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Beta-3 Adrenoceptors as New Therapeutic Targets for Cardiovascular Pathologies

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Abstract Catecholamines play a key role in the regulation of cardiovascular function, classically through ß$_{1/2}$-adrenoreceptors (AR) activation. After ß$_3$-AR cloning in the late 1980s, convincing evidence for ß$_3$-AR expression and function in cardiovascular tissues recently initiated a reexamination of their involvement in the pathophysiology of cardiovascular diseases. Their upregulation in diseased cardiovascular tissues and resistance to desensitization suggest they may be attractive therapeutic targets. They may substitute for inoperant ß$_{1/2}$-AR to mediate vasodilation in diabetic or atherosclerotic vessels. In cardiac ventricle, their contractile effects are functionally antipathetic to those of ß$_{1/2}$-AR; in normal heart, ß$_3$-ARs may mediate a moderate negative inotropic effect, but in heart failure, it may protect against adverse effects of excessive catecholamine stimulation by action on excitation-contraction coupling, electrophysiology, or remodelling. Thus, prospective studies in animals and patients at different stages of heart failure should lead to identify the best therapeutic window to use ß$_3$-AR agonists and/or antagonists.

Keywords ß$_3$-adrenoceptor · Adrenergic receptor · ß-blocker · Heart · Vessels · Nitric oxide · Nitric oxide synthase · NO · NOS · Remodeling · Electrophysiology · Contractility · Heart failure · Hypertension · Diabetes · Myocardial infarction · Diabetes · Catecholamine · Nebivolol · Cyclic guanosine monophosphate · cGMP · Cyclic adenosine monophosphate · cAMP · Messenger RNA · mRNA · Ventricular remodeling · Cardiomyocytes · Excitation-contraction coupling

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Introduction

Since its cloning in humans, ß$_3$-adrenoceptor (ß$_3$-AR) has been described in several tissues, such as white and brown adipose tissues, heart, blood vessels, gall bladder, gastrointestinal tract, prostate and urinary bladder detrusor, brain, and near-term myometrium. This important literature leads to propose ß$_3$-AR as a therapeutic target for several indications: obesity and metabolic disorders (ie, diabetes), heart failure (HF), prevention of preterm birth, depression, anxiety, and overactive bladder. In this latter indication, clinical proof-of-concept data are available.

In the cardiovascular system, it is now admitted that three ß-AR subtypes, $ß_1$, $ß_2$, and $ß_3$, are expressed and could regulate its function. Molecular structure and pharmacologic profile differentiate $ß_3$-AR from $ß_1$- and $ß_2$-AR subtypes. This review, based on the literature of recent years, presents an overview of $ß_3$-AR pharmacology and discusses the potential roles of $ß_3$-AR in normal and pathological cardiovascular systems and emphasizes their putative involvement as new therapeutic targets.

Pharmacology and Tools to Study $ß_3$-Adrenoceptors

Presently, several agonists and antagonists are available to study $ß_3$-AR, albeit their specificity could be discussed. Furthermore, the lack of highly specific tools limits the studies about the presence, function, and regulation of $ß_3$-AR [1].

Agonists

$ß_3$-AR is activated at higher concentrations of catecholamines than $ß_1$- and $ß_2$-AR, suggesting that $ß_3$-AR could be activated in situations where catecholaminergic tone is high. $ß_3$-ARs are activated by agonists belonging to two different classes. The first class comprises the phenylethanolamines, including BRL37344, SR58611A, and CL316243, but BRL37344 and SR58611A also possess low affinity for $ß_1$- and $ß_2$-AR. The second class includes the aryloxypropanolamines, such as CGP12177A and cyanopindolol, but also L-755507, which activates cloned human and rhesus monkey $ß_3$-ARs about 1000-fold more potently than the other two subtypes [2]. Recently, several papers reported that nebivolol, a third-generation ß-blocker, was a $ß_3$-AR agonist both in heart [3••] and in some vascular beds [4, 5].

