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Potential Use of β_3-Adrenoceptor Antagonists in Heart Failure Therapy

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ABSTRACT

Recently, a functional, negatively inotropic, β_3-adrenoceptor was characterized in the human heart. Several studies now suggest that this receptor might play an important role in the pathophysiology of heart failure, by counterbalancing the effects of a β_1- and β_2-stimulation. Therefore, this review summarizes the rationale and effects of β-adrenergic blockade in chronic heart failure and specifically addresses the question of the potential use of β_3-adrenoceptor antagonists in the treatment of heart failure and other pathophysiological conditions associated with a decreased cardiac contractility.

INTRODUCTION

Heart failure induces important adjustments in circulating levels of certain neurohormones in an attempt to improve myocardial function. One of the adjustments is an increase in the activity of the adrenergic nervous system resulting in an elevation of circulating levels of catecholamines. The severity of heart failure is in fact proportional to the plasma catecholamine concentration (54), with the highest plasma levels of norepinephrine predicting the most unfavorable outcome (15). This observation led to an early hypothesis that short-term activation of the sympathetic nervous system was necessary to support the circulation during heart failure and argued against the use of sympathetic antagonists (e.g., β-adrenoceptor antagonists) in patients with this disease (24). However, the realization that long-term activation of the sympathetic nervous system also exerts adverse effects that clearly contribute to the progression of heart failure (49) provided the rationale for conducting clinical trials to test the possible benefit of chronic β-blockade in heart failure. The results of large prospective, placebo-controlled, ran-

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domized trials have established the benefit of β-adrenoceptor antagonists in the management of chronic heart failure.

Recently, the characterization of a functional, negatively inotropic, β_3-adrenoceptor in the human heart provided new opportunities for research in this field (25,26). From a physiological point of view, the importance and role of this receptor, which is co-expressed with β_1- and β_2-adrenoceptors, needed to be clarified. Already, signaling pathways linked to β_3-adrenoceptors and the regulation of β_3-adrenoceptor gene expression in heart failure have been partially characterized. Linear studies with β_3-specific agonists or antagonists in animal models of heart failure will help to determine whether β_3-adrenoceptor agonists or antagonists are useful in the treatment of myocardial dysfunction.

This review summarizes the rationale and effects of β-adrenergic blockade in chronic heart failure and specifically addresses the question of the potential use of β_3-adrenoceptor antagonists in the treatment of heart failure and other pathophysiological conditions associated with a decreased cardiac contractility.

RATIONAL FOR β-ADRENOCEPTOR ANTAGONIST THERAPY IN CHRONIC HEART FAILURE

The failing human heart is characterized by an activation of the sympathetic nervous system (29,30,52). The resulting catecholamine overflow helps to maintain cardiac performance over the short-term by increasing contractility and heart rate. This increase in cardiac adrenergic drive is initially beneficial, but ultimately damaging to the myocardium (9,2,34).

Catecholamines, acting through their receptors, induce a positive inotropic and chronotropic response in the heart and promote growth of human cardiac myocytes (7,10). β_1- and β_2-Adrenoreceptors are coupled to stimulatory G proteins, G_s, which serve to increase the activity of adenylyl cyclase, thereby increasing intracellular levels of cAMP. In normal, non-failing human ventricles, the numerical β_1/β_2 ratio is clearly in favor of the β_1-receptor (20 – 30% of all β-receptors are β_1-receptors). In contrast, up to 35% to 40% of all β-receptors in failing ventricles are of the β_2-subtype due to selective downregulation of the β_1 subtype (6,7,10). α_1-Adrenoreceptors are coupled via a different G protein subclass (G_q) to phospholipase C, which, through the second messenger, diacylglycerol, activates certain isoforms of the protein kinase C family. In the failing heart α_1-adrenoreceptors are upregulated; thus the adrenergic receptor profile of cardiac myocytes changes from a predominately β_1 to a mixed α/β profile in end-stage heart failure (6). Finally, the β_3-adrenoreceptor is now known to be present in the human heart as a counterregulatory receptor exerting a negative inotropic effect, which appears to be coupled to the inhibitory G protein, G_i, and influenced by nitric oxide (NO) synthesis (25,26).

