertrophy	
Endothelin receptor antagonism	Bosentan, ambrisentan	Right atrial/ ventricular HF	Improved pulmonary arterial pressure	↓ PAH ↓ SNS tone (?) potential advantage
A1R (partial) agonism	Capadenoson	Chronic HF	Cardioprotection ↑ Survival	↓ arrhythmias, benefit in ischemic preconditioning are potential advantages; bradycardia is a disadvantage (although is less with partial agonism)
Adrenomedullin receptor agonism	Adrenomedullin, intermedin	Acute and chronic HF	↑ Contractility, CO Improved circulation parameters (BP, natriuresis)	Benefit in chronic HF unknown
Relaxin receptor agonism	Serelaxin (Rxn-2)	ADHF	↑ Contractility, CO Improved circulation parameters (BP, natriuresis)	Serelaxin currently in clinical trials for ADHF (RELAX-AHF)
CRFR2 agonism	Stresscopin, Ucn-1, Ucn-2	ADHF	↑ Contractility, CO Improved circulation parameters (BP, natriuresis, vasodilation)	Benefit in chronic HF unknown
Vasopressin receptor antagonism	Tolvaptan (V_2R-selective)	Chronic HF	Improved circulation parameters (BP, natriuresis, diuresis, vasodilation), ↓ Hyponatremia ↓ Cardiac hypertrophy (?)	Direct cardiac effects unknown, Tolvaptan approved in several countries for HF but not in the USA
Glucagon peptide receptor agonism	Glucagon, exenatide (GLP1R peptide agonist)	Chronic HF, diabetic cardiomyopathy	↑ Contractility, CO Cardioprotection ↑ Survival ↑ Myocyte proliferation	Glucagon is used to acutely raise CO in ADHF if patient is on β-blockers, Potential advantage in HF complicated with DM
RhoA/RhoK inhibition	Fasudil (RhoK inhibitor)	Chronic HF	↓ Adverse remodeling after MI	
Epac inhibition	None known as yet	Chronic HF	Cardioprotection (?) ↓ Arrhythmias (?)	
GRK2 blockade	Paroxetine, βARKct	Acute and chronic HF	↑ Contractility, CO Cardioprotection ↑ Survival ↓ Cardiac hypertrophy ↓ SNS (and RAAS) tones	βARKct is entering clinical trials for chronic HF soon
βarr2 stimulation (with concurrent βarr1 inhibition)	None known as yet, βarr1ct	Acute and chronic HF	↑ Contractility, CO Cardioprotection ↑ Survival ↓ SNS (and RAAS) tones	Exact role(s) of βarr1 versus βarr2 in the heart still await delineation, TRV027 (AT_1R βarr-biased agonist peptide) currently in development for ADHF

Abbreviations: A1R, adenosine type 1 receptor; ADHF, acute decompensated HF; AR, adrenoceptor; ARB, angiotensin receptor blocker; AT_1R, angiotensin II type 1 receptors; BP, blood pressure; cAMP, cyclic adenosine monophosphate; CO, cardiac output; CRFR2, corticotrophin-releasing factor receptor type 2; DM, diabetes mellitus; Epac, exchange protein directly activated by cAMP; GPCR, G protein-coupled receptor; G_{qI}, G_q protein inhibitor peptide; GRK, GPCR kinase; HF, heart failure; MI, myocardial infarction; PAH, pulmonary arterial hypertension; PKC, protein kinase C; RAAS, renin–angiotensin–aldosterone system; RELAX-AHF, Relaxin in Acute Heart Failure clinical trial; RhoA/K, ras homolog gene family member A/Rho-dependent kinase; Rxn-2, relaxin-2; SNS, sympathetic nervous system; T2DM, type 2 diabetes mellitus; Ucn, urocortin; V_2R, vasopressin receptor type 2; βarr, beta arrestin; ?, indicates effect currently under investigation; ct, C-terminal fragment; G_i/G_o, inhibitory/other G protein; GLP1R, glucagon-like peptide-1 receptor; βARK, beta-adrenergic receptor kinase.

