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INVITED REVIEW

Pathophysiology and pharmacology of G protein-coupled receptors in the heart

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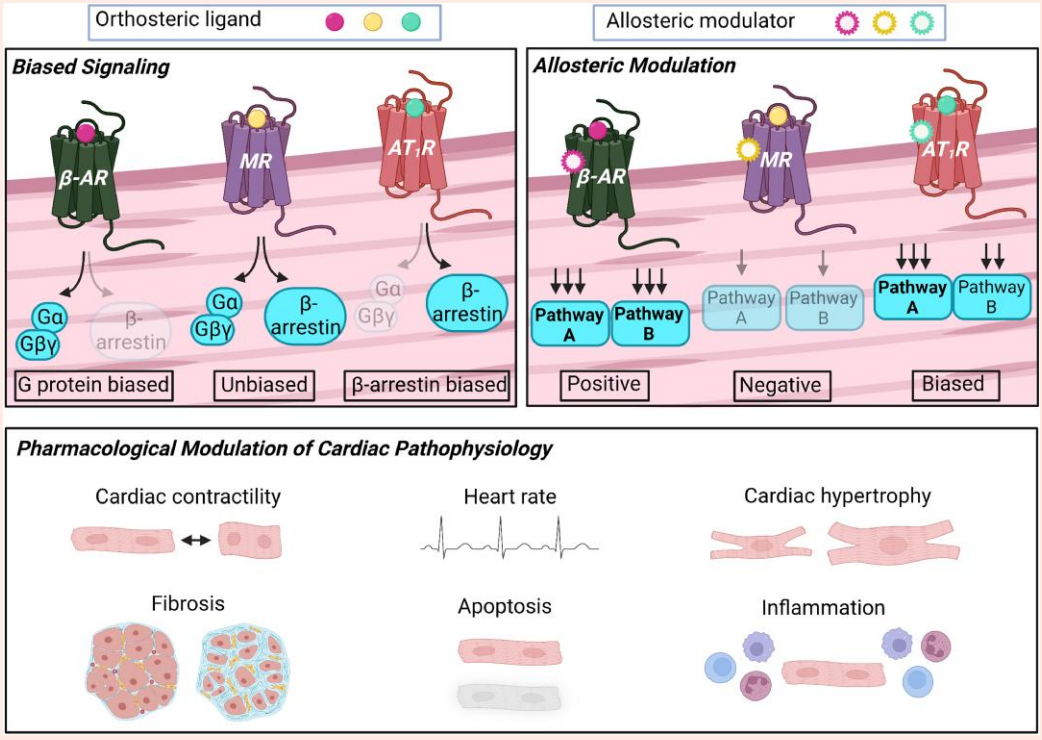
Abstract

G protein-coupled receptors (GPCRs), comprising the largest superfamily of cell surface receptors, serve as fundamental modulators of cardiac health and disease owing to their key roles in the regulation of heart rate, contractile dynamics, and cardiac function. Accordingly, GPCRs are heavily pursued as drug targets for a wide variety of cardiovascular diseases ranging from heart failure, cardiomyopathy, and arrhythmia to hypertension and coronary artery disease. Recent advancements in understanding the signalling mechanisms, regulation, and pharmacological properties of GPCRs have provided valuable insights that will guide the development of novel therapeutics. Herein, we review the cellular signalling mechanisms, pathophysiological roles, and pharmacological developments of the major GPCRs in the heart, highlighting the β-adrenergic, muscarinic, and angiotensin receptors as exemplar subfamilies.

Graphical Abstract



Keywords G protein-coupled receptors • Heart failure • Biased signalling • Allosteric modulators

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1. Introduction

G protein-coupled receptors (GPCRs), also known as 7 transmembrane domain receptors (7TMRs), encompass the largest and most extensively studied superfamily of cell surface receptors.[1] GPCRs are activated by a diverse array of ligands including hormones, peptides, and neurotransmitters, and serve as key regulators of a variety of cellular responses. Given their involvement in many different physiological processes, GPCRs are highly pursued pharmacologically and represent the primary targets of ~35% of all small molecule drugs currently approved by the Food and Drug Administration (FDA).[2] Of the nearly 800 different human GPCR genes,[3] more than 200 are expressed in the heart alone,[4] underscoring their prominent role in regulating cardiac function and highlighting their potential as therapeutic targets in heart disease.

1.1 GPCR signalling mechanisms

Canonically, GPCRs are activated via the binding of an agonist to the orthosteric ligand binding site on the extracellular surface (*Figure 1*). Agonist-bound GPCRs subsequently recruit heterotrimeric G proteins, consisting of Gα, Gβ, and Gγ subunits, and induce the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on Gα. This exchange stimulates the dissociation of Gα from Gβγ, generating two activated G protein units that independently transduce signals to downstream effectors.[5]

The Gα subunit is divided into four major classes including $G\alpha_s$, $G\alpha_i$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$, which each generate their own unique cellular responses (*Figure 1*).[5] The $G\alpha_s$ subunit, a stimulatory Gα protein, activates adenylyl cyclase (AC) to generate second messenger cyclic adenosine monophosphate (cAMP) which results in the activation of protein kinase A (PKA). In the heart, PKA phosphorylates sarcomeric proteins, calcium regulators, and ion channels resulting in enhanced contractile and calcium cycling dynamics thereby mediating positive inotropic and chronotropic responses.[6] In contrast, receptors that are coupled to $G\alpha_i$ inhibit AC, which leads to decreased production of cAMP and reduced PKA activity. $G\alpha_q$-coupled receptors promote the activation of phospholipase C (PLC), which generates the second messengers, namely diacylglycerol (DAG) and inositol triphosphate (IP_3) that stimulate protein kinase C (PKC) and calcium influx, respectively. Finally, $G\alpha_{12/13}$ activates the small GTPase Rho.