The poor prognosis for heart failure patients with high plasma levels of norepinephrine is attributed to the neurohormone's toxic effects on the heart. Norepinephrine has been shown to produce cardiomyocyte injury (42) at the 'physiological' concentrations found in the failing human heart and its cytotoxicity appears to be mediated largely through β- rather than α-adrenoceptors (42). In transgenic mice, overexpression of human β_1-adrenoceptors (21), G_s (31) or G_q (18) in the heart produces a cardiomyopathic phenotype, with left ventricular chamber dilatation and systolic dysfunction. Overexpression of G_s is also associated with an increase in markers of apoptosis, similar to the effect observed in adult rat cardiac myocytes exposed to β-agonists (16,17). In adult rat cardiomyocytes, stimulation of the

β_1-adrenoreceptor mediates apoptotic signaling, whereas the β_2-adrenoreceptor pathway is antiapoptotic through its coupling to G_i (14,17). However, overexpression of the human β_2-adrenoreceptor in the hearts of transgenic mice also results in depressed systolic function and a cardiomyopathic phenotype (39). In contrast, overexpression of a constitutively activated α_1-adrenoreceptor in the hearts of transgenic mice produces concentric hypertrophy (45). Taken together, these observations in transgenic mouse models suggests that chronic adrenergic signaling is a harmful process leading to cardiomyopathy.

In the failing heart, the β-adrenergic signaling pathway is characterized by desensitized β_1- and β_2-adrenoreceptors that are reduced in number, increased expression levels of G_i, and increased expression and activity of β-adrenergic receptor kinase (βARK) itself (57). βARK is a cytosolic enzyme that phosphorylates agonist-occupied β-adrenoceptors as well as some other G protein-coupled receptors, leading to their desensitization and functional uncoupling. These changes may be interpreted as an endogenous mechanism that protects the heart from further injury and progression to heart failure. In support of this view, homologous and heterologous models of β-adrenoceptor desensitization systems (53) suggest that β-adrenergic receptor pathway desensitization mechanisms are protective. In the end-stage failing heart, 50% to 60% of the total signal transducing potential is lost, but substantial signaling capacity remains (6). A potentially effective therapeutic strategy might be to reinforce this endogenous anti-adrenergic mechanism by inhibiting adrenoceptor signal transduction (5,8,23). This is one rationale for the use of adrenoceptor antagonists in the treatment of chronic heart failure.

THE ROLE OF THE β_3-ADRENOCEPTOR IN THE NORMAL HUMAN HEART

Until recently, activation of β-adrenoceptors has exclusively been associated with stimulation of cardiac contractility. However, the atypical effect of isoproterenol, a non-selective β-adrenoceptor agonist, on human ventricular endomyocardial biopsies in the presence of nadolol, a β_1- and β_2-adrenoceptor antagonist, (26) suggested the presence of a functional third β-adrenoceptor subtype in human ventricular muscle. Indeed, contrary to β_1- and β_2-adrenoceptor pathways, activation of this receptor by norepinephrine in the presence of α_1-, β_1- and β_2-antagonists, or by BRL 37344, a selective β_3-adrenoceptor agonist, decreases contractile force (25,26). BRL37344 also elicited a negative inotropic effect on human endomyocardial biopsies, an effect preserved in the presence of metoprolol (a β_1-adrenoceptor antagonist) or nadolol (a α_1- and β_2-antagonist), but antagonized by bupranolol (a non-specific α_1- β_2- and β_3-antagonist) (26). In addition, pharmacological evidence of a myocardial β_3-adrenoceptor was corroborated by the detection of β_3-adrenoceptor transcripts in the same biopsies (26). More recently, immunohistochemical localization of the β_3-adrenoceptor on cryostat sections of human endomyocardial biopsies was shown using a specific monoclonal antibody. The same antibody was also used in Western Blotting experiments to confirm protein expression of this β-adrenoceptor subtype in human cardiomyocytes themselves (46).

The intracellular signaling pathway relaying catecholamine stimulation of β_3-adrenoceptors in cardiac cells was recently characterized, at least in part. Similar to all other adrenoceptor subtypes, the β_3-adrenoceptor belongs to the superfamily of G protein-coupled receptors, each with seven hydrophobic transmembrane spanning domains. In the

human heart, the observation of a marked negative inotropic effect following β_3-adrenoceptor stimulation was difficult to reconcile with β_3-adrenoceptor coupling to G_s protein, as was previously documented in adipocytes. However, it was shown that under certain circumstances β_3-adrenoceptors could be linked to G_i proteins in adipocytes (3,13). In an attempt to define the possible involvement of G_i proteins in the human heart, Gauthier, et al. (26) tested the effects of BRL 37344 on pertussis toxin pretreated endomyocardial biopsies (pertussis toxin is a selective inhibitor of G_i or G_o activity). This treatment significantly reduced the effect of BRL 37344 both on cardiac contractility (26) and cyclic GMP (cGMP) production (25), suggesting the involvement of a G_i or G_o protein in the β_3-adrenoceptor signaling pathway.