Presently, some $ß_3$-AR agonists are evaluated in clinical studies. Solabegron and mirabegron are in phase 1 and phase 3 clinical trials, respectively, for the treatment of overactive bladder. SR58611A has been evaluated in phase 3 clinical trials for the management of major depressive disorder [2], but this study has been stopped due to adverse events.

Antagonists

$ß_3$-ARs are blocked by classical ß-AR antagonists, but with lower affinity than $ß_{1/2}$-ARs, and are blocked by selective $ß_3$-AR antagonists, such as SR59230A. However, this compound is a selective antagonist of the rodent $ß_3$-AR, but its usefulness as a selective antagonist of the human $ß_3$-AR is less certain. Furthermore, SR59230A could be an agonist in some tissues or cells. L-748328 and L-748337 are two selective antagonists of the human cloned $ß_3$-AR, but they show a weak affinity for rodent $ß_3$-ARs [6].

Thus, the characteristics of $ß_3$-AR ligands need to be taken into consideration in the interpretation of the functional data, particularly in studies where only a single agonist or antagonist concentration was used.

Determination of $ß_3$-Adrenoceptor Expression

To determine receptor expression density as well as location, it is possible to use binding assays that require selective radioligands. However, the lack of a selective $ß_3$-AR radioligand makes the $ß_3$-AR detection in tissues expressing mixed ß-AR population subtypes almost impossible. Thus, an alternative to evaluate the presence and the possible alterations of $ß_3$-AR expression in various pathophysiologic states is based on Western blot experiments and immunohistochemistry. Although some $ß_3$-AR antibodies are commercially available, few of them have been well validated. Thus, when possible, it is important to use the blocking peptide to strengthen the results. It also is possible to quantify $ß_3$-AR transcript expression. However, it has not been established whether alterations of $ß_3$-AR messenger RNA (mRNA) are strictly parallel to those of the proteins.

Finally, genetically modified animals, such as $ß_3$-AR knockout mice, mice overexpressing human $ß_3$-AR in cardiomyocytes, and rats overexpressing human $ß_3$-AR specifically in endothelium, could be used. The latter model, developed in our lab, should lead to identification of the putative paracrine effects of $ß_3$-AR. The functional ß-AR response remaining in $ß_1/ß_2$-AR double-knockout mice can be ascribed to $ß_3$-AR. However, it should be noted that compensatory $ß_3$-AR expression may occur in tissues from those mice, which may not be prominently expressed in wild-type (WT) mice.

$ß_3$-Adrenoceptors in the Heart

Electrophysiologic Effects

In rabbit cardiomyocytes, we have shown that $ß_3$-AR stimulation decreased Ca^{2+} transients and L-type calcium current ($I_{Ca,L}$) amplitude and enhanced I_{Ks} amplitude through activation of the nitric oxide (NO) pathway. The enhanced I_{Ks} amplitude, an outward repolarizing K^+ current, and the decreased $I_{Ca,L}$ amplitude led to an accelerated repolarization of rabbit ventricular cardiomyocytes [7]. In rabbit cardiomyocytes, BRL37344 and CL316243 stimulated the cardiac Na^+/K^+ pump by a decrease of $ß_1$ Na^+/K^+-pump subunit glutathionylation. Notably, this protection from glutathionylation previously was shown to be mediated by NO production, consistent

with NO synthase (NOS) activation by ß3-ARs. The cardiac Na^+/K^+-pump stimulation decreases intracellular Na^+, leading to enhanced Ca^{2+} export via Na^+-Ca^{2+} exchange and decreased cytosolic Ca^{2+} available for uptake into sarcoplasmic reticulum (SR) [8]. Thus, the Na^+/K^+-pump stimulation by ß3-AR agonists could contribute to the negative inotropic effect described by Audigane et al. [7] in the same model of healthy rabbit cardiomyocytes. In human atria, Skeberdis et al. [9] have shown that, at room temperature, several ß3-AR agonists can activate $I_{Ca,L}$. This effect is blocked by a high concentration (1 μM) of L-748337, a ß3-AR antagonist, which also inhibits ß1/ß2-AR at this concentration. Recently, similar results were obtained on $I_{Ca,L}$ at 24°C, but to a lesser extent, and they were lost at 37°C [10]. Taken together, these findings demonstrate that in human atrium, ß3-AR stimulation increases $I_{Ca,L}$ at lower temperatures, but incompatible with mammalian life, whereas at a physiological temperature, ß3-AR stimulation did not modify $I_{Ca,L}$.