pressure.[37,38] The release of NE from cardiac sympathetic nerve terminals is controlled by both presynaptic α_{2A}- and α_{2C}ARs,[39,40] and secretion of Epi (mainly) and NE from the adrenal medulla is also controlled (ie, inhibited) by α_{2C}ARs present in chromaffin cell membranes (Figure 1).[41,42] Genetic deletion of both α_{2A}- and α_{2C}AR subtypes leads to cardiac hypertrophy and HF due to chronically enhanced cardiac NE release, as well as enhanced NE and Epi secretion from the adrenal medulla.[40–42] In addition, the human α_{2C}AR Del322–325 genetic variant that displays impaired signaling and sympatho-inhibitory function is associated with increased risk of HF in homozygous African-American carriers, especially when co-carried with the hyperfunctional cardiac β_1AR Arg389 genetic variant, with the most probable mechanism being attenuated auto-inhibitory feedback of, and thus enhanced NE release from, the cardiac sympathetic nerves.[43] In fact, even in healthy humans, the α_{2C}AR Del322–325 variant is associated with increased sympathetic nervous and adrenomedullary hormonal activities, during both supine rest and pharmacologically evoked catecholamine release.[44] Thus, presynaptic inhibitory α_2-adrenergic autoreceptors crucially regulate SNS cardiac nerve activity and NE release into the heart, and any dysfunction of these receptors, either due to genetic polymorphisms or enhanced desensitization/downregulation (see GRK targets for HF therapy), translate into increased morbidity and mortality in chronic HF (Figure 1). Perhaps the crucial role of presynaptic α_2ARs in regulating NE release from cardiac SNS nerves stems from the fact that they are the only presynaptic ARs that can inhibit NE release;[9] presynaptic βARs (of the β_2AR subtype, mainly) are facilitatory autoreceptors enhancing NE release at sympathetic nerve terminals,[9] a phenomenon whose inhibition may contribute to the therapeutic benefit of β-blockers in HF (see βAR signaling targets for HF therapy) (Figure 1).

One of the hallmark abnormalities of chronic HF, contributing significantly to its morbidity and mortality, is chronically elevated SNS activity/outflow, as reflected by enhanced NE release in the heart (increased NE spillover) and enhanced adrenal catecholamine secretion leading to elevated circulating catecholamines.[45–47] Initially an adaptive mechanism aiming to compensate decreased contractility following cardiac insult, it becomes progressively maladaptive, contributing to HF establishment and progression and to its morbidity and mortality.[48,49] Based on this, sympatholysis (ie, reduction of SNS outflow and of circulating NE and Epi) is among the desirable goals of chronic HF therapy, and α_2AR agonism has been employed in HF clinical trials as one such strategy (Figure 1 and Table 1). However, one such drug, moxonidine, failed to provide any survival or hemodynamic benefits in its clinical trial (Sustained Release Moxonidine for Congestive Heart Failure [MOXCON]),[50] an unexpected and unfortunate outcome attributed (somewhat paradoxically) to excessive, incompatible with life sympatho-inhibition caused by the drug (Table 1). Unfortunately, this misfortune essentially ended further interest by industry in developing sympatholytic strategies for chronic HF, although bucindolol, a β-blocker with strong sympatholytic properties, is currently being promoted for chronic HF treatment based (in part) also on its SNS activity-lowering effects.[51] Of note, chronic HF is also accompanied by enhanced α_2AR desensitization and downregulation (see GRK targets for HF therapy and Table 1),[52] which might be another reason that moxonidine failed in its clinical trials for HF therapy.

