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Beta 3-adrenoreceptor Regulation of Nitric Oxide in the Cardiovascular System

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Abstract

The presence of a third β -adrenergic receptor (β_3 -AR) in the cardiovascular system has challenged the classical paradigm of sympathetic regulation by β_1 - and β_2 -adrenergic receptors. While β_3 -AR's role in the cardiovascular system remains controversial, increasing evidence suggests that it serves as a "brake" in sympathetic overstimulation - it is activated at high catecholamine concentrations, producing a negative inotropic effect that antagonizes β_1 - and β_2 -AR activity. The anti-adrenergic effects induced by β_3 -AR were initially linked to nitric oxide (NO) release via endothelial NO synthase (eNOS), although more recently it has been shown under some conditions to increase NO production in the cardiovascular system via the other two NOS isoforms, namely inducible NOS (iNOS) and neuronal NOS (nNOS). We summarize recent findings regarding β_3 -AR effects on the cardiovascular system and explore its prospective as a therapeutic target, particularly focusing on its emerging role as an important mediator of NO signaling in the pathogenesis of cardiovascular disorders.

Introduction

The β -adrenergic system plays an important role in the regulation of cardiovascular structure and function. In addition to the classical β_1 - and β_2 -adrenoceptors, the existence of two more subtypes of β -adrenoceptor (β -AR) has been suggested, although the putative β_4 -AR subtype is now believed to be a low-affinity state of the β_1 -AR¹. The β_3 -AR, however, mediates catecholamine effects independent of the stimulation of β_1 - and β_2 -AR and has now been demonstrated to have a significant role in many organ systems. Messenger RNA (mRNA) of β_3 -AR is abundantly expressed in human urinary bladder, gall bladder, small and large intestine, adipose tissue, near-term myometrium². It has also been shown to be expressed in the heart tissue of various species including canine, rodent, porcine, and human, though the effects of its activation with preferential agonists have shown considerable variability across species³. The functional presence of human cardiac β_3 -AR was observed by detection of

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mRNA encoding this subtype⁴. In addition, it was observed that β 3-AR protein expression levels were increased in the ventricles of failing human hearts⁵ compared to nonfailing. This observation strongly suggests that cardiac β 3-AR is up-regulated during pathological conditions.

β 3-AR belongs to the superfamily of G protein-coupled receptors like the β 1- and β 2-AR. In the cases of β 1- and β 2-AR, coupling to G_i is dependent on the initial coupling to G_s , PKA activation, and receptor phosphorylation⁶. β 3-AR is unique in that it can couple interchangeably to both G_s and G_i without the requirement for receptor phosphorylation^{7, 8}. β 3-AR activation occurs at catecholamine concentrations that are higher than those required for β 1-AR and β 2-AR^{9, 10}, supporting its likely role as a protective counter-mechanism during sympathetic over-stimulation. As β 3-AR lack the phosphorylation sites for protein kinase A and b-adrenoceptor kinase (bARK) that are important for receptor desensitization¹¹, its mediated response is thought to be preserved following prolonged sympathetic nervous system activation, whereas β 1- and β 2-AR mediated responses are diminished in this setting. Some clinically used β -blockers are associated with effects related to the β 3-AR. For example, carvedilol diminishes β 3-AR expression in the failing ventricles¹².

Comparison of the amino acid sequences predicted from the genomic nucleotide sequences and the corresponding cDNA sequences from different species revealed variations mainly in the carboxy-terminal regions of the β 3-AR. The existence of several exons raises the possibility of alternative splicing and thus of different receptor isoforms with putative distinct pharmacological properties. In a recombinant system, the mouse β 3a-AR isoform was shown to couple solely to G_i , while the β 3b-AR isoform coupled to both G_s and G_i ¹³. Results from studies using Chinese hamster ovary (CHO-K1) cells overexpressing mouse β 3a-AR, suggested that the C-terminal tail of the β 3a-AR isoform may have an important role in receptor localization and internalization¹⁴. Though β 3-AR is expressed in mouse heart tissue, there was no detection of distinct isoforms within the mouse heart¹³. However, recent studies show that mouse β 3-AR of ventricular cardiomyocytes is localized to the nuclear membrane to mediate transcription¹⁵. The mode of mechanism required for nuclear localization of cardiac β 3-AR is currently unknown.