Regulation of the Contractility

At the ventricular level, several papers confirmed the negative inotropic effect induced by the ß3-AR stimulation both at cellular level and whole heart. In rabbit ventricular cardiomyocytes, we demonstrated that several ß3-AR agonists produced a concentration-dependent negative inotropic effect correlated to the decreases of Ca^{2+} transients and $I_{Ca,L}$ amplitude. This effect at least partly results from the activation of $G_{i/0}$ and NO pathways [7], which previously have been described in human and canine ventricles [11, 12]. In rat Langendorff-perfused heart, BRL37344 also produced a concentration-dependent negative inotropic effect. This effect was not altered by nadolol, a ß1- and ß2-AR antagonist, but was completely suppressed by the addition of SR59230A or L-748337 [13, 14]. The negative inotropic effect induced by BRL37344 involved an NO–cyclic guanosine monophosphate (cGMP)–dependent pathway [14]. Interestingly, in this last study, the authors show that BRL37344 also produced a negative lusitropic effect independent of ß1/ß2-AR stimulation through the activation of the NO-cGMP–protein kinase G (PKG) pathway. In addition, BRL37344 counteracted the positive lusitropic effect induced by isoproterenol. These data suggest that the ß3-AR–mediated lusitropic control would oppose the effects of excessive ß1/ß2-AR stimulation, thereby preserving a normal cardiac function [14]. However, these results need to be completed to explain the mechanisms underlying the dose-dependent reduction in the maximal rate of left ventricle relaxation, which remain unclear [15]. In frog heart, a recent study reports the functional presence of ß3-AR, the stimulation of which induced a negative inotropic effect through $G_{i/o}$ protein and endothelial endocardium-NO-cGMP-PKG/phosphodiesterase 2 cascade. The involvement of endothelial endocardium is supported by the absence of BRL37344 effect after pretreatment of the heart by Triton X-100 (Dow Chemical Company, Midland, MI) [16].

During the past few years, it has been demonstrated that regulation of contractility by ß3-AR stimulation differs markedly between human atria and ventricles. As mentioned above, in human atrial myocytes, a first study performed at room temperature showed that the ß3-AR stimulation increased contractility through activation of $I_{Ca,L}$ via a cyclic adenosine monophosphate (cAMP)-dependent pathway [9]. Recently, this result has been put in doubt because SR58611A did not increase human atrial force at 37°C and the increase in human atrial force by BRL37344 resulted from $ß_{1/2}$-AR activation [10]. It is important to note that the negative inotropic effect induced by the ß3-AR stimulation involved NO synthases, which are not activated at room temperature.

In human ventricle, we recently confirmed the negative inotropic effect of ß3-AR stimulation. Using ventricular biopsies from transplanted hearts, we have shown a concentration-dependent attenuation of developed contraction force similar to BRL37344 with nebivolol, a vasodilating ß-blocker. This effect was maintained in the presence of nadolol, ruling out the implication of ß1/ß2-AR. Conversely, it was strongly reduced by pretreatment with L-748337. Nebivolol-induced negative inotropic effect was strongly reduced by L-NMMA (L-NG-monomethyl arginine citrate), an NOS inhibitor. Together, these data demonstrated that nebivolol activated ß3-AR in human ventricle through NO-pathway activation [3••].

Altogether, the ß3-AR effect observed in human ventricular tissue strongly differs from that obtained in human atrial tissue. Several explanations could be proposed. First, the differences in patient medical history and treatment could at least partly explain some discrepancies observed between atrial and ventricular contractility. Indeed, human endocardial biopsies are obtained from transplanted heart and cannot be considered "healthy tissue," whereas the atrial samples are harvested during cardiac surgery on patients with valvular or coronary artery disease. Second, functional differences may perhaps be explained by a different coupling in human atrium versus ventricle or differences in ß3-AR expression in right atrium versus left ventricle, where studies are classically performed.

