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Targeting β_3-Adrenergic Receptors in the Heart: Selective Agonism and β-Blockade

Alessandro Cannavo, PhD and Walter J. Koch, PhD

Abstract: Cardiac diseases, such as heart failure, remain leading causes of morbidity and mortality worldwide, with myocardial infarction as the most common etiology. HF is characterized by β-adrenergic receptor (βAR) dysregulation that is primarily due to the upregulation of G protein–coupled receptor kinases that leads to overdesensitization of β_1 and β_2ARs, and this clinically manifests as a loss of inotropic reserve. Interestingly, the "minor" βAR isoform, the β_3AR, found in the heart, lacks G protein–coupled receptor kinases recognition sites, and is not subject to desensitization, and as a consequence of this, in human failing myocardium, the levels of this receptor remain unchanged or are even increased. In different preclinical studies, it has been shown that β_3ARs can activate different signaling pathways that can protect the heart. The clinical relevance of this is also supported by the effects of β-blockers which are well known for their proangiogenic and cardioprotective effects, and data are emerging showing that these are mediated, at least in part, by enhancement of β_3AR activity. In this regard, targeting of β_3ARs could represent a novel potential strategy to improve cardiac metabolism, function, and remodeling.

Key Words: β-adrenergic receptor, G proteins, heart failure, β-blockers

(*J Cardiovasc Pharmacol*™ 2017;69:71–78)

INTRODUCTION

G protein–coupled receptors (GPCRs) are nodal regulators of mammalian cell physiology because they transduce cell signals from diverse ligands such as neurohormones, sensory stimuli, and ions through heterotrimeric G proteins.[1] In the heart, they represent the major modulators of both function and morphology with β-adrenergic receptors (βARs) representing "the heads of the line", and for this reason they are considered the most important molecular targets in the cardiovascular system.[2–4] Currently, 3 βAR subtypes (β_1AR, β_2AR, and β_3AR) have been identified in the myocardium, with β_1 and β_2ARs the most expressed and studied.[5–7] However, since its discovery in 1989,[7] it soon seemed clear that β_3ARs, the isoform with minor expression, can influence cardiovascular physiology. In particular, β_3ARs seem to have multiple roles that go from regulation of metabolism,[8,9] vasodilation, and relaxation[8] to cardiac contractility.[9] Thus, this receptor is of high interest especially for new potential therapeutic approaches for heart disease.

In this review, we will discuss what is known about the cardiac role of β_3ARs and how not only their activation but also the blockade could be beneficial or not in cardiac physiology and in disease.

β_3-Adrenergic Receptor (β_3AR) Structure

The mammalian β_3AR sequence consists of about 400–408 amino acids in a protein that has the typical structure of all GPCRs.[10] The β_3AR has 7 transmembrane domains (7-TMDs) with an extracellular N-terminal that is glycosylated, and an intracellular C-terminal domain.[10] Further, the Cys361 residue in the fourth intracellular domain is palmitoylated, a feature that has been shown to be associated with G protein-coupling and adenylyl cyclase stimulation following agonist stimulation of the receptor[11] (Fig. 1). As shown in Figure 2, the protein sequence alignment between different mammalian species demonstrates that most of the homology between the β_3AR amino acid sequences is concentrated in the 7-TMDs and in the membrane-proximal regions of the intracellular loops. Interestingly, when the β_3AR protein sequence is compared with other βAR (β_1 and β_2AR) isoforms, it is still possible to observe a high level of homology in the 7-TMD sequence, but a significant divergence is present both in the third intracellular loop and in the C-terminal domain (Fig. 3). This difference probably represents the major factor affecting the pharmacologic regulation of the receptors and their response to a ligand. In this regard, the C-terminus of both β_1 and β_2ARs is rich in serine and threonine residues, and is subjected to GPCR kinase (GRK)-mediated regulation through phosphorylation.[10] Further, these receptors also harbor a consensus sequence for protein kinase A (PKA).[10] Of note, the β_3AR lacks all of these sites, and is more resistant to agonist-induced desensitization/

Received for publication August 29, 2016; accepted October 16, 2016.
From the Department of Pharmacology, Center for Translational Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA.
Supported by American Heart Association 16POST30980005 (A.C.).
The authors report no conflicts of interest.
Reprints: Walter J. Koch, PhD, W.W. Smith, Chair in Cardiovascular Medicine, Department of Pharmacology, Center for Translational Medicine, Lewis Katz School of Medicine, Temple University, 3500 North Broad St, MERB 941, Philadelphia, PA 19140 (e-mail: walter.koch@temple.edu).