AT_1R signaling targets for HF therapy

Landmark studies have shown that antagonism of RAAS provides HF patients with a substantial symptomatic and survival advantage.[53,54] These observations are supported by studies unequivocally showing activation of the RAAS in both clinical and experimental HF.[55] Furthermore, the pivotal role of angiotensin II, acting through AT_1Rs, in the process of cardiac adverse remodeling has been clearly documented in both clinical and experimental HF models.[56] Thus, both angiotensin-converting enzyme (ACE) inhibitors, which inhibit synthesis of angiotensin II, and AT_1R antagonists (the so-called "angiotensin receptor blockers" [ARBs]) (Table 1) ameliorate HF-associated LV adverse remodeling, such as fibrosis, hypertrophy, dilatation, myocardial stiffness, and oxidative stress, particularly after myocardial infarction, and RAAS antagonism is the cornerstone of all pharmacotherapeutic regimens currently employed in HF treatment.[57] Further adding to the importance of RAAS inhibition for HF therapy, antagonism of aldosterone, which is produced by the adrenal cortex (and possibly also in the heart per se) in response to angiotensin II activation of AT_1Rs and is the last hormone activated in the RAAS hormonal axis, has recently emerged as a very effective therapeutic strategy for advanced-stage HF, promoting patient survival and ameliorating LV adverse remodeling (Figure 1).[58] AT_1R is a classic $G_{q/11}$-coupled receptor, ie, it signals through the very same pathway as do α_1ARs (see α_1AR signaling targets for HF therapy) (Figure 1), although it can also couple to $G_{i/o}$ and G_s proteins in certain cell types.[8] In addition, it also signals via β arrestins (βarrs), independently of G proteins (see βarr targets for HF therapy).[8] Finally, in the central nervous system (CNS), the AT_1R can elevate SNS activity/outflow, which also contributes to the adverse hemodynamic and LV remodeling responses to myocardial infarction that angiotensin II activation of this receptor elicits.[9] Thus, part of the benefit of RAAS inhibitors (and of AT_1R antagonists in particular) in HF might also derive from centrally mediated suppression of SNS activity (Figure 1 and Table 1).

Targets for HF therapy in signaling from other GPCRs

Endothelin receptors

Unlike blockade of the SNS and RAAS, which have been successful in HF therapy, strategies targeting the endothelin system have largely failed.[1] Endothelin is another cardiotoxic hormone, the plasma concentrations of which are considerably increased in patients with HF and correlate with disease severity.[59] Additionally, endothelin is the most potent endogenous vasoconstrictor substance produced in the body (much more potent than angiotensin II).[59] Therefore, development of endothelin receptor antagonists for HF therapy made perfect sense on paper, but, although the hemodynamic profile of various endothelin receptor inhibitors has been favorable and these drugs have proven to be of significant therapeutic value for pulmonary arterial hypertension (PAH) and might also be of value in coronary artery disease (CAD), they have not provided any substantial benefit for HF patients in terms of survival or disease progression.[59,60] This might indicate that endothelin receptor signaling has only a minimal impact on cardiac function and certainly negligible compared with the impact of adrenergic or angiotensin II receptor signaling in the heart. The only HF indication for which endothelin receptor antagonists might hold promise right now appears to be right atrial/ventricular HF, given the benefits they provide in PAH (Table 1).

Adenosine receptors

Adenosine is a purine nucleoside that exerts a variety of physiological actions by binding to four adenosine cell surface GPCR subtypes, namely A1, A2a, A2b, and A3. The cardioprotective effects of adenosine have been extensively studied and are primarily mediated by activation of the A1-receptor (A1R) subtype in ischemic preconditioning.[61] However, activation of A1R, which primarily couples to (inhibitory or other) $G_{i/o}$ proteins, also slows HR, which is therapeutically exploited in treatment of certain supraventricular arrhythmias, but, in the context of chronic HF, it might constitute an undesirable effect, as it can lead to bradycardias and atrioventricular blocks.[61] A partial A1R agonist, capadenoson,[62] was very recently shown to improve LV function and prevent progressive cardiac adverse remodeling in a canine chronic HF model.[63] Importantly, improvement of LV systolic function seemed to occur early after treatment initiation with capadenoson, and, since the compound is not a full agonist at the A1R, it appears devoid of the HR-lowering complications with which full A1R agonism is hampered.[63] Although the precise signaling mechanism(s) that mediate these beneficial effects of partial A1R agonism in chronic HF remain to be worked out, this study strongly indicates that A1R-selective (partial) agonists might have a place in the chronic HF drug armamentarium in the future (Table 1).