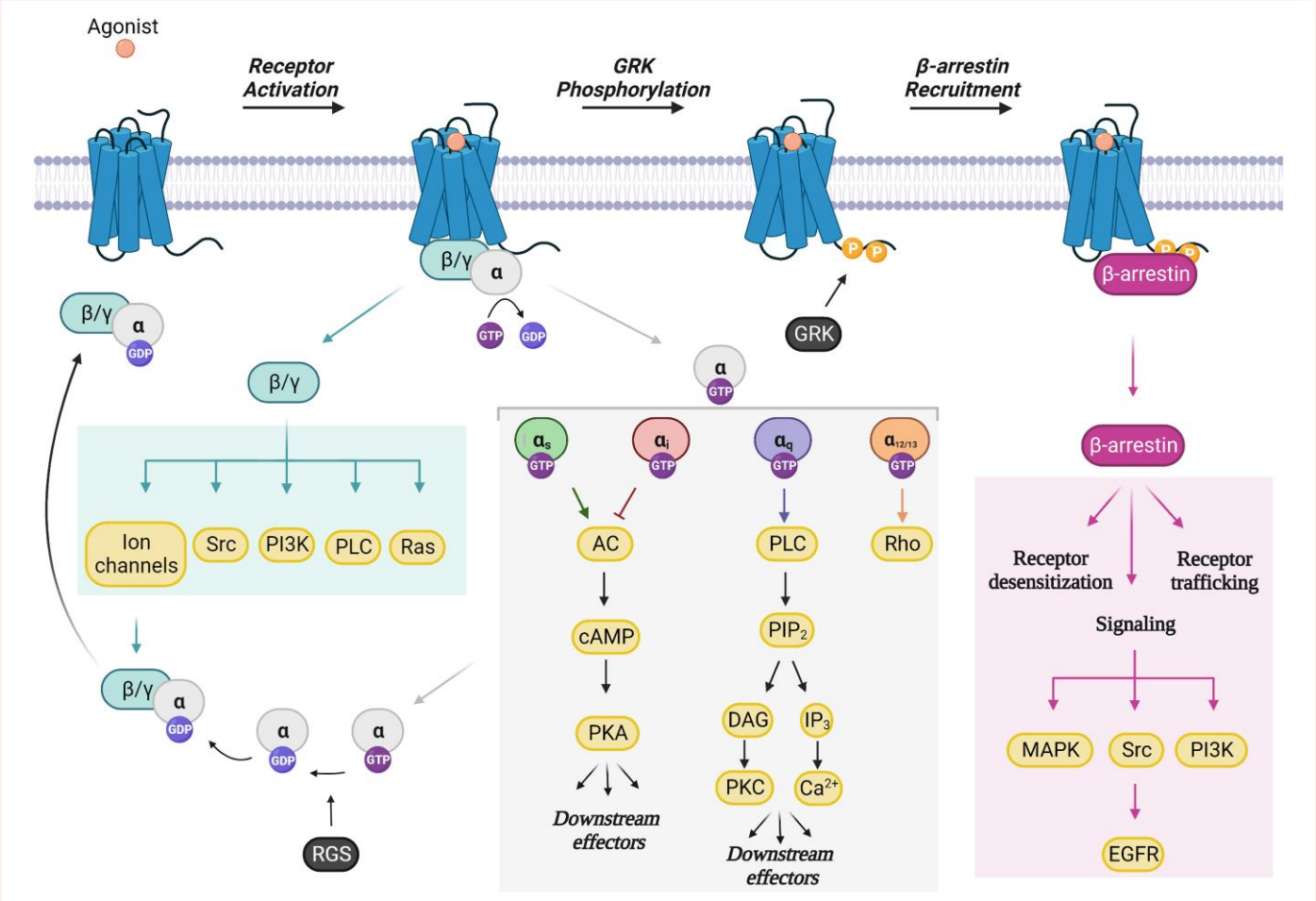

Figure 1 GPCR activation and downstream signalling. Following agonist binding, GPCRs recruit heterotrimeric G proteins (Gα, β, γ) and induce the exchange of GDP for GTP on Gα. The Gα and Gβγ subunits subsequently dissociate forming two activated G protein units that independently transduce signals to downstream effectors. The four families of Gα ($G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12/13}$) activate distinct signalling cascades, thereby generating unique cellular responses. RGS proteins accelerate the GTPase activity of Gα, thereby leading to the inactivation of G protein signalling. GRK-mediated phosphorylation of the COOH-terminus of the receptor facilitates the recruitment of β-arrestin, which in turn, promotes receptor desensitization, receptor trafficking/recycling, and β-arrestin-dependent signalling mechanisms. GDP, guanosine diphosphate; GTP, guanosine triphosphate; PI3K, phosphoinositide 3-kinase; PLC, phospholipase C; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PIP_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; PKC, protein kinase C; IP_3, inositol trisphosphate; MAPK, mitogen-activated protein kinase; EGFR, epidermal growth factor receptor; GRK, GPCR kinase; RGS, regulators of G protein signalling. Figure generated with Biorender.com.

To mediate signal termination, regulators of G protein signalling (RGS) and GPCR kinases (GRKs) are recruited to GTP-bound Gα subunits or agonist-activated GPCRs, respectively. RGS proteins serve to accelerate Gα GTP hydrolysis activity, causing the reassociation of Gα with Gβγ and the inactivation of downstream G protein signalling (*Figure 1*).[7] In contrast, GRKs are recruited to agonist-activated GPCRs to phosphorylate the COOH-terminal tail of the receptor (*Figure 1*).[8] This in turn, facilitates the recruitment of β-arrestin, a multifunctional adaptor protein, which promotes receptor desensitization via sterically uncoupling G proteins from activated GPCRs.[5] β-arrestins also serve as scaffolds for cyclic nucleotide phosphodiesterases (PDEs) and diacylglycerol kinases (DGKs) that degrade second messengers cAMP and DAG, respectively, providing another mechanism to dampen signalling.[9,10] In addition to their desensitization function, β-arrestins facilitate receptor internalization and trafficking to endosomes via binding clathrin and its adaptor protein AP2.[11] Importantly, β-arrestins function as signal transducers via scaffolding major signalling complexes such as mitogen-activated protein kinases (MAPKs), Src, and phosphoinositide 3-kinase (PI3K).[12]

The discovery that β-arrestins serve as functional signal mediators has led to the emergence of a fundamental concept in GPCR biology termed biased agonism. Compared to the cognate endogenous ligands for each GPCR which activate multiple signalling pathways in an unbiased fashion, biased ligands stabilize distinct receptor conformations to preferentially activate either G protein or β-arrestin pathways (*Figure 2A*).[13] The concept of biased agonism provides important implications in the development of therapeutics since drugs can be developed to target specific signalling pathways that will generate more beneficial physiological outcomes and/or reduce adverse side effects.

1.2 Allosteric modulation of GPCR function

Aside from the orthosteric binding region, ligands can target allosteric sites of the receptor (i.e. regions that are topographically distinct from the orthosteric binding pocket). These ligands, termed allosteric modulators, most often do not possess intrinsic activity on their own, but can enhance (positive allosteric modulator) or reduce (negative allosteric modulator) the activity of receptors stimulated by orthosteric ligands (*Figure 2B*).[14] The identification of allosteric modulators that possess intrinsic activity in the absence of an orthosteric ligand has additionally established 'ago-allosteric modulators' as an entity.[15] Importantly, both allosteric and ago-allosteric modulators can potentially act in a biased fashion by enhancing the activation of a particular signalling pathway while either having no effect or antagonizing alternative pathways (*Figure 2B*).[16] Given that the orthosteric binding pocket is typically highly conserved among GPCR subtypes, while allosteric regions exhibit greater sequence and structural diversity, allosteric modulators have a higher potential for being subtype-specific and/or generating biased cellular effects.[14]

Herein, we review the cellular signalling mechanisms, physiological functions, clinical implications, and current pharmacological developments of the major subtypes of GPCRs that regulate cardiac physiology. Given the large number of different GPCRs expressed in the heart,[4] we chose to focus on the receptors that have shown the greatest pharmacological potential and/or highest efficacy in the treatment of heart disease, including the β-adrenergic, muscarinic, and angiotensin receptor families.

2. β-Adrenergic receptors

Adrenergic receptors were initially subclassified into two distinct receptor families (α and β) by Ahlquist in 1948.[17] Within the β-adrenergic (βAR) family, there are three major subtypes that include the β_1AR, β_2AR, and β_3AR, which are encoded by the genes *ADRB1*, *ADRB2*, and *ADRB3*, respectively. All three βARs are endogenously activated by the catecholamine hormones epinephrine and norepinephrine (*Figure 3*). For the β_1AR, norepinephrine has a slightly higher potency than epinephrine, while the agonist potency is reversed for the β_2AR.[18]

2.1 Tissue expression and localization

The β_1AR is the most common subtype in the heart, as it comprises about 80% of total βARs while the remaining 20% is mainly the β_2AR.[19] The β_3AR, in contrast, is the least characterized to date and only constitutes about 3% of all cardiac βARs.[20] Consistent with its increased abundance, the β_1AR subtype displays a more widespread cellular distribution as it localizes to both the outer plasma membrane and T-tubule membranes of cardiomyocytes,[21] whereas β_2AR and β_3AR are confined to the T-tubular network.[21,22] In contrast to the healthy heart, failing human hearts exhibit a substantial reduction in βAR receptor density due to selective downregulation of β_1AR.[19] As a consequence, this shifts the β_1AR:β_2AR ratio from 80:20 to 60:40.[19]

2.2 Cellular signalling mechanisms

Under normal physiological conditions, the β_1AR couples to $G\alpha_s$ as the signalling transducer, while the β_2AR couples to both $G\alpha_s$ and $G\alpha_i$, and the β_3AR predominantly signals via $G\alpha_i$.[23,24] In addition to PKA activation via $G\alpha_s$, stimulation of β_1ARs increases calcium/calmodulin-dependent protein kinase II (CaMKII) activity in a β-arrestin-dependent manner.[25] β_2AR coupling to $G\alpha_i$ in the heart can activate cell survival signalling via Gβγ-mediated activation of PI3K and Akt.[23] Furthermore, the dissociated Gβγ subunit from $G\alpha_i$ can also activate the Src-family tyrosine kinases and G protein Ras.[26,27]