Regulation of Gene Expression

In neonatal rat cardiomyocytes, the chronic ß1- and ß3-AR stimulation reduces ß1-AR–mediated cAMP enhancement associated with a decrease in ß1-AR expression. In contrast, both treatments increase ß3-AR expression and ß3-AR-

inhibited forskolin response. ß$_1$-AR downregulation and ß$_3$-AR upregulation could involve the activation of transcription factors like inducible cAMP early repressor (ICER) or cAMP response-element binding protein via mitogen-activated protein kinase (MAPK) stimulation. Further studies are required to investigate the possible transcriptional mechanism involved in the cross-regulation between ß$_1$- and ß$_3$-AR [17•]. Additional experiments must be conducted to evaluate whether a similar cross-regulation exists in adult cardiomyocytes.

Glucocorticoids are known to increase the density and mRNA levels of ß-AR in many tissues. The administration of dexamethasone, a synthetic glucocorticoid, in rats significantly increased ß$_1$-AR transcripts in left ventricle, whereas it did not modify ß$_3$-AR mRNA [18].

Recently, evidence has accrued showing that functional G protein–coupled receptors are not solely localized at the plasma membrane, but also can signal from different endogenous membrane compartments, including the nuclear membrane [19]. These intracellular receptors may have the capacity to regulate signaling pathways that differ from those of their plasma membrane counterparts. ß$_1$-AR and ß$_3$-AR have been identified in the rat nuclear membrane [20]. Recently, ß-AR and endothelin-1–receptor type B located in the nuclear membrane have been shown to regulate RNA synthesis in opposing ways. In addition, isoproterenol, a nonselective ß-AR agonist, could modulate RNA synthesis by activation of MAPK and protein kinase B (PKB) and regulates specific mRNA targets, such as nuclear factor κB [21]. Unfortunately, in this work, no selective ß$_3$-AR agonist was tested to evaluate specific transcription factor regulation by this receptor.

Modification of ß$_3$-Adrenoceptors Expression and Function with Aging

Among different age-related changes in the senescent heart, such as contraction and relaxation dysfunction, the cardiovascular effects of adrenoceptor stimulation are attenuated even though plasma catecholamine concentration increases with age. In senescent rat heart, both ß$_1$- and ß$_2$-AR are downregulated [22]. Recently, it has been suggested that ß$_3$-AR expression tended to increase gradually with age [23] without a clear link between ß$_3$-AR upregulation and cardiac dysfunction. In a similar way, Birenbaum et al. [24] demonstrated an alteration of the positive inotropic effect induced by ß-AR stimulation in senescent rat heart that could be explained, at least in part, by a downregulation of ß$_1$-AR and an upregulation of ß$_3$-AR. Unfortunately, in this study, the evaluation of senescent rat papillary muscle contractility was performed at 29°C, compromising the coupling of ß$_3$-AR to NOS or other effectors. Although the study by Birenbaum et al. [24] shows undesirable short-term effects of enhanced ß$_3$-AR signaling on inotropy in aged hearts, the biologic consequences of long-term activation of this pathway remain elusive [25]. Finally, additional work must be conducted to evaluate ß$_3$-AR expression in human senescent heart and the consequences on cardiac dysfunction.

ß$_3$-Adrenoceptors in Blood Vessels

In vessels, the stimulation of ß-AR located in both endothelial and smooth muscle cells leads to a relaxation of the vascular smooth muscle, thereby controlling the blood flow distribution in different organs. Classically, ß$_2$-ARs are predominant, but the other subtypes also could participate, and their involvement varies according to the vascular bed and species.

Oliver et al. [26] reported unexpected results showing that the main ß-AR subtype in rat aorta was ß$_3$-AR, followed by ß$_1$-AR and a slight expression of ß$_2$-AR. Although these results are only based on transcript expression, they contrast with classic pharmacologic studies that attribute the ß-AR–mediated relaxation in vessels to ß$_2$-AR [27]. However, they are in accordance with more recent evidence of a role for ß$_1$-AR [28] and ß$_3$-AR in rat aorta [11].