Adrenomedullin receptor

Adrenomedullin is a peptide hormone released from multiple tissue types, including the kidneys and the adrenal medulla, in response to pressure and volume overloads, and its plasma levels have been shown to be elevated in acute decompensated HF.[64] In chronic HF, its levels appear to be independently predictive of 2 year mortality, especially in non-ischemic and in New York Heart Association (NYHA) class II or lower HF.[64] Its receptor is also a GPCR, albeit an unusual one: the receptor protein is encoded by the calcitonin receptor-like receptor (CRLR) gene, but, on its own, it is not functional. The receptor protein has to structurally couple to one of the receptor activity-modifying proteins (RAMPs) in order to become functional and capable of signaling.[65] The adrenomedullin receptor thus consists of the CRLR bound to RAMP2 (or RAMP3) and primarily couples to the G protein G_s, ie, activates, similarly to the βARs, the classic AC–cAMP–PKA signaling pathway, which, in the cardiac myocyte, leads to positive inotropy (increased contractility) (Figure 1).[65] Therefore, adrenomedullin and its analogs exert potent positive inotropic effects in the heart, which, coupled with their other beneficial effects on the circulation, such as hypotension and natriuresis,[66] make adrenomedullin receptor agonists (adrenomedullin non-peptide analogs, for instance) attractive possibilities for future HF drug development (Table 1). What's more, these drugs might prove useful for both acute and chronic HF treatments.

Relaxin receptors

The relaxins are a multi-member peptide hormone family comprising several relaxin-like and insulin-like peptides.[67] They are primarily involved in functional regulation of the reproductive and neuroendocrine systems, but they are also present in the brain and in the cardiovascular system, where relaxin-1 and -2 exert potent vasoactive (vasodilatory) effects.[67] Their cellular effects are mediated by four different types of relaxin family peptide (RXFP) receptors, which are all GPCRs. RXFP1 and RXFP2 receptors primarily couple to the G_s protein–AC–cAMP–PKA signaling pathway, which leads to positive inotropy in the heart and vasodilatation in vascular smooth muscle (Figure 1).[67] The RXFP1 receptor has also been shown to activate the phosphatidylinositol 3-kinase (PI3K) and extracellular signal-regulated kinase (ERK) signaling pathways.[67] Given that RXFP1 and RXFP2 receptors, which are both activated by relaxin-2, can lead to positive inotropy in the myocardium,[67] recombinant relaxin peptides and peptide analogs are currently pursued for HF drug development, and especially for acute decompensated HF (Table 1). One such agent, recombinant human relaxin-2 or serelaxin, is currently in clinical trials by Novartis for acute HF treatment, and the results have so far been more than encouraging.[68] Thus, development of relaxin peptide analogs and, specifically, of RXFP1 or RXFP2 receptor peptide or non-peptide agonists for acute HF is another area of HF drug development research that currently holds great promise, even though the chances of these agents also proving useful for chronic HF are low (Table 1).

Corticotrophin-releasing factor receptors

Corticotrophin-releasing factor (CRF) receptors (CRFRs) are GPCRs for the CRF family of peptide hormones, which includes urotensin-1, urocortins (Ucns), and CRF (or corticotrophin-releasing hormone [CRH]) itself, of course.[69] CRFR type 2 (CRFR2) is abundantly expressed in the human heart and primarily couples to G_s proteins, thereby activating the AC–cAMP–PKA (ie, the positive inotropic) signaling pathway of the cardiomyocytes (Figure 1).[69] This means that CRFR2 agonism can have cardiostimulatory effects and, indeed, in sheep HF, CRFR2 activation by Ucn1 induces sustained reductions in cardiac preload and afterload, improvements in cardiac output, and inhibition of a variety of cardiotoxic neurohormonal systems (eg, RAAS, endothelin, vasopressin, etc).[70] Antagonism of this receptor produces the mirror opposite effects in the same animals.[70] On the other hand, in human HF, CRFR2 activation by Ucn2 or stresscopin (a CRF-like peptide that selectively activates CRFR2) induces increases in cardiac output and LV ejection fraction, along with a fall in systemic vascular resistance.[71,72] Thus, similarly to the adrenomedullin and relaxin receptors above, Ucn-dependent CRFR2 activation produces positive inotropic, vasodilatory, and diuretic effects simultaneously, thereby making CRFR2 agonism another possible avenue for future HF drug development (Table 1).