Although β_3ARs canonically signal via $G\alpha_i$ functioning to inhibit activation of AC and PKA, administration of the β_3AR-selective agonist, L755-507, generated positive inotropic responses in transgenic mice overexpressing human β_3AR.[28] Moreover, stimulation with L755-507 resulted in increased AC activity in a pertussis-toxin insensitive fashion, indicating overexpressed β_3ARs couple to $G\alpha_s$.[28] Unlike β_1ARs and β_2ARs that have rich serine/threonine residues that can be phosphorylated by GRKs, β_3ARs lack these residues and therefore are more resistant to agonist-induced desensitization.[24]

Aside from their desensitization and internalization functions, β-arrestins also serve as signal transducers following βAR activation. Specifically, in response to an agonist, β-arrestin recruits c-Src to βAR, which subsequently induces the activation of extracellular signal-regulated kinases (ERK).[29] Furthermore, β-arrestin-mediated c-Src stimulation leads to the activation of matrix metalloproteases (MMPs), which in turn, initiate epidermal growth factor receptor (EGFR) transactivation.[30] Importantly, *in vivo*, βAR-mediated EGFR transactivation confers cardioprotection by preventing the development of ventricular dilation and contractile impairment under conditions of chronic catecholamine stimulation.[30]

2.3 Physiological functions and contribution to disease

Increased sympathetic nervous system activation and the adrenergic response are essential in maintaining circulatory support during acute heart failure, yet are associated with worse survival.[31] Indeed, prolonged activation of catecholamine signalling is detrimental to the heart since this causes βAR desensitization, excessive CaMKII activation, and cardiomyocyte hypertrophy and apoptosis.[25,32–34] Consistent with this, transgenic mice overexpressing β_1AR exhibit depressed cardiac function, progressive hypertrophy, and fibrosis.[35,36]

Similarly, mice with cardiac-specific overexpression of β_2AR display severe left ventricular dysfunction following aortic stenosis,[37] and spontaneous tachyarrhythmia related to increased interstitial fibrosis.[38] Furthermore, β_2AR overexpressing mice are more susceptible to ischaemic injury than their wild-type counterparts, which is further exacerbated by pertussis-toxin treatment suggesting that the pathological manifestations of β_2AR overexpression are mediated primarily by $G\alpha_s$ rather than $G\alpha_i$.[39] Indeed, β_2AR activation is considered anti-apoptotic through its ability to couple to $G\alpha_i$.[40,41] Therefore, β_2AR signalling may be either protective or deleterious in the heart depending on transducer coupling.

In addition to the evaluation of βAR overexpression, the generation of different βAR knockout mouse models revealed that the absence of βAR is also pathogenic. Particularly, β_1AR knockout mice ($\beta_1AR^{-/-}$) die

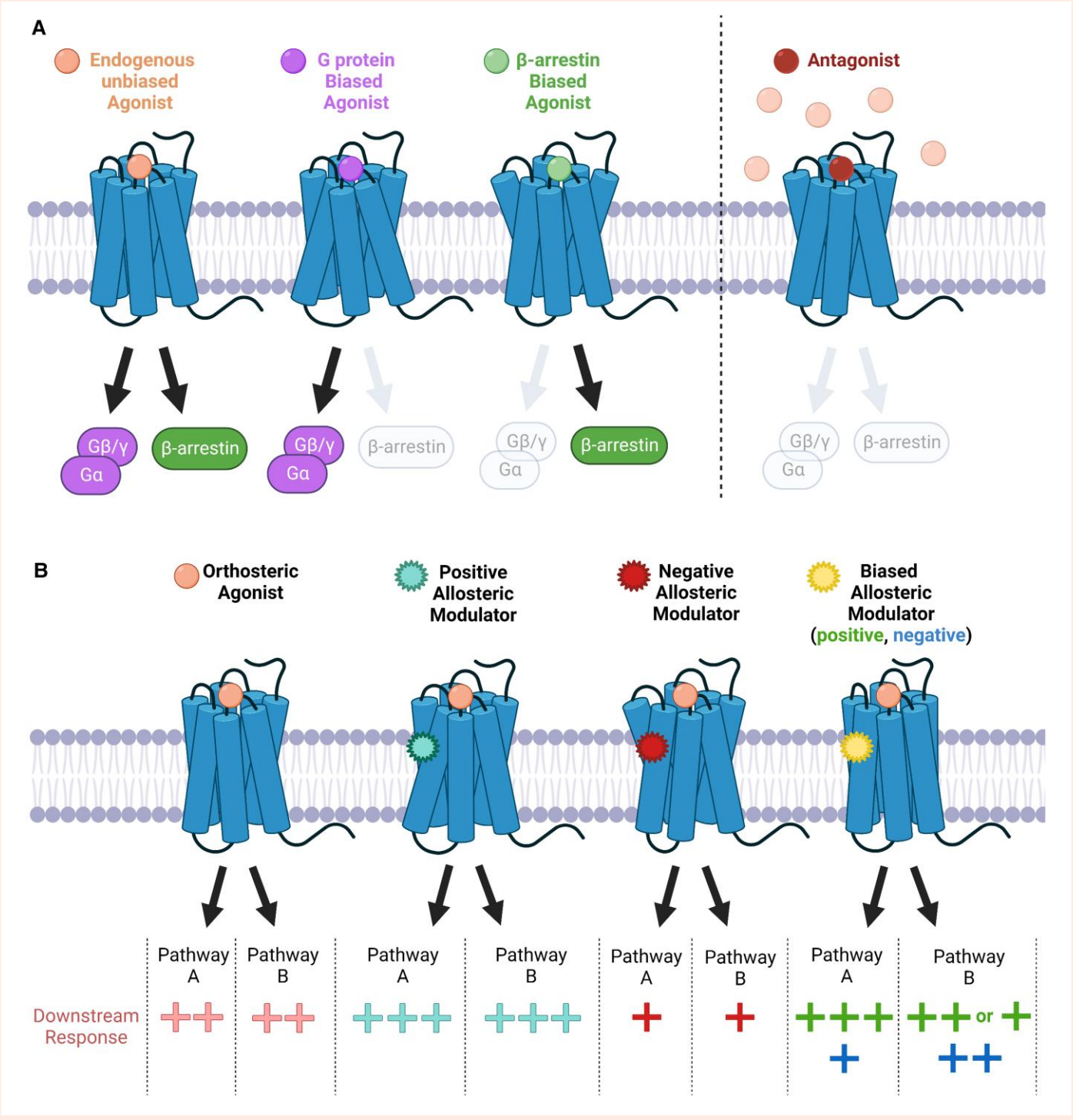

Figure 2 Biased agonism and allosteric modulation of GPCR function. (*A*) Unbiased orthosteric agonists non-selectively activate G protein and β-arrestin signalling pathways, while biased orthosteric ligands preferentially activate one pathway over the other. Conversely, antagonists competitively inhibit agonist binding and thereby inactivate both G protein and β-arrestin signalling in a balanced fashion. (*B*) Allosteric modulators bind to sites that are topographically distinct from the orthosteric binding pocket of the receptor. Functionally, positive allosteric modulators enhance (+++) the activity of an orthosteric agonist, while negative allosteric modulators reduce (+) orthosteric activity. Biased allosteric modulators can preferentially enhance (positive; +++) or reduce (negative; +) the activity of a particular pathway (i.e. pathway *A*) while having no effect (++) and/or antagonizing (+) an alternative pathway (i.e. pathway *B*) in the presence of an orthosteric agonist. Figure generated with Biorender.com.