In mesenteric artery from WT and endothelial NOS (eNOS) knockout mice, SR59230A abolished BRL37344-induced relaxation, whereas it only marginally reduced isoproterenol-induced relaxation [29]. These results suggest that ß$_3$-AR are present in this vascular bed, and their stimulation produces a vasodilatation independently of eNOS. Similarly, in rat mesenteric artery, the stimulation of ß$_3$-AR by various agonists induced an increase of intraluminal NO production. This effect was associated with the activation of the phosphatidylinositol 3–kinase (PI-3)/Akt pathway and the phosphorylation of eNOS at serine 1177. In anesthetized rats, the bolus administration of BRL37344 produced an NO-dependent reduction in systolic blood pressure [30]. The presence of functional ß$_3$-AR in rat vessels was confirmed in the liver, where the perfusion CGP12177A used as a ß$_3$-AR agonist produced a weak decrease of intrahepatic resistance [31]. Recently, Mori et al. [32] reported the first pharmacologic evidence of functional ß$_3$-AR in the rat retinal arterioles. However, this study did not evaluate ß$_3$-AR transcripts or proteins levels.

In human umbilical arteries, formoterol- and BRL37344-induced relaxations were mediated by a mixed population of ß$_{2/3}$-AR through cAMP increases [33]. Nevertheless, it is important to note that the authors used endothelium-denuded artery rings, whereas many studies performed in human and animal models suggest a preferential endothelial location of ß$_3$-AR. Finally, ß$_3$-AR also was described in

human hepatic artery [31], and its presence was confirmed in umbilical and placental arteries [34]. Surprisingly, although nebivolol has been described as a ß$_3$-AR agonist in human [12] and rat vessels [5], a recent study reports that nebivolol was not a ß$_3$-AR agonist in rat and human urinary bladders [35]. However, in this study, the effects of nebivolol were compared only to those of isoproterenol in the presence of SR59230A, which is known to have ß$_3$-AR agonistic properties at the high concentration used.

Cardiovascular Pathologies

Modulation of Ventricular Remodeling by ß$_3$-Adrenoceptors

Because of their distinctive pharmacologic properties as described above, ß$_3$-AR are likely to mediate long-term alterations in cardiovascular tissue remodeling such as those induced by circulating neurohormones, including catecholamines. Preliminary evidence shows that mice with cardiac-specific overexpression of human ß$_3$-AR are protected from hypertrophic remodeling after chronic infusion of catecholamines; this also was observed in primary cardiomyocytes with adenovirally overexpressed human ß$_3$-AR in response to α-adrenergic stimulation. In both models, this protective effect was dependent on NOS activation, as it was lost upon treatment with an NOS inhibitor in vitro and in vivo (Hammond J, Belge C, unpublished). Moreover, treatment of mice with nebivolol conferred a better protection against cardiomyocyte hypertrophy, postinfarction remodeling, and mortality than a selective ß$_1$-AR antagonist (metoprolol), which has been linked to the distinctive property of nebivolol to activate ß$_3$-AR and inhibit oxidant radicals production [36••].