Vasopressin receptors

Vasopressin is another very potent vasoactive peptide hormone that exerts its cellular actions via GPCRs (three different vasopressin receptor types: V_1R, V_2R, V_3R).[73] The human cardiac myocyte expresses (albeit to a limited extent) V_1R, which is a $G_{q/11}$ protein-coupled receptor, ie, activates the PLCβ–IP_3–DAG–PKC signaling pathway, similarly to the α_1ARs (see α_1AR signaling targets for HF therapy).[73] This signaling pathway leads to vasoconstriction in vascular smooth muscle cells and to hypertrophy in the myocardium, again similarly to the α_1ARs (Figure 1). Thus, vasopressin receptor antagonism poses as a possible therapeutic strategy in HF to combat cardiac hypertrophy, high blood pressure and systemic vascular resistance, overactivation of other cardiotoxic neurohormonal systems (eg, RAAS, SNS), and, of course, to reduce volume overload of the heart, along with its accompanying water and electrolyte abnormalities, which stem from the excessive V_2R-dependent anti-diuresis in the kidneys that causes hyponatremia.[74,75] Nevertheless, the main indication for vasopressin receptor antagonist drugs currently is hyponatremia (along with some other renal indications), and only in very few countries (eg, Japan but not in the USA) are they currently approved for congestive HF (Table 1).[76] Pretty much like α_1AR and endothelin receptor antagonism, cardiac V_1R antagonism, as well as antagonism of vasopressin receptors in general, clearly warrants further investigation before it can be considered a legitimate therapeutic strategy for HF.

Glucagon peptide hormone receptors

Glucagon receptor (GCGR) and glucagon-like peptide (GLP)-1 receptor (GLP1R) are class B GPCRs mediating some of the cardiovascular effects of glucagon and GLP1, respectively, both members of the glucagon family of peptide hormones.[77] GCGR is a G_s protein-coupled receptor and is present in cardiac myocyte membranes, where it can activate the positive inotropic AC–cAMP–PKA signaling pathway, pretty much like the β_1AR does (Figure 1).[77] This constitutes the molecular basis for the clinical use of glucagon to acutely raise cardiac output in acute decompensated HF patients who are on β-blockers,[77] a situation in which use of adrenergic positive inotropes (eg, dobutamine, dopamine) would be ineffective (Table 1). Glucagon receptor agonism for chronic HF has not been studied but is probably not recommended, given that it is, in essence, a positive inotropic therapy that raises workload and oxygen and metabolic demands of the heart.

The GLP1R is also expressed in the heart, where it seems to mediate some of the beneficial cardiac effects of GLP1, such as inhibition of apoptosis, myocyte proliferation, and even positive inotropy.[78,79] The signaling pathways initiated by GLP1R in the heart underlying these GLP1 effects are not entirely clear, but probably involve activation of the PI3K-Akt and the ERK1/2 signaling cascades (see Relaxin receptors), pathways known to result in cell survival and proliferation.[78,79] Given these beneficial effects of GLP1R in the heart, GLP1 analogs have been tried as potential therapies in a number of experimental HF studies, including some with concurrent diabetes mellitus but also some independent of diabetic complications, and the results are overall promising.[78,79] Thus, GLP1R agonism represents another potential avenue for future HF drug development, especially for those types of HF that are of diabetic etiology or are complicated by diabetes (Table 1).

G protein targets for HF therapy

With regards to heterotrimeric G proteins as potential targets for HF therapy, the reader is referred to the preceding sections of this article (specifically, the sections on ARs). In addition to the heterotrimeric G proteins, small (or Ras-like) G proteins also exist and provide an important link between the cell surface and intracellular signaling pathways.[80] Among these, the small G protein "Ras homolog gene family member A" (RhoA) activates a protein kinase, the Rho kinase, which can drive the cardiac hypertrophic process (Figure 1).[80] Inhibition of Rho kinase with the drug fasudil has been shown to reverse LV remodeling in experimental myocardial infarction, thus posing Rho kinase (or perhaps even RhoA itself) as a novel molecular target for HF therapy (Figure 1 and Table 1).[81,82]

Moreover, the second messenger cAMP can activate, in addition to PKA (see βAR signaling targets for HF therapy), another effector in the cardiac myocyte, the "Exchange protein directly activated by cAMP" (Epac) (Figure 1).[83] Epac was initially discovered as a cAMP-dependent and PKA-independent guanine nucleotide exchange factor (GEF) for the small G protein Rap1 (ie, Rap1 activator) but it is now known to be a multi-purpose adapter protein mediating a plethora of protein–protein interactions in the cell.[83] Epac2 was recently shown to mediate β_1AR-dependent SR Ca^{2+} leak and arrhythmias in the heart, upon its activation by cAMP generated by cardiac β_1AR stimulation.[84] This suggests that Epac inhibition might be of therapeutic value for cardiac arrhythmias and potentially also for HF, but further studies are needed to delineate the precise role(s) of Epac in the heart and in HF (Figure 1 and Table 1).