In several mammalian species the positive inotropic effect of catecholamines is attenuated by activation of endothelial nitric oxide synthase (eNOS), which is constitutively expressed in cardiomyocytes, and leads to an increase in NO production (2). Gauthier, et al. (25) examined the role of eNOS and NO in mediating the β_3-adrenoceptor effect on endomyocardial biopsies. In the presence of methylene blue, a non-specific inhibitor of NO-stimulated guanylyl cyclase activity, or in the presence of both L-NAME and L-NMMA, both NOS inhibitors, the negative inotropic effect of BRL 37344 was strongly reduced. Furthermore (25), the authors showed that the inhibition of NOS could be reversed by L-arginine, the substrate for the enzyme, but not by its enantiomer, D-arginine, which cannot be used as a substrate by NOS. Moreover, under similar experimental conditions, changes in intracellular cGMP levels paralleled the effects on cardiac contractility, supporting a role for this cyclic nucleotide in mediating attenuation of cardiac muscle contraction.

At least two intracellular signaling pathways may downregulate cardiac contractility in response to cGMP elevation: (1) activation of cGMP-dependent protein kinases, which decrease calcium current by regulating L-type calcium channels (43,58) or decrease cardiac myofilament sensitivity to calcium (51) (possibly through phosphorylation of troponin I), and (2) activation of cGMP-stimulated phosphodiesterases (PDE II), which decrease cAMP levels (44). Alternatively, NO may regulate cardiac function in a cGMP-independent manner by covalent modifications of key proteins such as cytochrome C oxidase (55), creatine phosphokinase (28), or L-type calcium channels (11). However, the evidence suggests that the relative importance of each pathway, as well as the final effect on contractility, may be profoundly influenced by the experimental preparation and species used, the region of the heart studied, and the concentration of NO or cGMP generated (for reviews, see ref. 1,35).

The regulation of cardiac electrical activity by β_3-adrenoceptors has not been extensively investigated. However, experiments with human endomyocardial biopsies demonstrated that the negative inotropic effect was associated with a decreased amplitude and acceleration of the repolarization phase of ventricular action potentials (26). These effects were not observed in ventricular tissues obtained from patients with cystic fibrosis (37), a genetic disease caused by a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein (50). The electrophysiological effects of β_3-adrenoceptor stimulation could, therefore, result, in part, from the activation of a chloride repolarizing current flowing through CFTR channels. In an heterologous expression system co-expressing human β_3-adrenoceptor and CFTR, β_3-adrenoceptor agonists induced CFTR activation through a cAMP/protein kinase A independent pathway (37). Further experiments showed that β_3-adrenoceptor agonists regulate CFTR conductance through a pertussis toxin-sensitive G protein (38). The recent discovery of functional coupling of β_3-adrenoceptors to the $Kv_{(s)}$ (KvLQT1/minK) K^+ channel in Xenopus

oocytes (32) suggests the additional possibility that β_3-adrenergic regulation of cardiac action potentials is mediated by activation of repolarizing K^+ currents. However, more electrophysiological studies on isolated cardiomyocytes are needed to clarify the role of the β_3-adrenoceptor in cardiac excitation-contraction coupling.

THE REGULATION OF THE β_3-ADRENERGIC PATHWAY IN DISEASED MYOCARDIUM

An important step in understanding the role of the β_3-adrenoceptor in the failing human heart is to determine how its expression is regulated. In a recent study of myocardial samples from human patients (46), a significant increase in β_3-adrenoceptor expression in failing cardiac tissues from either ischemic or dilated cardiomyopathic hearts was observed in comparison to non-failing, non-denervated hearts. Notably, immunohistochemical analysis revealed that the β_3-adrenoceptor was expressed principally in cardiomyocytes, supporting the paradigm that it is directly coupled to eNOS in this cell type. Interestingly, the increase in β_3-adrenoceptor expression occurred in parallel to an increase in $G_{\alpha i-2}$ protein expression, as previously reported in the literature (4,22).

Cardiac β_3-adrenoceptors, in comparison to α_1- and β_2-adrenoceptors, show distinctive features of potential interest in the context of cardiac failure. First, as mentioned above, is their coupling to a G_i or G_o; probably $G_{\alpha i-2}$, which is the main G_i isoform in the human ventricle, that is clearly upregulated during heart failure (4,22). Second, following agonist activation the β_3-adrenoceptor is relatively resistant to desensitization following activation with agonists (49). The β_3-adrenoceptor is also refractory to short-term agonist-promoted uncoupling of the signaling pathway (36) partly because it does not contain protein kinase A and βARK phosphorylation sites that are located in the third cytoplasmic loop and the C-terminal region of β_1- and β_2-adrenoceptors. Third, the β_3-adrenoreceptor is resistant to long-term downregulation. Finally, the β_3-adrenoceptor is activated at higher catecholamine concentrations than β_1- and β_2-adrenoceptors (20). Taken together, these data suggest that following prolonged activation by the sympathetic nervous system the β_3-adrenoceptor-mediated response is likely to be preserved while the β_1- and β_2-adrenoceptor-mediated responses are attenuated. In addition to an increase in receptor abundance, the evidence supports the view that the β_3-adrenoceptor plays a dominant role in regulating cardiomyocyte function during the high adrenergic tone that is typical of heart failure.