Activation of the β 3-AR pathway in the ventricular myocardium is accompanied by decreased contractility in humans⁴ due to β 3-AR's ability to generate NO. In heart failure, researchers have observed alterations in the expression levels of enzymes that regulate NO production¹⁶⁻¹⁸, which may act as a counter-regulatory mechanism that protects the heart from functional deterioration¹⁹. Neuronal NOS (nNOS) and inducible NOS (iNOS) derived NO production has been shown to increase in human failing hearts^{16, 17}, whereas endothelial NOS (eNOS) activity is depressed¹⁸. Enhanced nNOS activity observed in cardiomyopathy is thought to be due to increased expression of the NOS activator Heat shock protein 90 (Hsp90), as well as the increased interaction between NOS and Caveolin-3 (Cav-3), a scaffolding protein that complexes with post-translocated nNOS in the sarcolemma prior to nNOS activation²⁰. Elevated expression of iNOS in the failing heart has been associated with increased activation of cytokines, such as tumor necrosis factor- α (TNF- α), and interleukin-1 and -6²¹. The precise role of the different NOS isoforms in the cardiovascular system is still being debated, although evidence suggests that signaling by endogenous NO is restricted to intracellular effectors co-localized with different NOS in specific subcellular compartments²², leading to coordinated signaling by the three NOS isoforms on different aspects of cardiomyocyte function. Initial studies on the effects of NO were conducted using NO donors, which do not necessarily mimic the localized myocardial effects of endogenous NOS. Furthermore, results obtained using non-selective inhibitors of NOS were attributed to eNOS due to a lack of appreciation of constitutive expression of nNOS. Similar to the development of knowledge regarding the NOS isoforms, β 3-AR-induced NO production was initially linked to eNOS-dependent production of NO in

the human ventricle²³. Subsequent studies have focused on β_3 -AR signaling through this isoform. However, new data indicate that β_3 -AR can modulate NO signaling via the other two isoforms, nNOS and iNOS, in the heart and vasculature under either pathological conditions or pharmacological stimulation^{24, 25}. How β_3 -AR modulates NO via these two pathways is currently unknown. Therefore, it is of great interest to re-examine β_3 -AR-mediated NOS isoform regulation of NO bioavailability and possible alterations of this mechanism in the pathogenesis of cardiovascular diseases.

Pharmacology and Therapeutic Potential

β_3 -AR is physiologically activated by catecholamines at higher concentrations than β_1 -/ β_2 -ARs, with a K_i of 1 μ M²⁶. Agonists of β_3 -ARs generally belong to two classes. The first class is phenylethanolamines including BRL 37344²⁷, SR 58611A²⁸, and CL 316243²⁹. Radioligand binding studies with human and murine β_3 -AR have reported BRL 37344 affinity in the high nanomolar range (pK_i 5.8-6.8)³⁰⁻³², while the affinity at rat and bovine receptors could be much higher^{32, 33}. Its interaction with the other β -AR subtypes and its antagonistic action on muscarinic acetylcholine receptors are of concern when used in cardiac pharmacological studies^{34, 35}. CL 316243 is another popularly used agonist with higher selectivity for β_3 -AR relative to β_1 / β_2 -AR³⁶, though it also appears to be more selective in rodents than in humans³⁷. The other class of β_3 -AR agonists are aryloxypropanolamines, which include L 755507, a preferential β_3 -AR activator 1000-fold more potent at β_3 -AR activation than β_1 / β_2 -AR but generates an increase in cardiac contractility in transgenic mice with cardiac overexpression of the human β_3 -AR^{38, 39}. Another commonly used aryloxypropanolamine β_3 -AR agonist is CGP 12177A^{33, 40}, which activates β_3 -AR at a concentration 20-fold higher than that used to antagonize β_1 / β_2 -AR^{31, 36}. Other compounds in this class include ICI 201651 and pindolol.

Stimulation of β_3 -AR causes an increase in fat oxidation, enhancement in energy expenditure and improvement in insulin-mediated glucose uptake in rodents. Therefore, the potential of β_3 -AR agonists as antiobesity and antidiabetic agents has been explored⁴¹. After the first identification of β_3 -AR agonists in the early 1980s and the observation that they have a remarkable potential for correcting both obesity and diabetes in rodents, optimism was raised for developing β_3 -AR agonists for use in humans⁴². However, all the β_3 -AR agonists tested in clinical studies over the past 15 years ultimately failed due to either a lack of potency or deleterious side effects such as tachycardia (β_1 -AR effect) and muscle tremor (β_2 -AR effect) arising from a lack of β_3 -AR selectivity.