GRK targets for HF therapy

The majority of GPCRs are subject to agonist-promoted (homologous) desensitization and downregulation, a regulatory process that diminishes receptor response to continuous or repeated agonist stimulation.[85,86] At the molecular level, this process is initiated by receptor phosphorylation by a family of kinases, termed GPCR kinases (GRKs), followed by binding of βarrs to the GRK-phosphorylated receptor. The βarrs then uncouple the receptor from its cognate G proteins, sterically hinder its further binding to them (functional desensitization), and subsequently target the receptor for internalization.[85,86] Across all mammalian species, GRK2 and GRK5 are the most physiologically important members of the GRK family because they are expressed ubiquitously and regulate the vast majority of GPCRs. They are particularly abundant in neuronal tissues and in the heart.[87,88] The elevated SNS outflow and NE and Epi levels in chronic HF lead to chronically elevated stimulation of the cardiac βAR system, which has detrimental repercussions for the failing heart. Extensive investigations over the past 3 decades have helped delineate the molecular alterations afflicting the cardiac βAR system that occur during HF, and it is now well known that, in chronic human HF, cardiomyocyte βAR signaling and function are significantly deranged and the adrenergic reserve of the heart is diminished.[9,48] Cardiac βAR dysfunction in human HF is characterized at the molecular level by selective reduction of β_1AR density at the plasma membrane (downregulation) and by uncoupling of the remaining membrane β_1ARs and β_2ARs from G proteins (functional desensitization).[23,24] Importantly, myocardial levels and activities of GRK2 and GRK5 are elevated, both in humans and in animal models of HF.[89,90] This GRK elevation possibly serves as a homeostatic protective mechanism aimed at defending the heart against excessive catecholaminergic toxicity. However, several studies soon refuted this assumption, demonstrating that GRK2 upregulation is detrimental for the heart and causes the functional uncoupling of βARs in vivo.[91] This finding prompted investigations of the role GRK2 plays in cardiac function, which revealed that cardiac GRK2 is an absolutely critical regulator of cardiac βAR-dependent contractility and function. Specifically, cardiomyocyte-restricted overexpression of GRK2 to the same level of upregulation found in human HF (ie, three- to four-fold) markedly attenuated βAR signaling and contractile reserve, showing that GRK2 is the main culprit for the functional desensitization of cardiac βARs in HF (Figure 1).[92] The proof for this was provided by studies of the in vivo inhibition of cardiomyocyte GRK2, which were enabled by the development of the βARKct (beta adrenergic receptor kinase C-terminal fragment) mini-gene, which blocks cell membrane translocation and hence activation of GRK2, and its cardiomyocyte-specific expression in vivo in transgenic mice by virtue of the αMHC (myosin heavy chain) gene promoter.[90] Indeed, GRK2 inhibition in vivo in the heart with βARKct (or its partial genetic deletion) enhances cardiac contractility both at baseline and after adrenergic stimulation, reverses the contractile and βAR dysfunctions, and preserves or even augments cardiac function and survival in HF.[90] Of note, the antidepressant paroxetine was recently shown to inhibit GRK2, thus providing a lead for the development of GRK2-specific small molecule inhibitors.[93] In summary, elevated SNS activity in chronic HF causes enhanced GRK2-mediated cardiac β_1- and β_2AR desensitization and β_1AR downregulation, which leads to the progressive loss of the adrenergic and inotropic reserves of the heart, the hallmark molecular abnormality of this disease (Figure 1).[94]