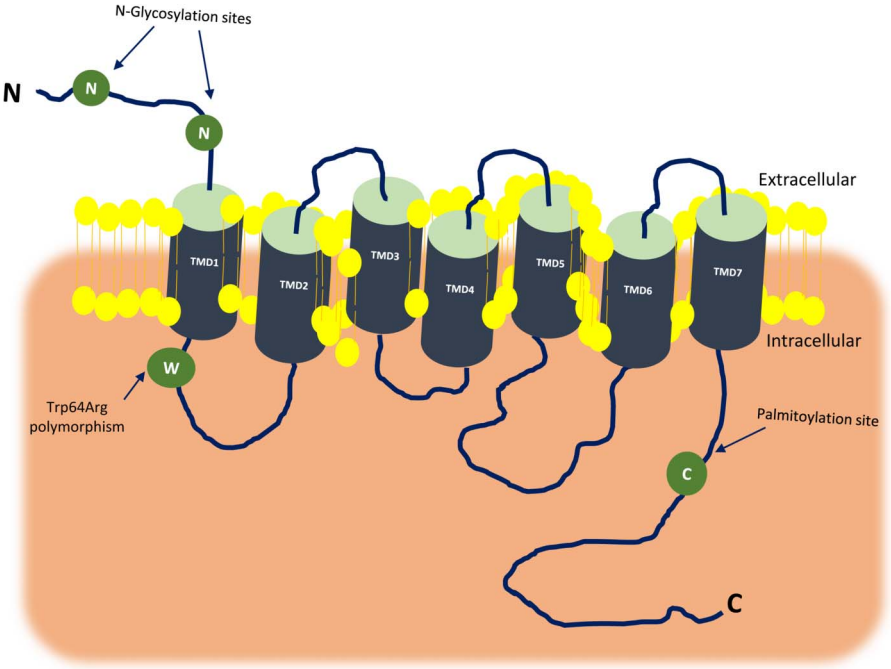

FIGURE 1. Human β_3AR structure. Shown here is the structure of the human β_3AR. The receptor is a GPCR with 7-TMDs, an extracellular N-terminal domain (exD1), and an intracellular C-terminal domain (inD4). The receptor presents also 6 loops, 3 are intracellular (inD1, inD2, and inD3), and 3 are extracellular (exD2, exD3, and exD4). Indicated with arrows are the asparagine (N) residues, in the exD1, that are sites of N-glycosylation; tryptophan (W) in position 64 that is the location of β_3AR-polymorphism (Trp64Arg) and the cysteine (C) in position 361 that is a site subjected to palmitoylation.

downregulation. Finally, these sequence divergences also support differential and intracellular signaling (including G protein-coupling) between the 3 βAR isoforms, which may determine their relative roles in physiology and in the disease.

β_3AR: in Search of Signaling and Function

The human β_3AR was cloned in 1989,[7] and the studies demonstrated that this new βAR isoform was mainly implicated in lipolysis and thermogenesis regulation in adipose tissues.[8,12] However, over the last 2 decades, different reports have also clearly shown that β_3ARs are present in the cardiovascular system, mainly in myocardium and endothelium, where they have a prominent role in modulating cardiac function and angiogenesis, respectively.[13,14] In this context, determining the specific pathways associated with these effects represents a tough challenge and remains a largely unresolved question regarding β_3AR function. This is due, at least in part, to the fact that the role of the cardiac β_3AR has not been studied with the same intensity as the β_1- and β_2ARs. Moreover, all the studies concerning β_3AR function have not been focused on similar cell types, and the agonist and the doses used are significantly different between most studies.[14,15] Another important difference is represented by the experimental model used in key studies.[15] In fact, it is known that in the mouse, the gene encoding for the β_3AR undergoes alternative splicing and gives rise to β_3aAR and β_3bAR variants.[16,17] These 2 β_3AR isoforms are coupled to adenylyl cyclase stimulatory Gα (Gαs) or to the inhibitory Gα (Gαi) protein subunits (β_3bAR), or exclusively to the Gαs protein (β_3aAR).[17,18] By contrast, in humans, although some reports have proposed that β_3AR can activate Gαs signaling in CHO/K1 cells[19] and in adipocytes,[20] it is a general assumption that, at least in ventricular myocardium, β_3ARs are mainly coupled with Gαi proteins.[21,22]