Myocardial Infarction and Heart Failure

It is admitted that the circadian clock controls numerous important physiological functions at molecular, cellular, and whole-body levels. The onsets of myocardial infarction (MI) and sudden cardiac death show obvious diurnal patterns, occurring mostly in the early morning, possibly due to higher sympathetic nerve activity and humoral factors in the same time window. Interestingly, Zhou et al. [37] report that the expression and function of ß$_3$-AR exhibited circadian rhythm in normal heart, that this rhythm was blunted in acute healed MI, and that ß$_3$-AR activation was associated with decreased occurrence of ventricular tachycardia and arrhythmias. Those data suggest an important role of ß$_3$-AR in the pathogenesis of cardiac circadian rhythm disorders after MI, and propose ß$_3$-AR as a therapeutic target in MI [37]. Chronic ß$_3$-AR stimulation reduced body weight, heart weight, and the incidence of ventricular tachycardia in canine MI. However, ß$_3$-AR stimulation did not prevent sudden cardiac death in this model [38], suggesting that ß-blockers with ß$_3$-AR–agonist properties may be useful to prevent cardiac arrhythmia after MI. In this context, nebivolol, which possesses these characteristics, could be a suitable candidate. In a similar way, in rabbits with aortic valvular chronic HF, BRL37344 inhibited ventricular arrhythmia induced by ß$_{1/2}$-AR agonist. The cellular mechanisms involved in this effect could result from the inhibition of ß$_1$/ß$_2$-AR stimulation on Na/Ca exchanger or the reduction of Ca^{2+} transient and SR Ca^{2+} load and SR Ca^{2+} leak in cardiomyocytes. The authors suggest that ß$_3$-AR activation decreases the susceptibility to ventricular arrhythmias through modulation of Ca^{2+} handling. Therefore, ß$_3$-AR may be a novel pharmacologic target among current strategies for treatment of fatal ventricular tachycardia in chronic HF [39]. A recent study performed on failing and nonfailing sheep hearts indicated a differential effect of BRL37344 consistent with Na^+/K^+ pump stimulation and a decrease in intracellular Na^+ that may have a negative inotropic effect in the normal heart but not in the failing heart. Interestingly, the authors suggest that any upregulation of the ß$_3$-AR with HF would not accentuate a negative inotropic effect of ß$_3$-AR–receptor activation because preservation of the Na^+/K^+ pump activity would prevent Na^+ overload, which is classically associated with altered excitation-contraction coupling and arrhythmia in HF; accordingly, there was no adverse acute effect on hemodynamic variables of BRL37344 in this large-animals model of severe HF [8]. Recently, ß$_3$-AR overexpression in human HF has been confirmed [40•]. Under basal conditions, Akt and $eNOS^{Ser1177}$ phosphorylation were reduced. Under BRL37344 stimulation, further dephosphorylation of $eNOS^{Ser1177}$ and Akt was observed, whereas $eNOS^{Ser114}$ phosphorylation was increased. These results would suggest a deactivation of eNOS via ß$_3$-AR stimulation, although NO production was not directly measured. The preserved ß$_3$-AR negative inotropic effect without evidence for eNOS activation in cardiac myocytes in combination with evidence for a predominant expression of ß$_3$-AR in endothelium would suggest a ß$_3$-AR paracrine signaling in human HF.

As mentioned above, nebivolol attenuated left ventricular dysfunction and cardiomyocyte hypertrophy early after MI in mice and improved survival, effects that were not attributed to ß$_1$-AR blockade because they are not observed under metoprolol. The nebivolol effects were largely blunted in eNOS-deficient mice, supporting a critical role of eNOS in this respect. The beneficial effects of nebivolol could be due to actions on NO-mediated endothelial function, early endothelial progenitor cells, and inhibition of myocardial NADPH (nicotinamide adenine dinucleotide

phosphate) oxidase [36••]. In addition, nebivolol, by activating ß3-ARs both in human heart and microcoronary arteries, produces an NO-dependent negative inotropic effect and a vasodilation, respectively, leading to improved energetic balance in heart. Those effects could explain the improvement of hemodynamic parameters obtained in patients with HF after nebivolol administration, as previously described in clinical trials (Fig. 1) [41].

Hypertension

In spontaneously hypertensive rats (SHR), nerve-activated $ß_{1/3}$-AR-mediated vasodilation was not present. However, in spite of enhanced epinephrine secretion and subsequent augmented norepinephrine release in SHR, epinephrine functioned as an antihypertensive agent by upregulating $ß_2$- and $ß_3$-AR–mediated vasodilation [42]. In this model, the previously described [43] $ß_3$-AR upregulation was confirmed and associated to G protein–coupled receptor kinase 2 (GRK2) increase [26], which seems to be the main factor involved in diminishing ß-AR signaling in hypertension [44]. Thus, as $ß_3$-AR resists the GRK2-mediated desensitization, its functional role in hypertension could be increased comparatively to $ß_1/ß_2$-AR.