prenatally, and those that do survive lack chronotropic or inotropic responses after administration of isoproterenol.[42] Although endogenous β_2ARs are present in β_1AR$^{-/-}$ hearts, they do not functionally compensate for the absence of β_1ARs, suggesting that the β_1AR subtype predominantly mediates the chronotropic/inotropic response in the heart.[42] As further evidence for this, while β_1AR/β_2AR double knockout and β_1AR$^{-/-}$ mice

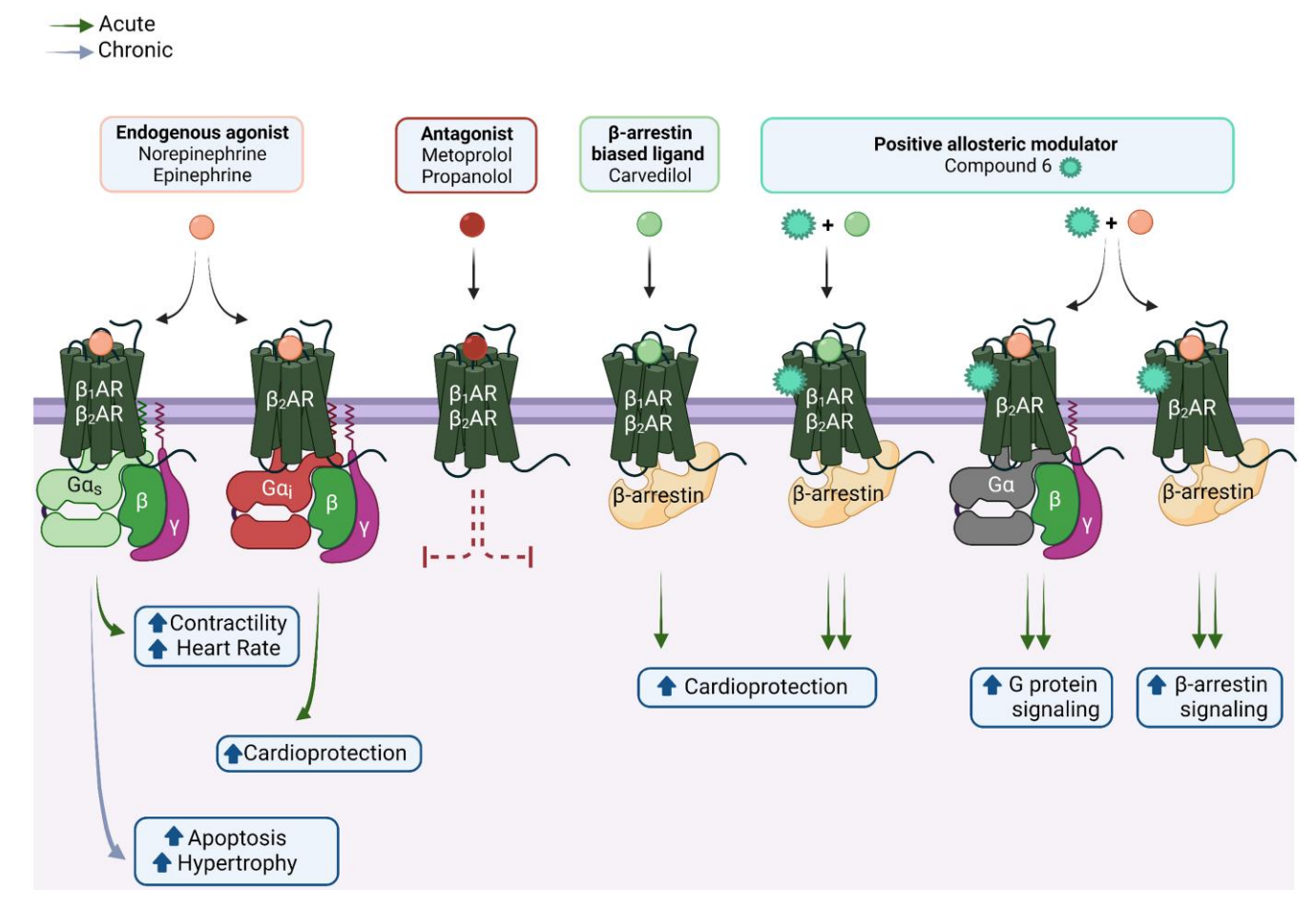

Figure 3 Physiological and pathological effects of cardiac βAR signalling. Norepinephrine and epinephrine, the endogenous βAR agonists, activate both β_1AR and β_2AR subtypes, albeit with different potencies. In general, β_1AR is coupled to $G\alpha_s$ and β_2AR is coupled to both $G\alpha_s$ and $G\alpha_i$. In the short term, the activation of β_1AR/β_2AR through $G\alpha_s$ generates positive chronotropic and inotropic responses; however, chronic activation through $G\alpha_s$ can induce cardiomyocyte apoptosis and hypertrophy. In contrast, $G\alpha_i$-mediated signalling via β_2AR is thought to be cardioprotective due to its anti-apoptotic and anti-fibrotic effects. Furthermore, both receptor subtypes can couple to β-arrestin, which also induces cardioprotective signalling cascades in the heart. Carvedilol, a clinically utilized β-arrestin-biased ligand, preferentially stimulates β-arrestin signalling while antagonizing G protein signalling. Notably, carvedilol-mediated β-arrestin-dependent cardioprotection is potentiated by Compound 6, a positive allosteric modulator of the β_1AR and β_2AR. Additionally, in response to agonist, Compound 6 potentiates G protein and β-arrestin signalling mediated by β_2AR, but not β_1AR. As expected, traditional antagonists such as metoprolol or propranolol, block βAR signalling via both G protein and β-arrestin. Figure generated with biorender.com.

lack heart rate and contractile responses to catecholamine infusion,[43,44] adult $\beta_2AR^{-/-}$ mice respond normally.[43,45] Moreover, progressive cardiac dysfunction following myocardial infarction is attenuated in $\beta_1AR^{-/-}$ and β_1AR/β_2AR double knockout mice, but not $\beta_2AR^{-/-}$ mice, demonstrating that regulation of cardiac function in normal hearts and following injury is predominantly mediated through β_1ARs.[43]

On the other hand, the role of β_3AR in cardiac function is relatively unclear. Several studies have reported that pharmacological activation and/or overexpression of β_3ARs reduced contractility in healthy hearts,[46–48] while others have suggested that β_3AR overexpression will augment contractility.[28] Moreover, overexpression or selective stimulation of β_3AR by BRL 37 344 is protective against cardiac remodelling and dysfunction in response to pressure overload, myocardial infarction, or neurohormonal stimulation through a nitric oxide synthase (NOS)-mediated pathway.[49–51] However, other studies have revealed that β_3AR antagonism via administration of L-748 337 improved the cardiac function and exercise performance in a canine model of pacing-induced heart failure.[52] Therefore, additional studies are needed to further interrogate the potential protective or detrimental impacts of β_3AR in the heart.

2.4 Pharmacological perspectives

β-Blockers competitively antagonize the binding of endogenous catecholamines to the orthosteric site of βARs and are a mainstay in the treatment of chronic heart failure. Among the many different clinically utilized β-blockers,[53] carvedilol possesses unique properties by virtue of its ability to recruit and activate cardioprotective β-arrestin signalling at both the β_1AR and β_2AR.[54–57] Specifically, carvedilol stabilizes βARs in a particular conformation that initiates β-arrestin-mediated signalling while remaining uncoupled from $G\alpha_s$.[54] While the evidence that carvedilol is more effective as a therapeutic agent comes exclusively from experimental studies, a clinical trial revealed that carvedilol was superior to metoprolol in reducing all-cause mortality in patients with heart failure.[58]

Although β-blockers effectively reduce mortality, patients often experience side effects including fatigue and reduced functional capacity, which may limit their maximal effectiveness.[59] This has led to an effort to identify new βAR ligands, particularly β-arrestin-biased ligands[60] or allosteric modulators, aimed at preferentially enhancing the beneficial effects and minimizing the adverse effects of overall β-blockade.[61] Recently, a positive allosteric