THE USE OF β_3-ANTAGONISTS IN CHRONIC HEART FAILURE

In the myocardium, the co-expression of three β-adrenoceptor subtypes coupled to opposing inotropic effects somewhat recapitulates a paradigm long known to be operative in blood vessels: catecholamines activate both contracting (α-adrenoceptor-mediated) and relaxing (β-adrenoceptor-mediated) effects. As in blood vessels, the concurrent activation of cAMP-dependent positive (β_1- and β_2-adrenoceptors) and NO-dependent negative (β_3-adrenoceptor) inotropic pathways in the same cardiomyocyte probably provides a mechanism for fine tuning of adrenoceptor-mediated control of cardiac contractility. Stated another way, the β_3-adrenoceptor pathway could function as a counteracting "rescue" mechanism that prevents cardiomyocyte damage from excessive stimulation of α_1- and β_2-adrenoceptors. However, as heart failure progresses to a later stage, this compensatory mechanism might become maladaptive, providing a persistent negative

inotropic effect that leads to further myocardial dysfunction thereby exacerbating the condition. Either way, the available evidence supports the view that the β_3-adrenoceptor plays an increasingly important role in the pathogenesis of heart failure as circulating levels of catecholamines are increased. However, β_3-adrenoceptor stimulation may not be uniformly deleterious. In blood vessels, β_3-adrenoceptors have a relaxing effect on vessel tone leading to vasodilation that might contribute to decrease the peripheral vascular resistance and afterload in the failing heart. In addition, a local release of NO in the myocardium following β_3-adrenoceptor stimulation might enhance diastolic relaxation and reduce oxygen consumption, thereby improving cardiac function.

The discovery of functional β_3-adrenoceptors in the human heart offers another plausible mechanism for the proven protective effects of β-adrenoceptor blockade in patients with heart failure. Elucidation of the specific role of β_3-adrenoceptor in healthy and failing human cardiac tissue with the possibility of developing new therapeutic approaches requires the development of potent and selective β_3-adrenoceptor antagonists.

A class of aryloxypropanolaminotetralin β_3-adrenoceptor antagonists was developed in 1996 (41). A potent member of this class, SR59230A, (3-(2-ethylphenoxy)-1-[(1S)1,2,3,4-tetrahydronaphth-1-ylaminol]-(2S)-2-propanol oxalate), was shown to be selective for the β_3-adrenoceptor in rat brown adipocytes (48), rat colonic motility assays (41), and human colonic circular smooth muscle relaxation activity assays (19). This drug has not yet been studied in human cardiac tissue, but in rat cardiac tissue SR59230A did not antagonize the effect of CGP 12177, a non-conventional β_3-adrenoceptor partial agonist, causing a positive chronotropic effect in right atria and a positive inotropic effect in left atria (33). In contrast, in another study in rats (40) SR59230A attenuated the cardiostimulant and thermogenic effects (in brown adipose tissue) of CGP 12177 to a similar extent. In a third study, this time in rat thoracic aorta, SR59230A antagonized the β_3-dose-dependent relaxation evoked by SR58611, a preferential β_3-adrenoceptor agonist (56), suggesting that β_3-adrenoceptor antagonists might be deleterious in the failing heart by increasing afterload. Again, this hypothesis remains to be tested.

More recently, Candelore, et al. (12) described a new class of selective human β_3-adrenergic-antagonists developed by utilizing heterologously expressed cloned human receptors. These are L-748,328 ((S)-N-[4-[2-[[3-[3-(aminosulfonyl)phenoxy]-2-hydroxypropyl]amino] ethyl]phenyl]benzenesulfonamide) and L-748,337 ((S)-N-[4-[2-[[3-[3-(acetamidomethyl)phenoxy]-2-hydroxypropyl]amino]ethyl]phenyl]benzenesulfonamide). Recently we confirmed the selectivity of L-748,377 in human endomyocardial biopsies. In the presence of 0.1 μM of this antagonist, the negative inotropic effect of BRL37344 was clearly attenuated (47), suggesting that L-748,377 might be a good candidate for the direct demonstration of a pathogenic role of β_3-adrenoceptors in myocardial dysfunction in humans. We believe that such an *in vivo* approach is now necessary and could possibly lead to the development of new drugs for the treatment of heart failure.

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