In preeclampsia, a hypertensive gestational state, $ß_2$-AR and $ß_3$-AR protein levels in placentae were similar to those of normal patients, suggesting that aberrations in the ß-AR signaling, rather than in the regulation of ß-AR–subtype expression, may occur in preeclampsia [34]. This hypothesis is supported by the fact that fenoterol- and BRL37344-induced relaxations were partly reduced due to the attenuation of cAMP levels, suggesting that $ß_2$- and $ß_3$-AR agonists may have considerable future pharmaceutical implications in the clinical management of pregnancy-related disorders (eg, preterm labor) or other conditions (eg, intrauterine growth restrictions) in which improvement in fetoplacental exchanges may be of interest [33].

Diabetes

Few studies suggest that $ß_1$-AR decrease associated with $ß_3$-AR increase may be involved in the development of diabetes-induced cardiac dysfunction. In spontaneously diabetic rats (Goto-Kakizaki [GK] rat, a model nearly reproducing human type 2 diabetes), both pancreatic and islet blood flow are higher than in WT mice. Interestingly, the islet blood flow in GK rats decreased after acute inhibition of $ß_3$-AR with SR59230A [45]. This work opens new fields of investigation concerning the effects of chronic $ß_3$-AR inhibition on the islet and white adipose tissue blood flow and its consequences on the impaired glucose tolerance in GK rat. In streptozotocin-induced diabetic rats, intense exercise training normalized $ß_3$-AR expression [46]. However, the corrective effect of exercise training on $ß_3$-AR expression in diabetic rats and its functional consequences remain to be clarified.

One of the most common complications of diabetes is retinopathy. At the early stage of diabetes in rat, vasodilation of the retinal arterioles induced by $ß_3$-AR stimulation was unaffected, leading to propose $ß_3$-AR agonists as one candidate for preventing the development of diabetic retinal diseases by improving retinal circulation [32].

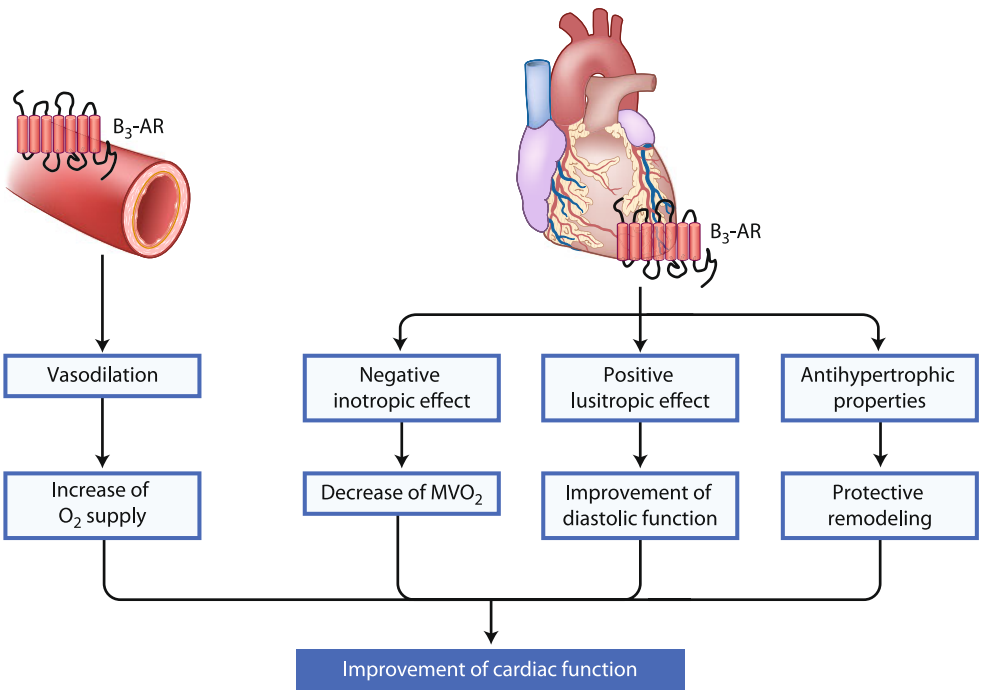

Fig. 1 Cardiovascular effects of ß3-adrenoceptor stimulation. ß3-AR—ß3-adrenoceptor; MVO2—myocardial oxygen consumption; O_2—oxygen