The latest human trials have focused on the β_3 -AR agonist L-796568^{43, 44}. Single-dose acute administration of 1000 mg of L-796568 increased lipolysis and energy expenditure in obese, nondiabetic young men. While systolic blood pressure also increased significantly (+12.2 mmHg vs. placebo +1.5 mmHg), no changes in heart rate, diastolic blood pressure, ear temperature, plasma catecholamine, potassium, or leptin were found in this study⁴⁴. Surprisingly, long-term treatment with L-796568 for 28 d had no major lipolytic or thermogenic effect in obese, nondiabetic young men but it lowered triacylglycerol concentrations. This lack of chronic effect on energy balance is supposedly explained by insufficient recruitment of β_3 -responsive tissues in humans, down-regulation of the β_3 -AR-mediated effects with chronic dosing, or both⁴³. There was no evidence in this study that L-796568 had effects on β_1 - and β_2 -AR, as indicated by L-796568's inability to elevate heart rate and blood pressure, or induce changes in plasma potassium. However, significantly more subjects in the L-796568 group had gastrointestinal side effects such as diarrhea, an effect which was also previously found in dogs but not in human studies of CL316243⁴³. In animal models, β_3 -AR agonists had anorectic effects, but L-796568 did not seem to have any effect on appetite parameters assessed during the subjects' stays in the respiration chamber⁴³. Not only have β_3 -AR been targeted as a

potential treatment for obesity and diabetes, but it has also been targeted as having important roles in blood vessel and urinary bladder function^{45, 46}. Stimulation of the β_3 -AR has also been proposed as a novel treatment strategy for anxiety and depressive disorders^{47, 48}. Bucindolol, a β_3 -AR agonist and α_1 -AR antagonist, was tested in a trial in patients with NYHA class III and IV heart failure⁴⁹, though mainly for its α_1 AR antagonistic properties. It did not produce significant overall survival benefit, possibly due to the fact that the patient population was in advanced chronic heart failure and had ejection fractions of 35% or less. On the other hand, β_3 AR antagonism has been shown to improve short term cardiac function in a rat model of heart failure⁵⁰.

The compound SR 59230A was administered intraperitoneally at 85nmol in 1 ml saline twice a day for 7 weeks to rats with isoprenaline-induced heart failure, and was shown to improve cardiac function relative to their counterparts with ejection fractions of 30-55%. However, even though SR 59230A has been used frequently to demonstrate β_3 -AR-specific effects, recent studies suggest that it is not selective for β_3 -AR and, if anything, has slightly lower affinity for this subtype than for β_1 - and β_2 -adrenoceptors^{31, 51, 52}. Additionally, SR 59230 can inhibit not only other β -adrenoceptor subtypes but also α_1 -adrenoceptors⁵³⁻⁵⁵. In some systems, SR 59230 has been reported to exhibit agonist rather than antagonist properties^{56, 57}. Therefore, additional evidence is necessary in order to sufficiently demonstrate that protection from isoprenaline-induced heart failure is a specific β_3 -AR antagonizing effect. Caution should be exercised in interpreting other studies that rely on a single agonist or antagonist to demonstrate β_3 -specific activity. More consistent with the idea of β_3 -AR limiting cardiac overexertion by excessive catecholamine stimulation, mice with cardiomyocyte specific overexpression of human β_3 -AR were shown to have attenuated LV hypertrophy compared to WT in response to chronic isoprenaline administration⁵⁸.

Two selective antagonists of the human cloned β_3 -AR, L-748328 and L-748337, have been described. L-748337 has been utilized in a study in dogs with pacing-induced chronic heart failure (CHF), where it produced a mild increase in LV contractile performance under normal conditions. Approximately 2 weeks after baseline measurements and CHF induction, L-748337 produced a larger increase in the slope of the LV pressure-volume relation and a greater decrease in the LV relaxation constant, indicative of improved LV function⁵⁹. This acute β_3 -AR antagonism is however different from chronic blockade of β_3 -AR signaling. Acute gain in inotropy does not correlate with long-term benefits.