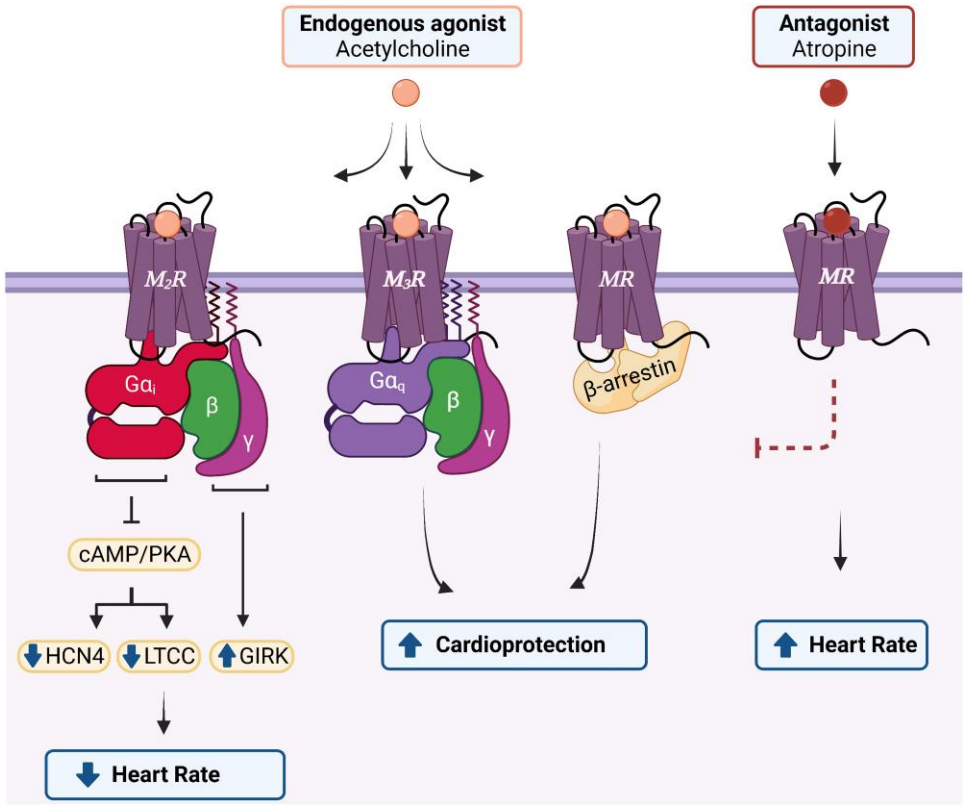

Figure 4 Physiological effects of cardiac MR signalling. The M_2R and M_3R, depicted herein as representative MR subtypes given that their physiological roles in the heart are the most extensively characterized, signal via distinct G protein classes. The M_2R, coupled to $G\alpha_i$, is the most abundant cardiac subtype and negatively regulates heart rate in response to the endogenous agonist, acetylcholine. In contrast, activation of M_3R, coupled with $G\alpha_q$, is considered cardioprotective due to its anti-arrhythmic/anti-fibrotic properties and protection against ischaemic injury. All MR subtypes couple to β-arrestin following agonist binding which initiates cardioprotective signalling cascades in the heart. In response to an antagonist, such as atropine, MR signalling is blocked and heart rate is increased. HCN4, hyperpolarization-activated cyclic nucleotide-gated 4 channel; LTCC, L-type Ca^{2+} channel; PKA, protein kinase A; GIRK, G protein-coupled inward rectifying K^+ channel. Figure generated with biorender.com.

modulator, Compound 6, was identified through a DNA-encoded small molecule library screen against the β_2AR.[62] Strikingly, Compound 6 increases the binding affinity of carvedilol for β_1ARs[57] and β_2ARs,[63] potentiates carvedilol-stimulated β-arrestin-dependent signalling,[57,63] and provides enhanced cardioprotection to ischaemic injury.[57]

3. Muscarinic receptors

Muscarinic receptors (MRs) comprise a family of GPCRs that are activated by the principal neurotransmitter of the parasympathetic nervous system, acetylcholine (*Figure 4*). The five major subtypes, M_1R through M_5R, encoded by genes *CHRM-1* to *CHRM-5,* respectively, range in size from ~460–590 amino acids in length and exhibit significant sequence homology to each other.[64,65] Given their ubiquitous expression across all organs,[66] they are implicated in a wide array of physiological processes ranging from the regulation of cognitive, behavioural, sensory, motor, and autonomic functions mediated by MRs in the central nervous system (CNS), to the regulation of heart rate, smooth-muscle contraction, and glandular secretion mediated by MRs in peripheral tissues.[67,68]

3.1 Tissue expression and localization

The M_2R is the predominant subtype in the heart[66] where its expression is almost 2.5-fold higher in the human atrium than in ventricles.[69] The other subtypes are present in the heart as well, although to a lesser extent than M_2R. While mRNA encoding all five subtypes has been reported in both atrial and ventricular human myocardium, immunoblotting experiments have only confirmed the expression of M_1R, M_2R, M_3R, and M_5R, but not M_4R, at the protein level.[70] Consistently, antibodies to all subtypes besides M_4R positively stain the sarcolemma of human ventricular cardiomyocytes, with M_3R and M_5R additionally exhibiting strong localization to the intercalated disc.[70] Although M_4R protein expression has not been reported in the human heart, both functional and binding assays have revealed its presence in atrial myocardium from canines.[71] This suggests that all five subtypes might be physiologically relevant in the heart but emphasizes that their functional significance may be species-dependent.

Evaluation of MR abundance in human patients with heart disease, on the other hand, has generated conflicting results. Early studies found a moderate, but not statistically significant, upregulation of M_2R in the left ventricles of patients with end-stage heart failure.[72] Corroborating this, positron emission tomography with radiolabeled 11C-MQNB, a muscarinic antagonist, revealed elevated MR levels in patients with idiopathic dilated cardiomyopathy perhaps as a compensatory mechanism to β-adrenergic signalling.[73] However, recent studies measuring the levels of MRs in atrial tissues from patients with chronic atrial fibrillation reported no change in M_2R expression, but an upregulation of the M_1R subtype.[74] This suggests that different cardiac disease states, chamber-specific differences, and/or receptor subtype-specific differences are important factors to consider when evaluating the impact of MRs in cardiac disease.

3.2 Cellular signalling mechanisms

Belonging to the Class A (rhodopsin-like) subtype of GPCRs, MRs undergo a stereotypical activation mechanism wherein agonist binding induces an outward movement in transmembrane helix 6 (TM6) that exposes a G protein binding cavity on the cytoplasmic surface.[75] Molecular dynamics simulations of different agonist-occupied receptor conformations[76] and cryo-EM structures of either M_1R or M_2R in complex with distinct heterotrimeric G protein families[77] further revealed that conformational rearrangements in TM5, 6, and 7 correlate with the selective coupling of MRs to different G protein classes. Specifically, M_2R and M_4R, mediate signalling via $G\alpha_i$, whereas M_1R, M_3R, and M_5R predominantly couple to $G\alpha_q$.[66]

The M_2R and M_4R receptors are coupled to the $G\alpha_i$ signal to inhibit AC leading to reduced PKA activity, reduced L-type calcium channel current, and suppression of the hyperpolarization-activated cyclic nucleotide-gated 4 (HCN4) channel. The HCN4 channel is a non-selective cation channel permeable to Na^+/K^+ that mediates the pacemaker or 'funny' current (I_f) in nodal cells and conduction tissues.[78] Together, these mechanisms collectively counteract the positive chronotropic effects mediated by β-adrenergic-$G\alpha_s$ signalling, thereby modulating the heart rate response. In contrast, $G\alpha_q$-coupled receptors M_1R, M_3R, and M_5R promote the activation of PKC and stimulate calcium influx via second messengers DAG and IP_3, respectively.