For these reasons, the β_3AR leads to effects that are either comparable or opposite to those elicited by β_1- and β_2AR stimulation. In fact, stimulation of β_3AR, through Gαs activation, increases the generation of cyclic AMP (cAMP) and the activation of the PKA, similar to β_1- and β_2ARs.[4,23] In the myocardium, after catecholamines stimulation of βARs, PKA phosphorylates many Ca^{2+} handling proteins and some myofilament components leading to positive inotropic, lusitropic, and chronotropic effects[4,23] (Fig. 4). However, because β_3ARs are also coupled with Gαi, they can act as a brake to prevent β_1 and β_2ARs overactivation, and this has been proposed as a mechanism in the heart[21,22] (Fig. 4). Moreover, in the heart, the stimulation of β_3ARs leads to increased endothelial nitric oxide (NO) synthase (eNOS)[22,24] or neuronal (nNOS) activation,[25,26] giving rise to NO generation and activation of soluble guanylate cyclase to produce cGMP and cGMP-dependent protein kinase G (PKG) activation[25–27] (Fig. 4). PKG is a serine/threonine kinase that mediates many of the biological effects of NO/cGMP.[28,29] In particular, PKG downstream of β_3ARs can enhance myocytes relaxation but cause negative inotropy, possibly through the phosphorylation of troponin I and L-type Ca^{2+} channel[26–29] (Fig. 4). Importantly, the β_3AR/NO-cGMP/PKG signaling axis seems to be a robust cardioprotective mechanism that can be beneficial in failing myocardium.[22,26,29] In fact, PKG activation downstream of cGMP has been proven to reduce Ca^{2+} oscillations which can cause ventricular arrhythmias, hypercontracture and sarcolemmal rupture as well as mitochondrial