β_3 -adrenoceptor and eNOS

Functional expression of β_3 -AR in human myocardium was first demonstrated by application of the preferential β_3 -AR selective agonist BRL 37344 to human endomyocardial biopsies^{4, 23}. Inhibition of contractility involves the inhibitory G protein and results from the production of NO and an increase in intracellular cGMP level. This negative inotropic effect was shown to be inhibited by non-selective NOS inhibitors L-NAME and L-NMMA and could be reversed by an excess of the NOS-substrate, L-arginine. Immunohistochemical staining of ventricular biopsies showed expression of eNOS but not iNOS, indicating an interaction between eNOS and β_3 -AR²³. β_3 -AR activation of eNOS was then shown to involve differential mechanisms according to anatomical region in the human heart. The primary mechanism of eNOS activation in the right atrium is via dissociation from caveolin proteins in the caveolae, subsequent binding to calcium-calmodulin and translocation into the cytoplasm. However, eNOS activation through phosphorylation of eNOS^{Ser1117} seemed to be of major importance in the left ventricle (LV)⁶⁰. Consistent results were observed in the murine myocardium⁶¹, it was also demonstrated that modulation of eNOS activity and increase in NO formation after the application of BRL 37344 is specifically coupled to a stimulation of the cardiac β_3 -AR, since activation of eNOS, either via translocation or via phosphorylation, was absent in $\beta_3^{-/-}$ mice

after the application of BRL 37344. eNOS activation has been demonstrated to potentiate postsynaptic muscarinic response and attenuate the effect of high concentrations of catecholamines⁶², which has been suggested to be consistent with eNOS coupling to β 3-AR.

Availability of substrate and cofactor in the NOS-catalyzed reaction of L-arginine to L-citrulline and NO is important in the regulation of NO bioavailability. Tetrahydrobiopterin (BH4) is important in this process. BH4 depletion results in NOS dimer instability causing a decrease in NO availability and an increase in NOS-dependent generation of superoxide and subsequent peroxynitrite, which further reduces BH4 availability⁶³. While BH4 is an essential cofactor for all three NOS isoforms, the process is best understood in eNOS. Given the vicinity of the Ser114 phosphorylation site in eNOS to the BH4 binding site, this residue may regulate the dimerization of eNOS by determining zinc binding or may act as a phosphoryl switch determining whether eNOS generates NO or superoxide anions⁶⁴, the so-called “eNOS-uncoupling”. A previous study showed no observable difference for Ser114 phosphorylation in the left ventricular of wildtype (WT) and β 3^{-/-} mice under baseline condition. However, after BRL37344 application, a significant increase in phosphorylation of Ser114 was observed in the WT, whereas a decrease was observed in β 3^{-/-} mice⁶⁰. The involvement of Ser114 phosphorylation in eNOS activity requires additional investigation as current results relating phosphorylation of this site to eNOS activity and NO release are limited and in conflict⁶⁵,⁶⁶. Additional evidence for eNOS coupling to β 3-AR came from results showing that BRL37344-induced reduction in sarcomere shortening and calcium transient are absent in cardiomyocytes isolated from eNOS^{-/-} mice²². However, many recent studies support a β 3-AR – nNOS connection as well.

eNOS/NO signaling has a pivotal role in regulating L-type Ca²⁺ channel activity in cardiac tissue⁶⁷,⁶⁸. Protein kinase G (PKG), a down-stream effector of the eNOS/NO signaling cascade, deactivates L-type Ca²⁺ channels via phosphorylation at residue Ser 533 of the channel⁶⁷. β 3-AR signaling has also been demonstrated to suppress L-type Ca²⁺ channel currents⁶⁹. Zhang et. al showed that increasing concentrations of BRL37344 in rat isolated ventricular myocytes lead to further suppression of L-type Ca²⁺ channel currents. This suppression was partially inhibited in the presence of NOS inhibitor L-NAME, suggesting that β 3-AR regulates L-type Ca²⁺ channel currents in a NOS/NO dependent manner⁶⁹.