In addition to the signalling mechanisms mediated by Gα, the Gβγ subunit of the $G\alpha_i$ complex directly couples and activates the G protein-coupled inward rectifying K^+ (GIRK) channel leading to K^+ influx, hyperpolarization, and slowed heart rate in response to MR activation.[79] Using atrial-specific or ventricle-specific GIRK knockout mice, Lee and colleagues demonstrated that MR-dependent regulation of heart rate is primarily mediated via atrial GIRK activation, rather than GIRK channels expressed in the ventricle.[80] Of note, this mechanism can be activated by the Gβγ subunits originating from $G\alpha_q$-coupled M_1R stimulation as well.[74] Finally, like other GPCRs, MRs are phosphorylated by GRKs in an agonist-dependent fashion resulting in the recruitment of β-arrestins which facilitate receptor desensitization, internalization, and downstream signalling.[81]

3.3 Physiological functions and contribution to disease

The lack of subtype-specific MR ligands and their heterogenous distribution across tissues has complicated the investigation of the individual roles of each member of the MR family. In order to study the unique function of each receptor subtype, mice constitutively deficient in each of the MRs have been generated via gene targeting.[67] In addition to a wide array of cognitive and behavioural phenotypes mainly mediated through the loss of MRs in the CNS,[67] mice deficient in MRs also exhibit profound abnormalities in cardiac autonomic regulation. In particular, while the application of carbachol, a muscarinic agonist, induced bradycardia in isolated atrial cells from wild-type mice, atrial cells from *CHRM2*$^{-/-}$ (M_2R knockout) mice remained unaffected.[82] In contrast, cardiomyocytes from M_4R knockout mice, *CHRM4*$^{-/-}$, show a very modest decrease in carbachol-induced bradycardia, suggesting that the M_4R could instead participate in maximizing the bradycardic effects of carbachol stimulation, at least in the species where it is expressed.[82] Although all other subtypes are detected to some level in the heart, the lack of compensatory effects mediated by the remaining subtypes in *CHRM2*$^{-/-}$ mice suggests that the M_2R subtype predominantly mediates the negative chronotropic response in the heart.[67,82]

A significant functional role has been established for the $G\alpha_q$-coupled M_3R in the heart.[83–88] In particular, pharmacological activation and/or overexpression of the M_3R have been associated with reduced cardiac hypertrophy and myocardial injury induced by angiotensin II,[83] reduced frequency of ventricular arrhythmias, cardiac fibrosis and electrical remodelling following chronic cardiac pressure overload,[84–87] and is protective against cardiac damage following ischaemic injury.[88] In contrast, the physiological roles of the remaining $G\alpha_q$-coupled receptors in the muscarinic family, M_1R and M_5R, remain largely unstudied in the heart.

Notably, several recent studies have investigated the protective potential of cardiac MR stimulation and downstream signalling. For example, modulating MR activity could be clinically useful in the treatment of doxorubicin-induced cardiotoxicity. Cancer patients being treated with anthracycline-based chemotherapies (such as doxorubicin) are susceptible to developing left ventricular systolic dysfunction and arrhythmia, likely arising from imbalanced cardiac autonomic signalling.[89,90] Notably, treatment with muscarinic agonist bethanechol improved overall cardiac function in a rat model of doxorubicin-induced cardiotoxicity by enhancing mitochondrial function, and reducing mitochondrial oxidative damage, cardiomyocyte apoptosis, and myocardial inflammation.[89] In addition, recent studies conducted in zebrafish embryos demonstrated that the MR antagonist, tolterodine, initiates transcriptional changes in the heart that promote pacemaker cell fate. These findings proposed a new role for MRs in cardiac development and underscore the potential utility of MR antagonists as a therapy for developmentally related cardiac conduction system disorders.[91]

3.4 Pharmacological perspectives

Excessive sympathetic signalling and attenuated parasympathetic activity manifest early in the development of cardiac disease. In fact, depressed parasympathetic activity can occur as early as three days after the development of cardiac dysfunction and precedes the upregulation of sympathetic activity.[92] Most current pharmacological interventions aim to suppress the over-active sympathetic nervous system (i.e. beta blockers). However, targeted modulation of parasympathetic pathways (i.e. via MR ligands) remains relatively under-exploited.

Aside from atropine, a muscarinic antagonist that is clinically utilized to acutely treat marked symptomatic bradycardia,[93] the majority of muscarinic agonists or antagonists characterized to date have been mainly developed for a variety of non-cardiac conditions such as glaucoma, asthma, smooth-muscle disorders, chronic obstructive pulmonary disease (COPD), peptic ulcer, Sjorgren's syndrome, cancer, and various neurological disorders.[68] Due to the lack of subtype-selective MR ligands, and the ubiquitous expression of MR subtypes across different tissues, the clinical utility of the current therapies is limited due to the presence of non-selective side effects.[68] For instance, the use of tiotropium, one of the six licensed MR antagonists used as a bronchodilator agent for the treatment of COPD,[94] has the potential for cardiovascular risk. As evidence for this notion, recent studies have revealed that perfusion of tiotropium in the rat heart leads to cardiomyocyte cell damage via pathological calcium signalling.[95]

A major barrier in the development of subtype-selective MR drugs is the high degree of conservation of the amino acids forming the orthosteric binding pocket across the different subtypes.[64] In particular, the five subtypes share 64–82% sequence identity overall and 82–92% identity within the transmembrane region which harbours the orthosteric binding pocket.[64,77] Importantly, the crystal structure of the M_3R revealed the presence of a relatively large cavity separate from the orthosteric binding pocket.[96] Although the structural feature is generally conserved among all subtypes, the amino acids surrounding this region are more diverse, which potentially makes it a target for subtype-selective allosteric modulators.[97] In line with this, allosteric modulators for the M_1R, M_4R, and M_5R receptors have been developed that are predicted to bind to this region.[98] To date, many subtype-specific allosteric modulators have been identified for all five MRs, however, they have predominantly been pursued for the treatment of CNS disorders.[99] This warrants the further discovery and characterization of subtype-selective muscarinic allosteric modulators that might be clinically useful in the treatment of cardiovascular disease as well.

4. Angiotensin II receptors

Angiotensin II type 1 (AT_1R) and Type 2 (AT_2R) receptors, endogenously activated by the peptide hormone, Angiotensin II (Ang II), are additional members of the GPCR superfamily that are fundamental for the regulation of cardiovascular physiology (*Figure 5*). Although the role of AT_1R is well

