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Beta3-Adrenoreceptors in Cardiovasular Diseases: New Roles for an "Old" Receptor

Jean-Luc Balligand*

Pole of Pharmacology and Therapeutics, Institut de Recherche Experimentale et Clinique, and Department of Medicine, Cliniques Saint-Luc, Universite Catholique de Louvain, Brussels, Belgium

Abstract: Beta3-adrenoreceptors (B3AR) are traditionally known as metabolic receptors in adipose tissue, but came into focus in the cardiovascular field after our demonstration of their expression in human cardiac myocytes and endothelial cells, where they mediate endothelium-dependent relaxation of coronary resistance vessels through production of both nitric oxide and endothelium-dependent hyperpolarization factor(s) (EDHF). B3AR are also expressed at the plasma membrane of rodent and human cardiac myocytes. Notably, their expression is increased in several forms of human cardiomyopathies, which raises questions about their adaptive or maladaptive role in myocardial remodelling. To test the hypothesis that they may counteract the adverse effect of B1-B2-AR overactivation, we set out to study the cardiac phenotype of transgenic mice expressing human recombinant B3AR under the cardiac-specific alpha-MHC promoter. While exhibiting no apparent phenotype at basal state, these mice seem protected from hypertrophic remodeling under a variety of stresses, without developing left ventricular dysfunction. Notably, this protection seems to depend on a functional nitric oxide synthase (NOS), as it is abrogated under NOS inhibition. These features can all be recapitulated in homotypic cardiac myocytes cultures *in vitro*. B3AR transgenic mice may also be protected from fibrosis through a paracrine cross-talk to cardiac fibroblasts. These data suggest a beneficial role of B3AR in myocardial remodeling through attenuation of fibrosis and of excessive cardiac myocyte hypertrophy, while at the same time optimizing perfusion. As B3AR are resistant to homologous desensitization, they are attractive targets for therapeutic interventions in the setting of chronic sympathetic stimulation, as it is prevalent in heart failure and several cardiomyopathies.

Keywords: Beta3-adrenergic receptor, Human, Catecholamines, Heart Failure, Hypertrophy, Myocardial remodeling, Nitric Oxide.

INTRODUCTION

Catecholamines are well-known regulators of cardiovascular function and tissue remodelling. Traditionally, their signaling has been rationalized through differential affinity for alpha- and beta-adrenergic receptors distributed in diverse cell types in heart and vessels. Among the latter, beta1 and beta2-adrenergic (B1- and B2AR) receptors classically mediate positive inotropic and trophic effects in cardiac muscle, as well as vasodilatation in several vascular beds. The interpretation of catecholamines'effects has recently been re-examined in light of the demonstration of the expression of the third isotype, or beta3-adrenergic receptor, in cardiovascular tissues in humans and several animal species [1]. This isotype was known to mediate lipolysis and thermogenesis in adipose tissue. In the heart, contrary to B1- and B2 isotypes, it mediates a negative inotropic effect when activated with high concentrations of agonist ex vivo. Given its differential expression in cardiac tissue in pathologic hearts and resistance to homologous desensitization, contrary to the other isotypes, it is an attractive target for therapeutic modulation in several cardiomyopathies.

*Address correspondence to this author at the Pole of Pharmacology and Therapeutics, Institut de Recherche Experimentale et Clinique, and Department of Medicine, Cliniques Saint-Luc, Universite Catholique de Louvain, Brussels, Belgium; Tel:/Fax: +3227645269;
E-mail: jl.balligand@uclouvain.be

Before this can be translated to the clinic, one must acquire sufficient background knowledge on the regulation of its expression, coupling mechanisms and their impact on long-term cardiovascular remodeling and function in experimental models. Accordingly, we will briefly review current data and draw perspectives with new pereferential agonists for the B3AR.

EXPRESSION OF B3AR IN CARDIAC AND VASCULAR TISSUES

B3AR proteins are detected in microvascular endothelial cells and cardiac myocytes from normal and diseased human hearts. In the ventricular myocardium, expression levels do not seem to differ between the sub-endocardium to sub-epicardial layers. Protein abundance was found to be higher in atrial compared with ventricular chambers [2]. The distinct cellular subtype expression was confirmed both by immunohistochemistry and RT-PCR amplification of transcripts from cardiac endothelial cells [3] or cardiac myocytes isolated by laser-capture microscopy [4].

Recently, we used subcellular fractionation techniques (isopycnic centrifugation) to examine the subcellular localization of B3AR in cardiac extracts and found B3AR to co-segregate with the caveolar protein, caveolin3 in light membrane fractions corresponding to caveolae/rafts. This

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corresponds to observations with a second technique using Proximity Ligation Assay (Hammond J, Dubois E, Balligand JL, 2012).

Notably, contrary to B1- and B2 isotypes, B3AR are upregulated in disease conditions, such as ischemic or dilated cardiomyopathies in humans [2]. This has been replicated in some rodent models under hemodynamic or neurohormonal stress. In human ventricular myocardium, this has been associated with an imbalance between B1-2 and B3AR effects on contractility, in favor of the latter [2].

This raises the question whether B3AR upregulation is an adaptative or deleterious phenomenon on the long term evolution of cardiac disease.