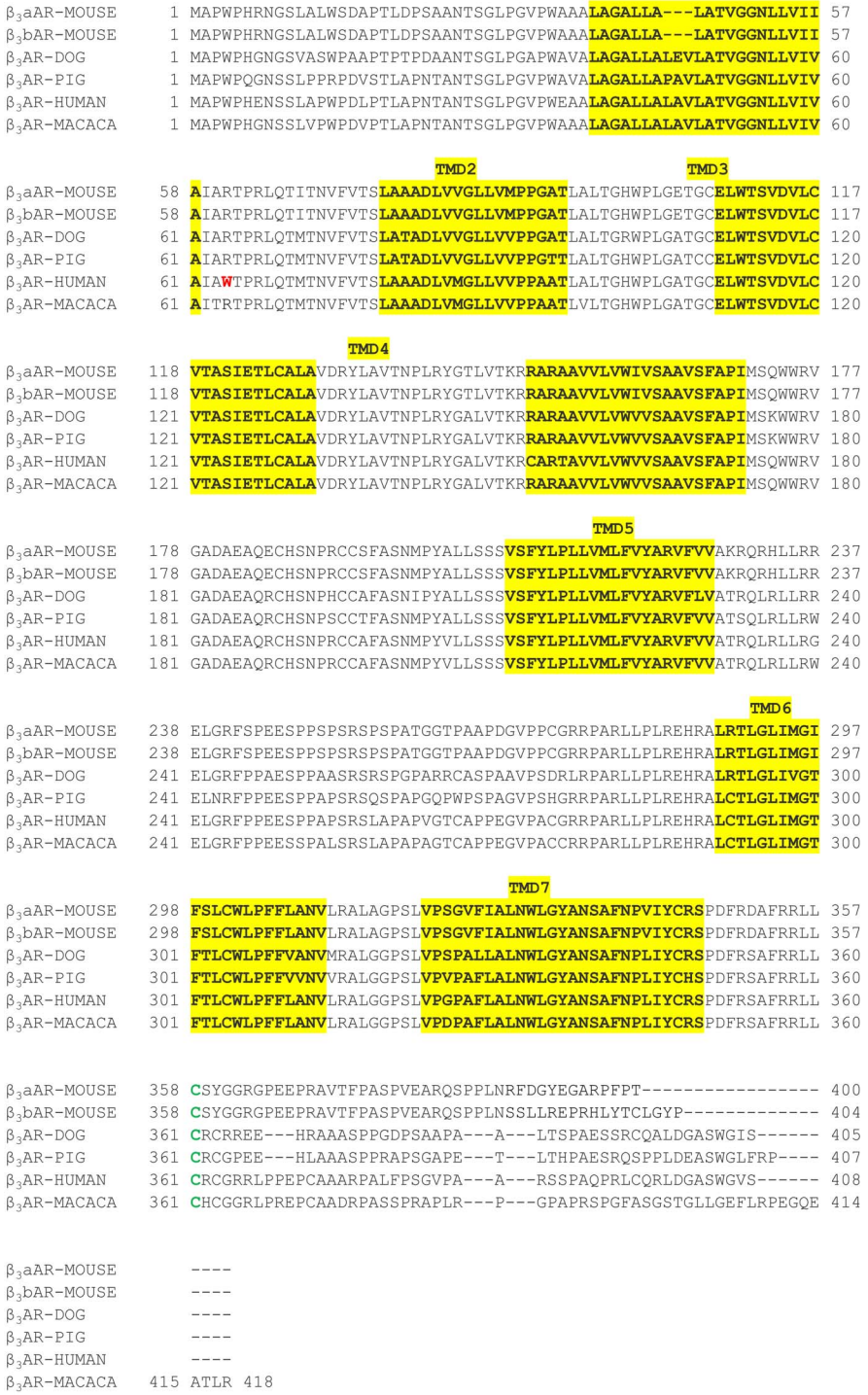

FIGURE 2. Multiple alignment of mammalian β_3AR. Protein sequence alignment of mouse, dog, pig, human, and *Macaca mulatta* β_3AR. The TMD is highlighted in yellow; the tryptophan (W) in position 64 is in red; the cysteine (C) in position 361 is in green.

permeability transition pore that is a cause of cell death.[30–32] Of note, both these phenomena occur during reperfusion of ischemic myocardium and can lead to progression toward heart failure (HF).[30–32]

Pertaining to specific species differences of the β_3AR, humans have a natural mutation. In fact, there is a nonsynonymous polymorphism at amino acid 64 where a tryptophan can exist instead of arginine (Figs. 1, 2). This mutation makes the β_3AR less responsive to catecholamine stimulation,[33] which has been associated with some pathophysiological conditions (ie, obesity)[34,35] (Box 1). This important difference suggests that nonhuman β_3AR and its signaling may not fully mirror the human system.

BOX 1. Pathologies Associated With β_3AR-Trp64Arg Polymorphism

1. Obesity, susceptibility to
2. Diabetes
3. Insulin resistance
4. Hyperuricemia

The β_3AR in Cardiac Disease

Accumulating evidence has revealed that the β_3AR, present in endothelium and myocardium, may have specific beneficial effects in the cardiovascular system including cardioprotection.[26] This becomes critically crucial in cardiac diseases such as HF, a syndrome characterized by decreased cardiac output, caused by deficits in contractility and/or relaxation. Importantly, after an injury such as ischemia, to preserve cardiac output, there is an increase in sympathetic activity and in catecholamine release to stimulate βAR-mediated inotropic capacity. However, chronic exposure of the heart to high levels of catecholamines can lead to further pathologic changes in the heart that can induce a progressive deterioration of cardiac function and structure. Of note, catecholamines directly stimulate βARs, and the sustained activation of these receptors correlate with left ventricular (LV) dysfunction and mortality.[36] In this regard, GRK2, the principal GRK involved in βAR regulation within the cardiomyocytes, phosphorylates the receptors attenuating their increased responsiveness.[4] This process, called desensitization, at early stage represents a protective mechanism, but in chronic stage can cause β_1 and β_2ARs dysregulation and signaling abnormalities (eg, downregulation and overdesensitization) and promote the progression of the disease.[4] Importantly, as discussed above, β_3ARs lack GRK recognition sites and are not subject to desensitization and downregulation, and in fact, their levels within human failing myocardium remain unchanged[37] or become upregulated.[38] Enhancement of β_3ARs could represent either a protective mechanism against the detrimental effects of chronic βAR stimulation or a detrimental mechanism that may lead to further deterioration of HF. In this regard, the role of this receptor in the heart has been debated for years, and some reports have suggested that due to its cardiodepressant effect, sustained activation of β_3ARs in HF could contribute to impaired cardiac function.[39] Consistently, the antagonism of this receptor has been proposed as a potential strategy against HF development.[39,40] However, by contrast with this hypothesis, it has been demonstrated that in the failing myocardium, β_3ARs are able to inhibit, through activation of Na^+-K^+ pump, the deleterious accumulation of Na^+ in cardiac myocytes[41,42] thus blocking cAMP generation,[43] and consequently, reducing the activation of the cAMP-downstream oxidative pathways. Mechanistically, this effect decreases the glutathionylation of the β_1 Na^+-K^+ pump subunit and enhances the Na^+-K^+ pump activity in presence of β_3AR agonists.[43]