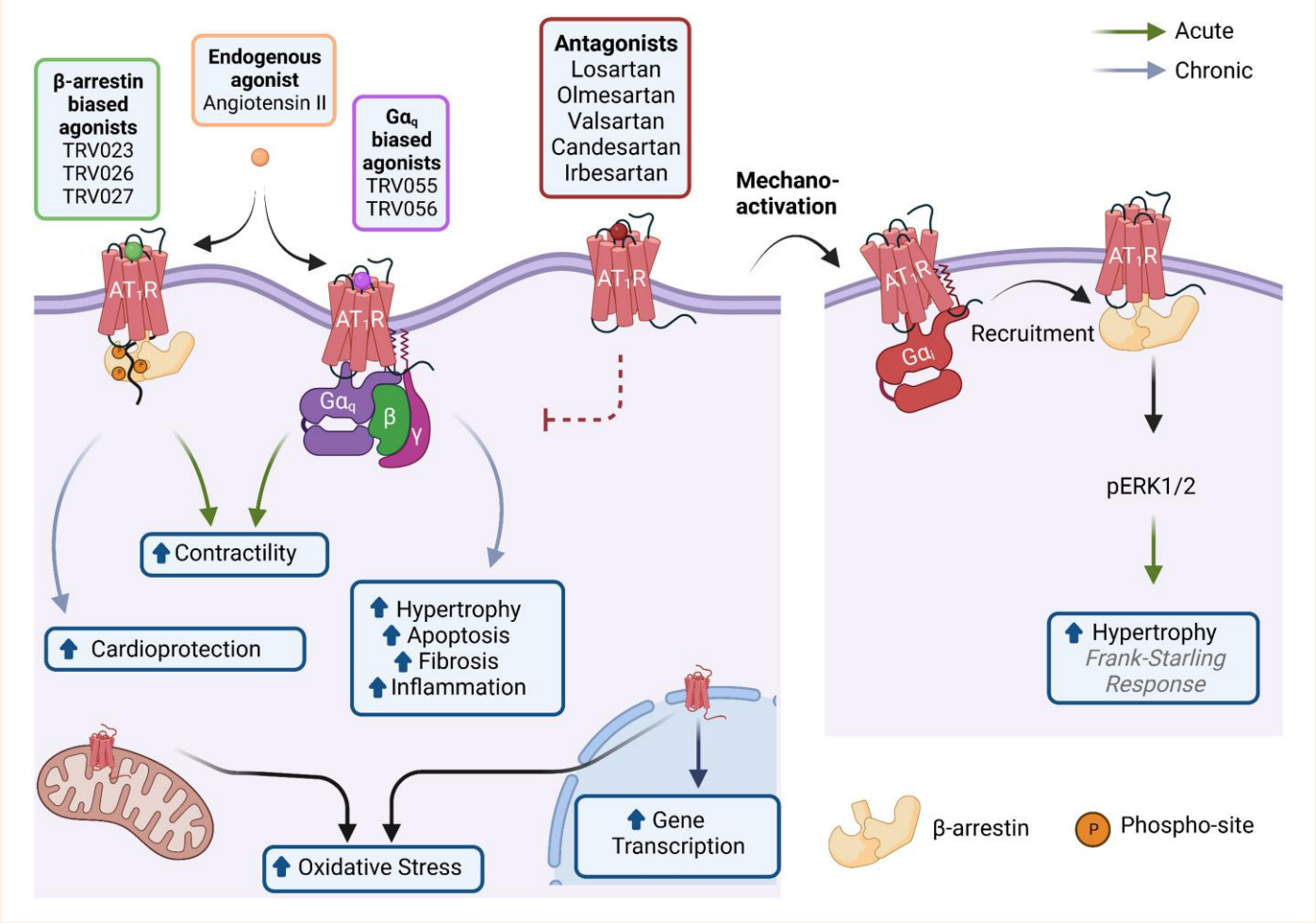

Figure 5 Physiological and pathological effects of cardiac AT_1R signalling. The AT_1R is a pleiotropic receptor that localizes to the cellular, mitochondrial, and nuclear membranes of cells. Ligands that preferentially stabilize the AT_1R/β-arrestin complex (TRV023, TRV026, TRV027) increase the contractility of cardiomyocytes and have cardioprotective effects under long-term use. Likewise, ligands that preferentially stabilize the AT_1R/Gαq complex (TRV055 and TRV056) increase the contractility of cardiomyocytes. However, chronic activation of this signalling pathway promotes cardiac hypertrophy, apoptosis, fibrosis, and inflammation. The endogenous ligand for the AT_1R, Ang II, is unbiased towards both signalling pathways. AT_1R antagonists such as Losartan, Olmesartan, and Valsartan, among others, stabilize the receptor in an inactive conformation thereby blocking the activation of any signalling cascade. Another activation mechanism of the AT_1R is via membrane stretch. In turn, β-arrestin is recruited in a process mediated by $G\alpha_i$ and activates ERK1/2 phosphorylation regulating the Frank–Starling mechanism and inducing hypertrophy. Finally, the activation of the AT_1R at mitochondrial or nuclear membranes induces oxidative stress and activates gene transcription. Figure generated with Biorender.com.

established in the heart, the significance of AT_2R remains highly controversial since altering the expression of AT_2R does not affect cardiac function[100,101] and findings regarding the role of AT_2R in cardiac pathology appear contradictory. Therefore, we mainly focus on the AT_1R while providing a summary of the most relevant aspects of the AT_2R in cardiac pathophysiology.

The translation of human AT_1R comes from a single gene, *AGTR1*, which shares ~95% homology to bovine and rat AT_1Rs.[102] Comparatively, two separate AT_1R genes are found in mice and rats, *Agtr1a* and *Agtr1b*, that encode for the $AT_{1A}R$ and $AT_{1B}R$, respectively. Both murine receptors share ~95% homology and are functionally identical, yet are expressed in a tissue-specific manner.[103] The $AT_{1A}R$ is the predominant isoform in rodent hearts and is therefore the main subtype addressed in this review when discussing AT_1R in rodent models. Multiple reviews with a specific focus on the structure, trafficking, function, and/or pathophysiology of the AT_1R are also available for complementary information.[4,104–106]

4.1 Tissue expression and localization

The AT_1R is expressed in all cell types of the heart including the endothelial cells,[107] vascular smooth-muscle cells,[108] fibroblasts,[109] myocardial cells,[110] and immune cells.[111] The AT_1R represents 59% of the total AT receptors in human ventricles, compared to its homologue subtype, the AT_2R, which comprises the remaining 41%.[112] Conversely, the human atrium shows an inverted relationship where the AT_1R:AT_2R ratio is 30:70.[113] Acute heart injury such as myocardial infarction upregulates AT_1R expression, whereas chronic cardiac damage such as dilated cardiomyopathy downregulates its expression.[112] Thus, the temporal nature of the cardiac disease is a key regulator of AT_1R levels. Beyond the cellular membrane, AT_1R further localizes to nuclear and mitochondrial membranes in the heart where it activates gene transcription and induces oxidative stress, respectively.[114,115] This suggests that the localization within the cell is also an important factor for the signal transduction and function of AT_1R.

4.2 Cellular signalling mechanisms

The ubiquitous expression of AT_1Rs along with the endocrine, paracrine, autocrine, and/or intracrine effects of its endogenous ligand, Ang II, are main contributors to the multiple cellular effects (i.e. growth, contractility, inflammation, fibrosis, and apoptosis, among others) regulating the cardiovascular system.[104] Independent from Ang II, AT_1Rs can also be activated by the mechanical forces exerted within the cardiovascular system.[116,117] Moreover, the AT_1R can couple to multiple intracellular transducers including several G protein subtypes (i.e. $G\alpha_{q/11}$, $G\alpha_s$, $G\alpha_{i/o}$, and $G\alpha_{12/13}$) and β arrestins to initiate different cellular responses.[118,119] The best-characterized signalling pathways for the AT_1R in physiological conditions are the canonical $G\alpha_q$ pathway and the β arrestin pathway.

Like other GPCRs, the dynamic structure of the AT_1R permits multiple active conformations that preferentially interact with distinct transducers and can be further stabilized by biased ligands.[120,121] Importantly, the development of biased ligands for the AT_1R has allowed for a more in-depth exploration of the signalling pathways downstream of the AT_1R and their biological consequences. This is clearly illustrated in a recent proteomics study utilizing proximity ligation assay to identify more than a thousand functional and structural proteins proximal to the AT_1R after activation with unbiased or biased ligands for the $G\alpha_q$ or the β-arrestin pathway.[122] Understanding the effects of these ligands in the heart will enable more precise modulation of AT_1R activity and will allow for the development of novel strategies to preferentially target beneficial signalling cascades in disease states.