β 3-AR, nNOS, and iNOS

Constitutive expression of nNOS in cardiomyocytes gained appreciation around the turn of the 21st century⁷⁰. Its role in modulation of myocardial contractile function has been increasingly established since its localization to the sarcoplasmic reticulum (SR), where n-NOS-derived NO open the probability of RyRs activation via S-nitrosylation. Myocardial nNOS expression and activity have been reported to be increased following experimental myocardial infarction in rats or mice, in human failing hearts and in spontaneously hypertensive rats. In these pathological circumstances, increased nNOS expression was accompanied by a translocation from the SR to the sarcolemma, where nNOS associated with caveolin-3 and the regulatory protein HSP90. These findings were associated with a reduction in the level of RyR/nNOS complexes, suggesting that both activity and target proteins of nNOS-derived NO may differ in the remodeled myocardium. More recently, publications have demonstrated that inhibition of myocardial nNOS results in decreased SR load due to phosphorylation of phospholamban, a protein that inhibits SR Ca²⁺ channels⁷¹⁻⁷³ in its dephosphorylated state. This decrease in SR Ca²⁺ load is rescued by β -adrenergic stimulation⁷².

Under basal conditions, pharmacological inhibition of nNOS increased basal LV inotropy and prolonged the time constant of LV isovolumic relaxation in control rat hearts, whereas in post-myocardial infarct failing hearts these effects were significantly blunted⁷⁴. In contrast,

inhibition of nNOS enhanced the inotropic and lusitropic response to β -adrenergic stimulation in failing hearts but had no significant effect in control rats, suggesting that myocardial nNOS overexpression may contribute to the depressed inotropic responsiveness to β -adrenergic stimulation observed in heart failure. Such an effect could be construed as a beneficial adaptive mechanism to protect the diseased heart from the harmful effects of excessive catecholamine stimulation. Consistent with an adaptive role for myocardial nNOS overexpression in the remodelled myocardium, Saraiva et al. and Dawson et al. found that nNOS^{-/-} mice developed more severe LV remodeling and impaired β -adrenergic reserve after myocardial infarction when compared to control mice with similar infarct size^{75, 76}. Unlike the effects of nNOS inhibition seen in rat hearts, we found no change in basal LV inotropy in nNOS^{-/-} mice as compared to WT mice^{22, 76}, although others have seen a decrease⁷⁵. This could be due to differences in species or the mechanism used to inhibit nNOS.

β 3-AR coupled NO production via nNOS has recently been demonstrated in diabetic and aged rat hearts^{24, 77}. Fourteen weeks of untreated type 1 diabetes has been shown to almost double both mRNA and protein levels of cardiac β 3-AR in rats. The estimated ratio of β 1-, β 2-, and β 3-AR proteins in the heart of control rats was found to be approximately 62:30:8, respectively. In streptozotocin-induced diabetic rats this ratio changed to approximately 40:36:23, with 2-week insulin treatment restoring the complement of β -AR to 57:33:10⁷⁸. *In vivo* and *in vitro* β -adrenergic activation by dobutamine lead to a positive inotropic response in healthy rat hearts that was attenuated in rats with diabetic cardiomyopathy. Interestingly, immunoblot of protein from isolated cardiomyocytes only detected nNOS within diabetic cardiomyocytes. A β 3-AR antagonist, nonselective NOS inhibitor L-NAME, or selective nNOS inhibitor L-VNIO partially restored the positive inotropic effect of dobutamine in diabetic rats but had no significant effect in healthy rats, consistent with the idea that β 3-AR induced nNOS-dependent NO production led to the attenuated β -AR response in diabetic cardiomyopathy.

The same group reported that hearts from 2-year-old rats have an impaired inotropic response to isoprenaline as compared to young (3-month-old) hearts in another study⁷⁷. The positive inotropic response was not significantly modified by L-NAME or the nNOS-selective inhibitor L-VNIO in the young group. In the older group, however, β 3-AR, nNOS, and iNOS protein expressions all increased; and the positive inotropic response to isoprenaline was partially restored by L-NAME and L-VNIO but not by the iNOS-selective inhibitor 1400W. Treating left ventricular papillary muscles with BRL 37344 decreased dibutyl cyclic adenosine monophosphate-induced increase in inotropy in the aged group. Taken together, these two studies suggest that while β 3-AR's functional significance may not be apparent in healthy subjects, it has the capability to signal through nNOS and can become important in altering contractile response to β -AR stimulation in conditions with increased β 3-AR expression.