In line with the notion that β_3AR activation in failing myocardium is not detrimental, studies strongly support the idea that overexpression or persistent activation of β_3AR is cardioprotective and can attenuate pathological LV hypertrophy induced by continuous infusion of isoproterenol and angiotensin II, or by transaortic constriction, in mice.[44,45] Importantly, as shown in these studies, the activation of NOS and subsequent NO generation represent the main mechanism responsible for β_3AR-induced cardioprotection. In agreement with this mechanism of action, another study, using small (mice) and large (pigs) animal models of ischemia/reperfusion (I/R) injury, demonstrated that administration of selective β_3AR agonist BRL 37344 positively affected infarct size (acutely) and LV function (chronically).[46] Further, this study showed that β_3AR/NO signaling decreased opening of the mitochondrial permeability transition pore, thus conferring protection to the cardiac cells against cell death.[46] Finally, it is well established that the role of exercise in cardioprotection,[47] and β_3ARs, has been also shown to be a mediator of this effect,[48] especially in a setting of cardiac I/R injury.[48] Of note, during exercise, it seems that Gαs is the key mediator of β_3AR-induced protection that leads to the PKA/Akt/eNOS activation, thus suggesting that, in some conditions, the β_3AR, similarly to the cardioprotective β_2AR,[49] is coupled with both Gαs and Gαi in cardiomyocytes (Fig. 4). In this regard, this signaling pathway activation has a multiple protective role in the injured myocardium like the promotion of revascularization of the ischemic tissue. In fact, activation of Akt and eNOS, and the consequent secretion of NO, has been proven to directly stimulate endothelial cell function and promote the neoangiogenesis.[50] Further, as discussed above, the eNOS-mediated generation of NO is also responsible for cGMP and PKG activation, thus directly conferring beneficial effects on the myocardium.[51]

β-Blockers and β_3ARs

As described above, β_3ARs have emerged as novel potential targets for the treatment of certain cardiovascular diseases including HF. The clinical relevance of this is further supported by the successful effects obtained with β-blocker treatment in patients with HF as they can block the noxious effects of catecholamines and prevent further β_1 and β_2ARs downregulation.[4] Importantly, as proposed by us and others, the use of β-blockers may influence the expression/activity of β_3ARs.[35,52,53] However, because there are some specific differences in β-blockers (β_1AR selective or nonselective), the full extent of whether any molecular changes in β_3ARs are significant contributors to the therapeutic mechanisms of βAR antagonism in HF still remains to be elucidated. Accordingly, answering these mechanistic questions is important and may lead to novel therapeutic advances in HF.

In 2007, it was first reported that a relationship between β-blockers and β_3AR may exist.[54] In particular, in an HF rat model induced by transaortic constriction, it has been shown that, although metoprolol (a selective β_1-blocker) treatment did not affect the expression levels of β_3AR which was increased after HF, the use of carvedilol (a nonselective β-blocker) resulted in a robust β_3AR downregulation.[54] Nevertheless, soon after this report, a number of studies proposed

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β3AR     1 --------MAPWPHENSSLAPWPDLP--------------TLAPNTANTSGLPGVPWEAA  38
β1AR     1 MGAGVLVLGASEPGNLSSAAPLPDGAATAARLLVPASPPASLLPPASESPEPLSQQWTA-  59
β2AR     1 ---------MGQPGNGS------------AFLLA----PNRSHAPDHDVTQQRDEVWVV-  34