Hypothyroidism

Thyroid hormone deficiency has been reported to decrease expression and function of both ß$_1$- and ß$_2$-AR in different tissues, including heart, without clear data on ß$_3$-AR expression. A recent study reports a significant increase in ß$_3$-AR mRNA expression, although BRL37344-induced negative inotropic effect was decreased [47]. The reduced BRL37344-induced negative inotropic effect could be at least partly explained by alteration of downstream ß$_3$-AR signaling pathway. Indeed, eNOS phosphorylation by the PI-3 kinase/Akt/PKB pathway was impaired by thyroid hormone deficiency.

Sepsis

Sepsis represents the systemic response to infection. In this disease, ß$_3$-AR were overexpressed in the myocardium of patients who died from sepsis. In mouse ventricular cells treated with macrophage-conditioned medium to mimic exposure to sepsis-related cytokines, the negative inotropic effect induced by ß$_3$-AR agonists was increased, suggesting a functionally upregulated ß$_3$-AR pathway upon cytokine exposure that may contribute to the cardiac depression observed during sepsis [48].

Cirrhosis

A recent study showed, for the first time, a possible role of ß$_3$-AR in the modulation of the increased intrahepatic resistance and portal pressure in cirrhosis. Indeed, a marked hepatic and mesenteric ß$_3$-AR upregulation has been reported in human cirrhosis and in two animal models. In addition, ß$_3$-AR agonists, in combination with nonselective ß-AR blockers, produced a stronger beneficial effect on portal pressure in cirrhosis than nonselective ß-AR–blockers alone [31]. Thus, ß$_3$-ARs may represent a new target for the therapy for portal hypertension in cirrhosis.

ß$_3$-Adrenoceptor Polymorphism

In the past 3 years, few papers have reported on a potential contribution of ß$_3$-AR polymorphism in the onset and the development of cardiovascular disease. A missense mutation of the human *ADRB3* gene replacing tryptophan with arginine at codon 64 (Trp64Arg) previously has been reported to be associated with some cardiovascular risk factors such as obesity, diabetes mellitus, and elevated blood pressure. It is important to note that in a heterologous expression system, this ß$_3$-AR polymorphism failed to alter interaction of the receptor with its ligand [49]. A recent cohort study combined with a meta-analysis of previous reports does not support a role of Trp64Arg human ß$_3$-AR polymorphism in coronary heart disease risk [50].

Conclusions

Definitive evidence for the expression of ß$_3$-AR in human cardiac and vascular tissues now opens new perspectives for the treatment of cardiovascular diseases. Nevertheless, we still need a more complete understanding of the human ß$_3$-AR pharmacology. Albeit cardiac negative inotropy and vascular relaxation induced by ß$_3$-AR appear subtle in healthy tissues, one should take into account ß$_3$-AR overexpression in cardiac disease, thereby reinforcing their influence relative to ß$_{1/2}$-AR [51•]. Although this would strengthen ß$_3$-AR vasorelaxation in the vasculature, the consequences on cardiac function are harder to predict. They probably vary depending on the stage of cardiac disease; at early stages, ß$_3$-AR activation may confer protection against catecholamine-induced tissue remodeling (with marginal effects on left ventricle contractility), but whether chronic activation remains beneficial in advanced HF is uncertain. Important other aspects should be thoroughly examined, such as ß$_3$-AR's influences on arrhythmia, metabolism, or polymorphism, which remain underexplored and should be resolved for a safer prediction. Prospective studies in animal models, or even in human patients, using the new specific ß$_3$-AR agonists currently under development for urologic or neurologic diseases are needed now to clarify this important issue.

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