Although earlier results showed that the depressed contractile response to BRL37344 remained unaltered in nNOS^{-/-} cardiomyocytes from WT²², a subsequent study demonstrated the contrary by using BRL37344 in the presence of β 1/2-AR blockade by nadolol. The negative inotropic response to β 3-AR stimulation seen in control cardiomyocytes was absent in both nNOS^{-/-} cardiomyocytes and control cardiomyocytes with acute nNOS inhibition⁷⁹. In addition to possible stimulation of nNOS production of NO by β 3-AR, this group proposed another intriguing paradigm in which nNOS may help to maintain eNOS coupling and thus allow β 3-AR signaling. In nNOS^{-/-} mice, the cardiomyocyte's unresponsiveness to β 3-AR stimulation was shown to be corrected with oxypurinol treatment, a xanthine oxidoreductase (XOR) inhibitor that reduced L-NAME-inhibited (eNOS-derived) superoxide production in LV homogenates of nNOS^{-/-} mice. As nNOS limits superoxide production by xanthine oxidoreductase (XOR)⁸⁰ in its absence increased XOR-derived superoxide may promote eNOS uncoupling and reduce β 3-AR signaling ability. Additional studies are necessary to determine the functional significances of increased β 3-AR and nNOS expressions in

myocardial remodeling. β_3 -AR stimulation of NO production, either via nNOS or eNOS, could both be seen as adaptive mechanisms in preserving myocardial contractile reserve in response to high catecholamine levels. Whether this indeed translates to augmented NO bioavailability, or instead reacts with increased reactive oxygen species to result in further oxidative and nitrosative stress will need to be clarified.

There is very limited information available on β_3 -AR's association with iNOS. The β_1 -blocker, nebivolol, which is also a β_3 -AR agonist, induces NO via an iNOS-dependent manner, not eNOS nor nNOS²⁵. In a recent publication, Maffei et al. demonstrated that β_3 -antagonist SR59230A inhibits nebivolol-induced NO in an in vitro Langendorff model, suggesting a possible role for β_3 -adrenergic receptors in regulating iNOS-dependent NO²⁵. There is a need for more research pertaining to this area of study.

β_3 -adrenoceptor in the Vasculature

β_3 -AR agonists have been seen to produce peripheral vasodilation in conscious rats and dogs^{81, 82}, anesthetized pigs⁸³, and anesthetized β_1 -/ β_2 -AR double knockout mice⁸⁴. Ignarro *et al.* demonstrated that nebivolol induces vasorelaxation of canine pulmonary arterial ring in an endothelium-dependent manner, while rat aortic ring studies showed that nebivolol induces vasorelaxation via both an endothelium-dependent and endothelium-independent mechanism⁸⁵. Both mechanisms involved the activation of the NO-cGMP signaling cascade, however, NO generated from the endothelium-independent rat aortic relaxation was not due to NOS activity⁸⁵, suggesting alternate pathways for β_3 -AR-induced NO production. In humans, a partial β_3 -AR agonist CGP12177A induced a weaker effect in the control of lipolysis and nutritive blood flow in human subcutaneous abdominal adipose tissue⁸⁶, though a placebo-controlled randomized trial showed that a 28-day treatment with the β_3 -AR agonist L-796568 did not induce any significant cardiovascular changes⁸⁷. Additionally, β_3 -AR agonists failed to induce vasodilation in nonhuman primates⁸¹.

In isolated canine pulmonary arterial rings, CL 316243 and BRL 37344 produced a concentration-dependent relaxation. The dilating effect was endothelium-independent and accompanied by increased intracellular cAMP levels^{88, 89}. In rats, BRL 37344 and isoprenaline produced significant relaxation in isolated common carotid arteries⁹⁰. SR 58611A, CGP 12177, and isoprenaline in the presence of nadolol produced slow-developing relaxation in rat thoracic aortas⁹¹. In rat thoracic aorta, endothelium removal strongly reduced the β_3 -AR-induced relaxation, indicating that the β_3 -ARs are mainly located in endothelial cells in rat thoracic aortas⁹². The relaxation is suggested to be the result of activating a NOS pathway and a subsequent increase in cGMP levels⁹¹. Several potassium channels including BKCa, KATP, and KV were activated by the β_3 -AR, leading to vasorelaxation⁹². In cultured endothelial cells, epinephrine-stimulated migration was completely attenuated by the β_3 -AR antagonist SR95230A and was also blocked by the transfection of siRNA constructs targeting Rac1 or PKA⁹³. These observations suggest that Rac1, an essential upstream modulator of receptor-regulated activation of kinase Akt and eNOS, acts as a key regulator of β_3 -AR signaling to eNOS in the vascular wall and that both Rac1 and PKA play an essential role in β_3 -AR regulation of endothelial cell migration.