                TMD1                                   TMD2
β3AR    39 LAGALLALAVLATVGGNLLVIVAIAWTPRLQTMTNVFVTSLAAADLVMGLLVVPPAATLA  98
β1AR    60 GMGLLMALIVLLIVAGNVLVIVAIAKTPRLQTLTNLFIMSLASADLVMGLLVVPFGATIV 119
β2AR    35 GMGIVMSLIVLAIVFGNVLVITAIAKFERLQTVTNYFITSLACADLVMGLAVVPFGAAHI  94

                               TMD3                              TMD4
β3AR    99 LTGHWPLGATGCELWTSVDVLCVTASIETLCALAVDRYLAVTNPLRYGALVTKRCARTAV 158
β1AR   120 VWGRWEYGSFFCELWTSVDVLCVTASIETLCVIALDRYLAITSPFRYQSLLTRARARGLV 179
β2AR    95 LMKMWTFGNFWCEFWTSIDVLCVTASIETLCVIAVDRYFAITSPFKYQSLLTKNKARVII 154

                                                             TMD5
β3AR   159 VLVWVVSAAVSFAPIMSQWWRVGADAEAQRCHSNPRCCAFASNMPYVLLSSSVSFYLPLL 218
β1AR   180 CTVWAISALVSFLPILMHWWRAES-DEARRCYNDPKCCDFVTNRAYAIASSVVSFYVPLC 238
β2AR   155 LMVWIVSGLTSFLPIQMHWYRATH-QEAINCYANETCCDFFTNQAYAIASSIVSFYVPLV 213

β3AR   219 VMLFVYARVFVVATRQLRLLRGELGRFPPEES-PPAPSRSLAPAPVG------------T 265
β1AR   239 IMAFVYLRVFREAQKQVKKIDSCERRFLGGPARPPSPSPSPVPAPAPPPGPPRPAAAAAT 298
β2AR   214 IMAFVYSRVFQEAKRQLQKIDKSEGRFHVQNLSQV------------------------- 248

                                    TMD6
β3AR   266 CAPPEGVPACGRRPARLLPLREHRALCTLGLIMGTFTLCWLPFFLANVLRALGGPSLVPG 325
β1AR   299 APLANGRAG-KRRPSRLVALREQKALKTLGIIMGVFTLCWLPFFLANVVKAFH-RELVPD 356
β2AR   249 --EQDGRTGHGLRRSSKFCLKEHKALKTLGIIMGTFTLCWLPFFIVNIVHVIQ-DNLIRK 305

                 TMD7
β3AR   326 PAFLALNWLGYANSAFNPLIYCRSPDFRSAFRRLLCRCGRRLPPEPCAAARP--ALFPSG 383
β1AR   357 RLFVFFNWLGYANSAFNPIIYCRSPDFRKAFQGLLCCARRAARRRHA---THGDRPRASG 413
β2AR   306 EVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCLRRSSLKAYGNGYSSNGNTGEQSG 365

β3AR   384 VPAARSSPAQP----------RLCQR--------LDGASWGVS----------------- 408
β1AR   414 CLARPGPPPSPGAASDDDDDDVVGATPPARLLEPWAGCNGGAAADSDSSLDEPCRPGFAS 473
β2AR   366 YHVEQ-----------EKENKLLCEDLP--GTEDFVGHQGTVPSDNIDSQGRNCSTND-- 410

β3AR       ----
β1AR   474 ESKV 477
β2AR   411 -SLL 413
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FIGURE 3. Multiple alignment of human βARs. Protein sequence alignment of human β_1, β_2, and β_3AR. The TMD is highlighted in yellow.

that β_1-blockers and their subsequent beneficial effects in HF could induce enhancement of the β_3AR expression and activity. For example, Sharma et al[53] showed that metoprolol treatment was able to improve cardiac function in diabetic rats mainly through β_3AR upregulation and NO generation. Analogous, in a canine model of mitral regurgitation, we recently found that metoprolol can promote β_3AR upregulation and enhance its protective signaling (ie, nNOS/NO/cGMP).[25] Moreover, correlative data have been recently shown for the potential of β_3AR agonistic activity of nebivolol, a highly selective β_1-blocker.[52,55] In particular, in a model of I/R injury, nebivolol administration activated cardiac