Additionally, GRK2 expression and activity are also increased in the adrenal gland during HF.[95] Specifically, our studies over the past few years have established that adrenal GRK2 upregulation is responsible for severe adrenal α_2AR dysfunction in chronic HF, which causes a loss of the sympatho-inhibitory function of these receptors in the adrenal gland, and catecholamine secretion is thus chronically elevated (Figure 1).[9,11,96] This emerging crucial role for adrenal GRK2 in HF is underlined by the fact that its specific inhibition, via adenoviral-mediated βARKct adrenal gene delivery (see above), leads to a significant reduction in circulating catecholamine levels, restoring not only adrenal, but also cardiac function in HF.[95] Additional evidence for the crucial role of adrenal GRK2-regulated α_2ARs in regulating adrenal SNS tone in HF comes from the phenylethanolamine-N-methyl transferase (PNMT)-driven GRK2 knockout (KO) mice.[97] These mice, which do not express GRK2 in their adrenal medullae from birth, display decreased SNS outflow and circulating catecholamines in response to myocardial infarction, which translates into preserved cardiac function and morphology over the course of the ensuing HF.[97] Of note, elevated GRK2-dependent α_2AR dysfunction during HF might also occur in other peripheral sympathetic nerve terminals of the heart (Figure 1) and of other organs, thus contributing to the increased NE release and spillover, as well as to the presynaptic α_2AR dysfunction in SNS neurons observed in chronic HF (see α_2AR signaling targets for HF therapy).[9,11] Thus, GRK2 inhibition poses not only as a positive inotropic therapy in the heart per se, but also as a novel sympatholytic strategy in HF, blocking catecholamine release at the sources of these hormones (ie, adrenals and cardiac SNS terminals) and preventing their toxic effects on peripheral organs, like the heart. In addition, adrenal βARKct expression might have a synergistic action with β-blockers, as both of these therapeutic strategies target adrenergic hyperactivity in HF (Figure 1 and Table 1).

βarr targets for HF therapy

βarrs comprise two ubiquitously expressed isoforms, βarr1 and βarr2 (arrestin-2 and -3 respectively), both of which are abundantly expressed in cardiac muscle.[98] As co-factors of GRKs in βAR desensitization/downregulation, they contribute to the diminished inotropic and adrenergic reserves of the failing heart and their inhibition should theoretically be beneficial in acute HF, as it would enhance the G_s–AC–PKA axis of pro-contractile signaling of cardiac βARs (see βAR signaling targets for HF therapy), thereby increasing cardiac contractility.[98] However, βarrs do not merely terminate G protein-mediated signaling by GPCRs. It is now well established that they promote signaling in their own right, independently of G proteins, and a number of recent studies point to a beneficial role played by them in the heart, especially when they engage the cardiac β_1AR.[99] More specifically, they have been reported to mediate epidermal growth factor receptor (EGFR) transactivation by the β_1AR.[99] Consistent with this, a mutant β_1AR lacking 14 GRK phosphorylation sites in its C-terminal tail that cannot undergo βarr-dependent desensitization, fails to transactivate the EGF receptors.[100] In response to chronic isoproterenol stimulation, transgenic mice expressing this β_1AR mutant develop severe dilated cardiomyopathy with significantly increased LV dilatation, decreased fractional shortening, and increased myocardial apoptosis compared with wild-type β_1AR-expressing transgenic mice.[100] In this model, inhibition of EGF receptors worsens the dilated cardiomyopathy, suggesting a protective rather than deleterious role for transactivated EGFRs in the heart and prompting the investigators to speculate that βarr-dependent EGFR transactivation exerts a cardioprotective effect and thus, βarr-mediated (in contrast to the classical G protein-dependent) β_1AR signaling might be of therapeutic benefit in HF (Figure 1 and Table 1).[99,100]