A generalized decrease of the β -AR response has been recognized in systemic hypertension in human and in various animal models⁹⁴. The main defects include β -AR downregulation, alteration of G protein levels and impairment of β -AR-G protein-effectors coupling⁹⁵. In a canine perinephritic hypertension model, β_3 -AR stimulation exerted a beneficial effect⁹⁶. In 12-week-old spontaneously hypertensive rats, increased β_3 -AR expression was observed, albeit not associated with an increase in β_3 -AR-induced vasorelaxation⁹⁷. Since all three

identified β -AR subtypes produce vasodilation, upregulation of β 3-AR expression in hypertension could compensate for the downregulation of β 1-/ β 2-AR in this condition.

β 3-AR involvement in cardiac function and heart failure

β 3-AR stimulated activation of NO is accompanied by decreased contractility in humans⁴. This decrease in contractility after β 3-AR stimulation has been confirmed in a transgenic mouse model with cardiac-specific overexpression of the β 3-AR protein⁹⁸, in isolated Langendorff perfused rat hearts⁹⁹, and in other species such as dog and guinea pig^{100, 101}. Positive inotropic effects have been seen after β 3-AR stimulation as well, best documented in the atrium, though the mechanism is not completely established and could be partly due to non-specific stimulation of β 1/ β 2-AR^{35, 102}. Positive chronotropic effects described *in vivo* in humans and the canine model are likely due to baroreflex activation in response to vasodilation¹⁰³. The paradigm regarding β 3-AR inotropic regulation in the ventricles is that it produces a negative inotropic effect opposite that of β 1- and β 2-AR at high catecholamine concentrations, serving as a “safety-valve” at high levels of sympathetic stimulation.

Imbrogno et al. demonstrated that BRL37344-induced negative inotropic effect in isolated working heart preparations of *Anguilla anguilla* (fresh water eel) was abolished by exposure to the Gi/o inhibitor pertussis toxin¹⁰⁴. Pre-treatment with either an inhibitor of soluble guanylate cyclase or an inhibitor of the cGMP-activated protein kinase G (PKG), abolished the β 3AR-dependent negative inotropism. Furthermore, the negative inotropic effect of β 3-AR stimulation is associated with shortening of action potentials via decreased Ca^{2+} transients¹⁰¹. BRL 37344 inhibits L-type Ca^{2+} channels and attenuates intracellular Ca^{2+} transients in canine ventricular myocytes with an associated dose-dependent decrease in contractility¹⁰⁰. A similar effect on basal $\text{I}_{\text{Ca,L}}$ is partly abolished by L-NAME¹⁰⁵.

The cardiodepressant effects mediated through β 3-AR stimulation have been proposed to contribute to impaired cardiac function in patients with heart failure^{106, 107}. β 3-AR proteins have been reported to show a 2- to 3-fold increase in left ventricular myocytes of failing human hearts compared to those from non-failing human hearts⁵. In the same study, the positive inotropic effect of isoprenaline was found to be decreased by 75% in failing hearts, though the negative inotropic effect of β 3-AR agonists was paradoxically mildly attenuated. It has been proposed that β 3-AR upregulation may become maladaptive by producing an imbalance between inotropic pathways in the later phases of disease³. The desensitization-resistant β 3-AR continues to mediate a negative inotropic effect at a time when the β 1- and β 2-AR are down-regulated or desensitized. As a net result, the negative inotropic action of β 3-AR may in theory overwhelm the impaired influence of β 1- and β 2-AR, tipping the balance in favor of a depression in contractility, which could exacerbate heart failure. Changes in the expression of this pathway may alter the balance between positive and negative inotropic effects of catecholamines on the heart, potentially leading to myocardial dysfunction. Curiously, a recent study observed that although β 3-AR expression was increased and BRL 37344 elicited a negative inotropic response in LV trabeculae isolated from failing dilated human myocardial tissue, phosphorylation of eNOS Ser1117 and Akt/PKB were actually decreased, along with increased eNOS Ser114 phosphorylation¹⁰⁸. This uncoupling between β 3-AR and eNOS phosphorylation in human failing myocardium in spite of preservation of β 3-AR-stimulated decrease in contractility suggests a more complex mechanistic paradigm than previously proposed.