PRE-CLINICAL MODELS OF CARDIAC-SPECIFIC OVEREXPRESSION OF B3AR

To answer this question, one can examine several animal models with B3AR overexpression, either endogenously or with transgenic techniques. Using the latter, our preliminary observations showed an apparently neutral phenotype in baseline conditions, including through hemodynamic and morphometric analysis. Under neurohormonal stress, B3AR-TG seem to be protected from remodeling, with less increase in LV mass but no functional degradation. This "resistance" to hypertrophy seems to involve signaling that is confined to the myocyte, since it can be reproduced in isolated B3AR-overexpressing myocytes *in vitro*. Another important feature is the apparent protection from fibrosis, that probably involves B3AR-mediated cross-talk between myocytes and neighbouring cells, including fibroblasts [5].

VASCULAR B3AR, VASODILATATION AND ANGIOGENESIS

An important component of cardiac remodeling is the adaptation of capillary density and function in the face of hemodynamic stress. In this regard, B3AR again seem to confer protection, as we found that B3AR agonists vasodilate coronary resistance vessels from human hearts; this involves both a NO-dependent pathway and an EDHF component [6]; the latter is particularly suitable to substitute for NO in conditions of prevailing oxidant stress, where the EDHF response seems preserved and would maintain coronary perfusion.

In other vascular beds, such as the retina, some evidence suggested a pro-angiogenic effect of B3AR activation. We could reproduce this effect in vascular rings from mice treated with various B3AR agonists, and confirmed specificity by demonstrating that angiogenic effects were abrogated in rings from B3AR KO animals [3]. Therefore, the vascular effects of B3AR expressed in the endothelium would promote coronary perfusion by maintaing vasodilatation as well as pro-angiogenic effects in the face of chronic neurohormonal stress [7]. Such effects would also be exprected to protect against post-hypoxic/ischemic remodeling, as suggested from initial experiments in mice [8]. Of note, B3AR have also been implicated in similar protective effects of exercise [9].

B3AR-COUPLED SIGNALING IN HEART AND VESSELS

In adipocytes, B3AR classically couple to G-alpha-s proteins and activate adenylyl cyclase to increase cAMP. We previously showed that in human heart muscle, B3AR couple to G-alpha-i and activate a nitric oxide synthase, with resultant increase in NO and cGMP [10].

We confirmed this in isolated myocytes from rodents with several approaches including direct measurements of NO and cGMP, which are sensitive to NOS inhibition. This is in line with the subcellular localization of B3AR in membrane caveolae/rafts where constitutive NOS have also been previously found.

In vessels, NOS activation and NO production was also observed after B3AR activation with various agonists or genetic overexpression in endothelial cells. There, eNOS is the effector, since NO-dependent angiogenic effects were again abrogated in vessel rings from eNOS KO mice [3].

B3AR, NOS AND REMODELING

The causality of NOS in mediating the protection against remodeling can be examined with inhibitors both *in vitro* and *in vivo*. In isolated cardiac myocytes, NOS inhibition seems to abrogate the protection from hypertrophy in response to several neurohumoral agonists. Similar results were obtained with PKG inhibitors, suggesting the protection to be mediated by cGMP/PKG downstream NOS. Among possible mechanisms for PKG-mediated protection, recent work has identified TRP-C6 as well as RGS2/4 modulation of the Ca-calmodulin NFAT pro-hypertrophic pathway [11].

Similarly, the beneficial effects of B3AR activation were shown to be mediated through activation of nitrosothiols [9] or nNOS and eNOS [8], as identified using the respective genetically-deficient mice, in exercise and ischemia/ reperfusion, respectively.

Finally, in the context of post-infarction remodeling, we recently demonstrated that a beta-blocker with combined B1AR antagonism and B3AR agonist properties (i.e. nebivolol) conferred superior protection compared with a pure B1AR blocker (metoprolol) in terms of LV remodeling, progenitor cells mobilisation, endothelial function, LV performance and mortality, at least in mice [12]. The B3AR agonist properties of nebivolol had previously been demonstrated by us in mouse vessels, and specificity confirmed using B3AR KO mice [3].

FUTURE THERAPEUTIC POTENTIAL

These pre-clinical data point to protective effects of overexpressed B3AR against LV remodeling in the setting of neurohormonal or post-ischemic stress. Theoretically, one could conceive the benefit of using B3AR agonists to prevent adverse remodeling in these conditions. Recently, a study showed preliminary encouraging data using BRL37344 in mice submitted to transortic constriction [13], although the specificity of this molecule as an agonist for the murine B3AR is somewhat disputed [14]. Nevertheless, our data with Nebivolol, a bona fide B3AR agonist in the mouse, go in the same direction; their relevance in humans is sup-

ported from the SENIORS trial that demonstrated the benefit of nebivolol use in elderly patients with heart failure [15].

Caution should nevertheless be used, as the long-term effects of B3AR agonists have been very little studied, in particular in terms of their impact on LV function in heart failure. A reassuring observation is that long-term overexpression of B3AR does not seem to affect mortality or LV function in mice. In a sheep model of heart failure, the use of B3AR agonists was suggested to even improve LV function through restoration of Na-K-ATPase function; the mechanism also involved NOS by preventing glutathionylation of the pump [16]. In the setting of heart failure this may reduce Na overload and indirectly contribute to improvement of Ca homeostasis, contractility and rhythm stability.

CONFLICT OF INTEREST

The author confirms that this article content has no conflicts of interest.

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PATIENT CONSENT

Declared none.

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