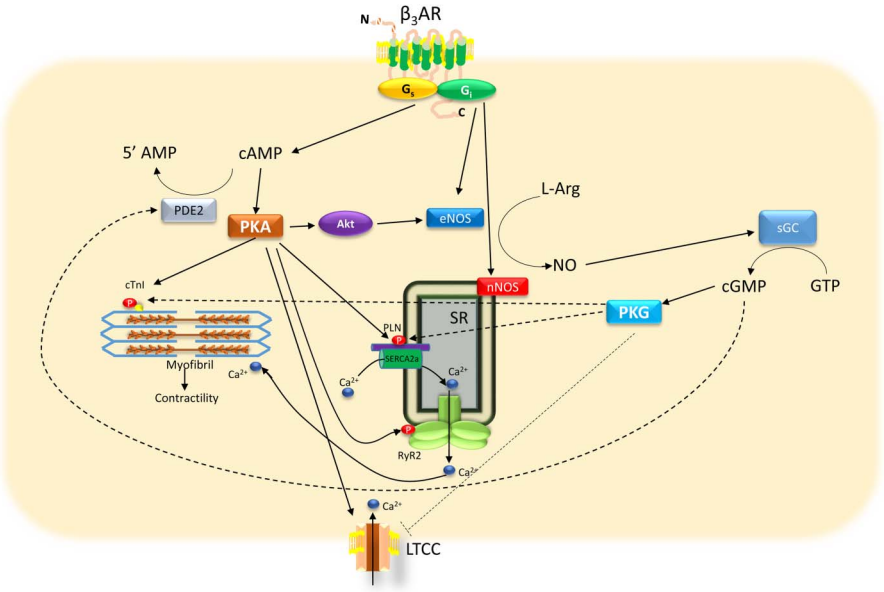

FIGURE 4. Schematic representation of β_3AR signaling activation in cardiomyocytes. β_3ARs are coupled to both stimulatory G proteins (Gs) and inhibitory G proteins (Gi). Although the Gs pathway induces the generation of cAMP and cGMP which, in turn, activates the PKA and PKG, respectively, the activation of Gi signaling pathway is able to stimulate only the generation of cGMP and the activation of PKG. PKA has multiple roles in cardiomyocytes and is able to induce the phosphorylation of several key factors involved in the regulation of contractility, such as cardiac troponin I (cTnI) and phospholamban (PLN). The latter affects Ca^{2+} cycling to the contractile proteins. Furthermore, PKA can phosphorylate the L-type Ca^{2+} channel (LTCC) increasing the influx of extracellular Ca^{2+}. Importantly, PKA can also activate protein kinase B (Akt) with the subsequent activation of the endothelial NO synthase (eNOS). Of note, eNOS activation increases the generation of NO which, in turn, activates the soluble guanylate cyclase to produce cGMP and PKG activation. Similar to PKA, PKG is able to phosphorylate PLN and cTnI. However, PKG can induce the inactivation of LTCC, thus reducing the extracellular Ca^{2+} influx. Moreover, cGMP can stimulate phosphodiesterase 2 (PDE2), reducing the cAMP levels and the activation of PKA. Of note, after the Gi signaling pathway activation, the β_3AR is able to give rise to NO through both eNOS and neuronal NOS (nNOS) located on the sarcoplasmic reticulum (SR).

β_3ARs leading to a significant reduction of infarct size.[55] Similarly, Zhang et al[52] demonstrated, in a setting of HF in mice, induced by left anterior descending artery ligation, that nebivolol was able to reduce cardiac fibrosis and apoptosis and improved cardiac function. Interestingly, this report showed also that after 4 weeks postmyocardial infarction, there was a significant reduction of cardiac β_3AR levels, and that nebivolol was able to restore the expression of this receptor.[52]

Importantly, further to the direct effect in cardiomyocytes, β_1-blockers, such as nebivolol have been also reported to act on endothelium.[56,57] Of note, enhancement of neoangiogenesis in the failing heart is considered one of the mechanisms of protection activated by this class of drugs.[58] In this context, nebivolol through β_3AR activation increases the generation of NO, a key mediator of endothelial function,[50] enhancing endothelial proliferation and increasing vasodilation.[56,57]