Earlier assertions of β 3AR overexpression leading to loss of function and exacerbation of heart failure may hold true during end-stage conditions; however, an important physiologic role of β 3AR may be protection of the myocardium from effects of adrenergic overstimulation. β 3^{-/-} mice have been shown to have increased SERCA 2a expression in combination with increased

phosphorylation of phospholamban, resulting in an increased SERCA 2a activity¹⁰⁹. Therefore, it has been proposed that β 3-AR overexpression in heart failure may depress SERCA 2a activity and reduce SR Ca^{2+} uptake and contribute to cardiac function loss. However, a decreased SERCA 2a may in fact be correlated to higher resistance against reduced oxygen supply¹¹⁰. As demonstrated in a β 2-AR overexpression model, increased SERCA 2a activity may actually be responsible for increase in energy consumption and ischaemic damage¹¹¹. Chronic stimulation of β 1-AR in an overexpression model also showed development of hypertrophy, interstitial fibrosis, and heart failure¹¹². In this light, β 3-AR is likely a compensatory mechanism in heart failure that aims to overcome injury elicited by prolonged sympathetic activity.

β 1-blockers, such as nebivolol, have been demonstrated to elicit anti-adrenergic effects through β 3-AR activation in the heart. A recent study in endomyocardial tissue from nonrejection transplanted human hearts showed that nebivolol induced a concentration-dependent decrease in peak tension in a manner similar to β 3-AR agonist, BRL-37334¹¹³. Nebivolol's induced suppression of peak tension was attenuated in the presence of β 3-AR antagonist, L-748377. In addition, negative inotropic effects induced by both nebivolol and BRL-37344 were NOS dependent and were inhibited by non-selective NOS inhibitors¹¹³. The NOS isoform(s) that nebivolol uses to achieve this effect in the heart remains unknown. Nevertheless, nebivolol induces a negative inotropic effect through a β 3-AR- and NOS-dependent mechanism.

Our recent results demonstrate a beneficial role of β 3-AR¹¹⁴. We have demonstrated that lack of β 3-AR signaling leads to imbalance in NO/superoxide production favoring subsequent NOS uncoupling in response to chronic pressure overload stress on the myocardium. Mild transverse aortic constriction that results in mild hypertrophy without fibrosis in the wild type mice led to a pronounced cardiac hypertrophic and fibrotic response in β 3^{-/-} mice, accompanied by decreased survival. Myocardial tissue from β 3^{-/-} mice demonstrated augmented NOS-dependent superoxide generation and increased nNOS and iNOS expressions evident at 3 weeks after pressure overload. By 9 weeks decreased eNOS Ser1117 phosphorylation and decreased calcium-dependent NOS-activity measured by arginine to citrulline conversion were observed, along with persistently increased superoxide production by NOS, which is indicative of NOS uncoupling. The expression of GTP cyclohydrolase 1, the enzyme catalyzing the first and rate-limiting step in BH4 biosynthesis, was also significantly reduced in β 3^{-/-} mice at 9 weeks after chronic pressure-overload compared to WT. The ratio of BH4 to other biopterins was decreased in β 3^{-/-} mice compared to WT both at baseline and after pressure overload, and more importantly, exogenous BH4 treatment rescued β 3^{-/-} mice from pressure overload-induced adverse modeling and suppressed harmful superoxide production by NOS. The shift from NO to superoxide production by NOS isoforms has many pathophysiological consequences in the cardiovascular system, and β 3-AR involvement in this intricate balance establishes another important role for this receptor.

Conclusion

While the presence and function of β 3-AR in the cardiovascular system across different species is somewhat variable, and caution against eager extrapolation of information obtained from animal models to humans is warranted, the β 3-AR has emerged to play an important role in the pathophysiology of heart failure. Its primary cardiovascular role is described as a “brake” on the sympathetic nervous system - it is activated at high catecholamine concentrations and produces a negative inotropic effect opposite that of β 1- and β 2-AR to limit over exertion. β 3-AR stimulation has been described under different conditions to produce NO via all three identified NOS-isoforms and has been shown to be essential in preventing pressure overload-induced myocardial NOS-uncoupling. The involvement of β 3-AR in NO signaling needs to be

further explored and has the potential to become an important therapeutic target in the treatment of heart failure and other oxidative stress-related disorders.

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