Effects of cardiac βarr-dependent signaling can be quite different when the AT_1R is bound by βarrs, An artificially constructed $AT_{1A}R$ mutant (AT1-i2m), which fails to activate G proteins but nonetheless interacts with βarrs, activates the mitogenic Src–Ras–ERK1/2 pathway in vitro.[101] In vivo, cardiomyocyte-specific overexpression of this receptor mutant leads to greater cardiomyocyte hypertrophy, bradycardia, and fetal cardiac gene expression than comparable overexpression of the wild-type receptor.[101] Conversely, overexpressed wild-type $AT_{1A}R$ produces greater cardiomyocyte apoptosis and interstitial fibrosis than the G protein-uncoupled mutant, suggesting that G protein-dependent and -independent $AT_{1A}R$ signals mediate different aspects of the hypertrophic response.[101] Of course, these studies do not directly implicate βarr signaling; another series of studies using the AngII peptide analog SII ([Sar^1-Ile^4-Ile^8]-AngII), which, when bound to the $AT_{1A}R$, elicits βarr signaling but no G_q protein signaling,[98] provide direct evidence for potential roles of βarrs in cardiac $AT_{1A}R$ signaling. Several studies have shown that AT_1R βarr-dependent signaling in cardiac myocytes leads to cardiomyocyte proliferation without hypertrophy (which requires $G_{q/11}$ protein signaling) and can even result in positive inotropy and lusitropy.[102] These effects require GRK6 and βarr2, whereas GRK2 seems to oppose them, consistent with the specialized role of GRK isoforms described in a transfected system.[102] On the other hand, AT_1R-bound βarrs do not produce inotropic or chronotropic effects in isolated Langendorff-perfused cardiac preparations despite activating ERK1/2.[103] Thus, it seems that, while cardiac AT_1R promotes hypertrophy and cardiomyocyte proliferation via the classical G_q protein–PKC pathway, it can increase cardiac contractility and function via βarr2-dependent signaling (Figure 1). Since βarr2 is bound to stop the G protein-mediated signaling of the receptor, and GRK2 also seems to oppose this pro-contractile signaling of βarr2, it follows that stimulation of βarr2 activity and/or GRK2/βarr1 inhibition at the cardiac AT_1R might be of therapeutic value in HF and/or cardiac hypertrophy treatments (Figure 1 and Table 1).[8] In fact, since βarr1 also mediates AT_1R-induced aldosterone production and secretion in the adrenal cortex (Figure 1),[8,104,105] βarr1 inhibition in both the heart and adrenals might be of therapeutic value in chronic HF (Table 1).

Conclusions and future perspectives

The tremendous progress of molecular biology, physiology, and pharmacology over the past 2 decades or so, coupled with the advent of the first GPCR structures and of the so-called "rational" (ie, target- and structure-based) drug design have provided clinicians and pharmacologists with a tremendous expansion of the therapeutic arsenal for HF. Nevertheless, HF still remains the most devastating, in terms of morbidity, mortality, quality of life, and health care costs, cardiovascular disease and the number one killer in the western world.[1–7] Thus, new and innovative drugs are desperately needed in order to, if not cure, at least improve quality of life of HF patients.[1] The field of GPCR signaling keeps providing exciting new possibilities and targets for HF drug development. For instance, targeting intracellular signaling components of the traditional cardiac GPCR drug targets, βARs and AT_1Rs, such as heterotrimeric G proteins per se (G_i protein activation or G_q protein inhibition), small G proteins like RhoA, and Epac, might produce novel useful HF drugs. Another exciting avenue for future HF drug development is targeting and exploitation of new GPCRs, the important roles of which in cardiac physiology and pathophysiology keep getting uncovered, such as select adenosine receptor agonism or agonism of certain vasoactive peptide hormone receptors, eg, adrenomedullin, relaxins, or Ucns (CRFR2). In addition, further studies on signaling of cardiac α_1ARs, endothelin, and vasopressin receptors, as well as on central and adrenal α_2AR signaling, might also finally yield some valid therapeutic targets. Finally, targeting molecules that regulate cardiac GPCR signaling, such as cardiac GRKs and βarrs, is perhaps the most exciting area for future HF drug development. For instance, GRK2 inhibition, which can provide a positive inotropic and sympatholytic therapy at the same time, has the potential to revolutionize current chronic HF therapy. On the other hand, as the physiological relevance of cardiac βarr signaling becomes fully elucidated, selective targeting of βarr1 or βarr2 in the heart or development of "biased" GPCR ligands, which selectively recruit βarrs over G proteins (or vice versa) at cardiac GPCRs,[98] will also offer attractive options for future HF drug development. The research conducted to date already indicates that inhibition of βarr1 or stimulation of βarr2 in the heart per se might be beneficial for cardiac function and in HF. Future studies will help clarify the picture regarding the potential of cardiac GRK2 and βarr targeting for HF treatment. Nonetheless, cardiac GPCR signal transduction appears to be among the research fields that currently hold the largest potential and the biggest promise for the future of HF drug design and development.

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Disclosure

The authors report no conflicts of interest in this work.

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