4.3 Physiological functions and contributions to disease

Upon activation, Ang II binds to the AT_1R and increases cardiomyocyte contractility by coupling to the canonical $G\alpha_q$ pathway as well as β-arrestin.[123] While the latter observation was first shown *in vitro* using [Sar1, Ile4, Ile8]-Angiotensin II (SII), the first β-arrestin-biased ligand available for the AT_1R,[123] this concept was later demonstrated *in vivo* using the β-arrestin-biased AT_1R ligands TRV023 and TRV027.[124,125] While the regulation of calcium is central to the contractile response induced by both Ang II and β-arrestin-biased ligands (TRV023 and TRV 027), the mechanisms by which they regulate intracellular calcium are distinct. Specifically, stimulation with Ang II leads to increased intracellular calcium concentration via enhanced calcium release through the AT_1R-$G\alpha_q$-IP_3/PKC signalling axis.[126] In contrast, β-arrestin-biased activation of the AT_1R increases myofilament calcium sensitivity without altering the global intracellular calcium transient, in part through modifying the phosphorylation status of myofilament proteins.[127–129]

In contrast to acute activation, chronic AT_1R stimulation by direct stimulation with Ang II,[130,131] overexpression of AT_1R, or expression of constitutively active mutants of the AT_1R[132,133] induces cardiac hypertrophy. Similarly, increasing the activity of the local renin angiotensin system (RAS) of the heart by angiotensinogen overexpression, the main precursor of Ang II, also induces cardiac hypertrophy.[134] The hypertrophic response induced by chronic AT_1R stimulation is largely mediated by $G\alpha_q$, since *in vivo* overexpression of $G\alpha_q$ or *in vitro* transfection of a constitutively activated $G\alpha_q$ mutant induces hypertrophy and apoptosis in cardiomyocytes.[135] Moreover, overexpression of an inhibitor of $G\alpha_q$ blocks the development of cardiac hypertrophy induced by pressure overload.[136] The alternative β-arrestin pathway might also contribute to this effect since cardiac-specific overexpression of the AT_1R mutant lacking $G\alpha_q/G\alpha_i$ coupling also induces hypertrophy.[131,137] Nevertheless, numerous studies have demonstrated that β-arrestin-biased activation of AT_1R is cardioprotective via promoting cardiomyocyte cell survival.[116,125,138]

Although the hypertrophic response mediated by chronic AT_1R stimulation is well established, it has not been consistently observed. Recent studies demonstrate that mice overexpressing AT_1R or a constitutively active AT_1R mutant do not undergo hypertrophy despite developing fibrosis and/or ventricular dysfunction.[139,140] Moreover, AT_1R knockout mice develop cardiac hypertrophy following pressure overload or myocardial infarction, suggesting that the AT_1R may not be critical for the development of hypertrophy in heart failure.[141,142] Accordingly, chronic stimulation with Ang II in mice lacking AT_1R in the kidney, yet expressing physiological levels of AT_1R in the heart, does not develop cardiac hypertrophy.[143] In line with this, selective expression of the AT_1R in the kidney or in resistance vessels mimics the cardiac hypertrophy induced by chronic Ang II treatment observed in wild-type mice.[143,144] Taken together, the development of cardiac hypertrophy following the manipulation of AT_1R could be secondary to the increased peripheral resistance that induces pressure overload in the heart.

Other paracrine, stretch, and transactivation mechanisms of the AT_1R are also contributors to cardiomyocyte growth. Indeed, Ang II-stimulated cardiac fibroblasts secrete exosomes and multiple cytokines such as transforming growth factor (TGF-1β) and interleukin-6 that stimulate cardiomyocytes to increase local Ang II production, AT_1R expression, and induce hypertrophy.[145–148] In addition, AT_1R can be activated by mechanical stretch independent from Ang II, leading to hypertrophic growth.[117] This mechano-activation mechanism appears to be dependent on $G\alpha_i$ and β-arrestin pathways.[116,149,150] Mechanical stretch also increases the expression of the AT_1R and other components of the RAS, while Ang II decreases AT_1R expression in the heart.[151] Therefore, mechano-activation of the AT_1R behaves as a positive feedback loop for the cardiac RAS. Notably, this mechanism underlies the Frank–Starling response which describes the length-dependent activation of contractility wherein increased cardiac filling, and thus increased sarcomere length, enhances the force of contraction.[127] Finally, AT_1R stimulation transactivates EGFR through multiple intracellular mechanisms including β-arrestin recruitment and direct association/dimerization between both receptors ultimately inducing hypertrophy.[152] Aside from cardiomyocyte growth, AT_1R activation also leads to apoptosis, hyperplasia, fibrosis, and oxidative stress, which further contribute to disease.[130,132,133,139]

4.4 Pharmacological perspectives

Eight AT_1R blockers (ARBs), which stabilize the receptor in an inactive state, have been approved by the FDA and are clinically utilized for the treatment of multiple cardiovascular diseases including heart failure.[153,154] The AT_1R β-arrestin-biased ligand TRV027 was also recently proposed as pharmacotherapy for acute heart failure. However, TRV027 failed to improve the clinical status of patients with this condition in clinical trials.[155] This unexpected outcome might be related to the short duration of the treatment (1 month). Accordingly, Ryba and colleagues showed that a 3-month treatment with the β-arrestin-biased ligand TRV067 improves the cardiac function of mice with dilated cardiomyopathy.[156] Alternatively, the developmental stage might also modify the efficacy of the treatment, since activation of the AT_1R-β-arrestin2 pathway in neonatal or immature cardiomyocytes induces sustained cardiac contractility[157] whereas this effect is short lasting in adult hearts of mice or rats.[124,158] Thus, refocusing these novel compounds to treat heart failure for a longer period or in the paediatric population might prove therapeutic.

4.5 Angiotensin II Type 2 receptor

The AT_2R subtype, only ∼34% identical to the AT_1R,[159] exhibits unique transducer coupling properties and complex pathophysiological effects. The first crystal structure available of the AT_2R showed a non-canonical position of helix 8 that blocked the binding sites of G proteins and β-arrestins.[160] This finding is consistent with multiple studies that reported a lack of signalling through G proteins or internalization.[161] The recently crystalized AT_2R bound to Ang II shows a rather canonical outward position of helix 8, raising the possibility of conformational selection by different ligands to induce multiple responses.[162] Besides Ang II, new endogenous ligands derived from Ang II, such as Ang 1–7 and Ang 1–9, have been described as agonists for the AT_2R that may be beneficial in the heart.[163,164]

In the human heart, AT_2R has been detected in cardiomyocytes,[165] fibroblasts,[112] and coronary arteries.[166] During heart failure or dilated cardiomyopathy, AT_2R levels are downregulated or upregulated, respectively,

suggesting that the regulation of the AT_2R depends on the aetiology of the disease, or perhaps reflects alterations in the relative proportions of different cell types during the pathological process of adverse cardiac remodelling.[112,165] Moreover, studies evaluating the contribution of AT_2R to cardiac disease show conflicting results. Some claim that AT_2R activation is associated with decreased hypertrophy and fibrosis.[167–169] Conversely, others report that AT_2R overexpression results in constitutive hypertrophy in neonatal rat cardiomyocytes, fibrosis, and heart failure,[170,171] whereas deletion of the AT_2R decreases hypertrophy following chronic Ang II administration or myocardial infarction.[172,173] These discrepancies might be explained by the expression levels of the AT_2R during the cardiac insult.[174] Thus, pharmacological activation of AT_2R during disease states when there are high expression levels of AT_2R might prove therapeutic. Indeed, multiple recent studies using AT_2R agonists in models of heart disease have shown cardioprotective effects in rodents.[164,175,176] In summary, the AT_2R is a potential therapeutic target in cardiac disease that needs further examination to better understand its pathophysiological significance.

5. Conclusion

GPCRs serve as excellent therapeutic targets for the identification of novel heart failure treatments given their diverse and essential roles in cardiac health and disease. The β-adrenergic, muscarinic, and angiotensin receptor families exemplify only 3 of the >200 types of GPCRs in the heart that fundamentally regulate cardiac function and demonstrate great pharmacological potential. Importantly, recent advancements in understanding the mechanisms of biased signalling coupled with the identification of novel allosteric modulators have enabled the discovery of more selective ligands possessing enhanced cardioprotective effects with reduced unwanted side effects. Nonetheless, the heterogeneous distribution of GPCRs across multiple cell types in the heart,[4,177] combined with their overlapping signalling networks, and localization to cellular compartments aside from the cell surface,[177–180] remain essential elements to consider in evaluating the physiological effects of these novel drugs. Further investigation of the biased signalling pathways, physiological functions, disease mechanisms, and pharmacological features of cardiac GPCRs will therefore prove impactful in designing novel therapeutics.

Conflict of interest: H.A.R. is a scientific cofounder of Trevena Inc., a company that is developing new GPCR ligands.

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