Clinical Perspectives of β_3AR Receptor Targeting

As described above, the β_3AR represents an emerging attractive target for pharmacological modulation in the injured heart that, for its compensatory effects, prevents the effects of excessive catecholamines stimulation on the heart.[43,44] In fact, selective β_3AR agonism has been proven to confer protection in the failing heart through a specific cGMP/NO signaling pathway.[26] A particular role for NO has been associated with the enhancement of endothelial cell proliferation,[50] and within failing myocardium it can lead to a beneficial effect on cardiac function and remodeling.[57,58] As described above, β_3ARs are expressed not only in cardiomyocytes but also in endothelial cells, thus supporting the idea that this receptor can also promote proangiogenic mechanisms.[9,56,57] Moreover, in adipocytes, the β_3AR is implicated in metabolic regulation [fatty acid (FA) oxidation, lipolysis, and thermogenesis],[59] that can also be a crucial mechanism to rescue the heart from failure. Importantly, this mechanism is particularly important in several pathological conditions that affect cardiac function, such as ischemia and pressure overload.[60] In fact, in these conditions, the heart, in the presence of limited oxygen supply, suppresses glucose and FA oxidation with a shift in cardiac substrate metabolism from FA oxidation to glycolysis.[61] Glycolysis without a concomitant increase in glucose oxidation results in an accumulation of different harmful catabolites such as lactate and protons that are the cause of intracellular acidosis and Na^+ and Ca^{2+} overload.[62] These effects strongly reduce the capacity of the heart to provide sufficient energy for contractile work because more energy is spent to restore ion homeostasis and lead to an increase in lipid accumulation within the cardiac cells with consequent lipotoxicity.[61]

In this context, as recently demonstrated in adipocytes, nebivolol, through β_3AR activation, is able to improve adipocyte metabolism.[63] Therefore, it is plausible that long-term β_3AR stimulation, through selective agonists and/or β-blockers (ie, nebivolol), can be used as a novel therapeutical approach also to improve metabolism within the failing myocardium.

Currently, 2 clinical trials, the *Beta 3 Agonist Treatment in Heart Failure* (Beat-HF; clinicaltrials.gov: NCT01876433), or in the prevention of HF development, the *Assessment of Efficacy of Mirabegron, a New Beta 3-adrenergic Receptor in the Prevention of Heart Failure* (Beta3_LVH; clinicaltrials.gov: NCT02599480), are trying to evaluate the effects of β_3AR agonism on HF progression and development.

In this context, patients will be treated with the selective β_3AR agonist, mirabegron (also known as YM-178), a drug already approved in the United States, Japan, and Europe, for the treatment of overactive bladder.[64] This drug, through the activation of β_3AR, exerts a myorelaxant effect in the detrusor muscle thus improving the bladder filling. These trials have recently started, so no results are available yet. Anyway, in the meantime that these trials will give us the proof-of-concept of the beneficial role of β_3ARs in human HF, the most important clinical evidence that we currently have regarding the role of this receptor remains the proved efficacy of β-blockers. The cardioprotective effect of βAR-blockade has been largely attributed, for decades, to antagonism of cardiac β_1- and β_2ARs and a resulting heart rate reduction.[65] However, as discussed above in this review, there is emerging evidence that this class of drugs may also act through β_3AR signaling pathway activation which is not blocked by the drugs currently used in clinical HF-therapy (Box 2). However, the main question that still remains unanswered is why not all patients respond favorably to these agents. For these reasons, further elucidation on specific mechanisms of action of these drugs is extremely interesting.[2] In particular, it will be important to evaluate how different β-blockers can impact, in a positive or a negative manner, β_3AR activity, and if this effect is a significant contributor of therapeutic mechanisms in HF.

BOX 2. β-blockers and β_3AR Expression/Activity

1. Both metoprolol and nebivolol enhance β_3AR expression and activity.
2. Carvedilol reduces the expression of β_3AR.

CONCLUSIONS

The β_3AR is a novel and intriguing receptor, with multiple functions within the cardiovascular system. In this review, we have discussed how many disparities have been generated around the signaling and the function of this receptor. However, the emerging concept in the literature is that β_3AR is mostly protective for the cardiovascular system and its agonism with selective ligands or activation during β_1- and β_2AR blockade could represent a future therapeutic strategy to prevent development of HF. Anyway, as discussed above, the β_3AR function in the heart is still poorly investigated, and for this reason, further investigations are required to clarify the causal mechanistic relationship between β_3AR expression and cardiac dysfunction and protection. More importantly, since presently, only β_1AR-blockers have been associated with improvement of the β_3AR signaling pathway, it will be crucial to define the specific signaling pathways associated with β-blocker–dependent activation/inhibition of β_3ARs because it can help in personalizing anti-HF therapy.

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