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β_3-Adrenergic stimulation in the human heart: Signal transduction, functional implications and therapeutic perspectives

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Next to β_1- and β_2-adrenoceptors, a third β-adrenoceptor population is expressed in the human heart, the β_3-adrenoceptor. In mammalian ventricular myocytes, β_3-adrenergic stimulation leads to a decrease in contractility via a release of nitric oxide (NO). Recently, different molecular mechanisms of β_3-adrenergic activation of endothelial nitric oxide synthase (eNOS) have been uncovered in cardiac myocytes. In the non-failing and especially the failing heart, β_3-adrenergic stimulation may offer protection against excessive catecholaminergic β_1-adrenoceptor stimulation. In this context, the β_3-adrenoceptor is discussed as a novel target for the pharmacological therapy of heart failure.

1. Introduction

β-Adrenergic stimulation is one of the most important mechanisms for the modulation of cardiac function. The cardiac β-adrenoceptor population is dominated by β_1-adrenoceptors (about 70%, Bristow et al. 1986) which mediate an increase in contractility and frequency upon catecholaminergic stimulation (Wallukat 2002). β_2-Adrenoceptors are expressed in far lower numbers in myocardium. Though their physiological role has not been fully uncovered yet, there is evidence, that they may have positive inotropic effects via stimulating G-proteins (G_s) but as well may oppose β_1-adrenergic stimulation by interacting with inhibitory G-proteins (G_i) and endothelial nitric oxide synthase (eNOS) (Xu et al. 2000; Dedkova et al. 2002; Malan et al. 2004) (Fig. 1).

The observation that catecholamines mediate cardiostimulant effects even in the presence of $\beta_{1/2}$-adrenoceptor blockade has led to the suggestion of a putative β_4-adrenoceptor population (Kaumann et al. 1998). Lately, this hypothesis has been questioned as it was shown that these effects are indeed mediated by an atypical so called "low affinity" activation state of the classical β_1-adrenoceptor (Kaumann et al. 2001).

By contrast, the β_3-adrenoceptor differs distinctly from classical $\beta_{1/2}$-adrenoceptors concerning its molecular struc-

Abbreviations:

cAMP	cyclic adenosinmonophosphate
cGMP	cyclic guanosinmonophosphate
eNOS	endothelial nitric oxide synthase
G_I-protein	inhibitory G-protein
G_s-protein	stimulating G-protein
L-NMA	N-nitro-L-arginine
L-NAME	N-Nitro-L-arginine methylester hydrochloride
NO	nitric oxide

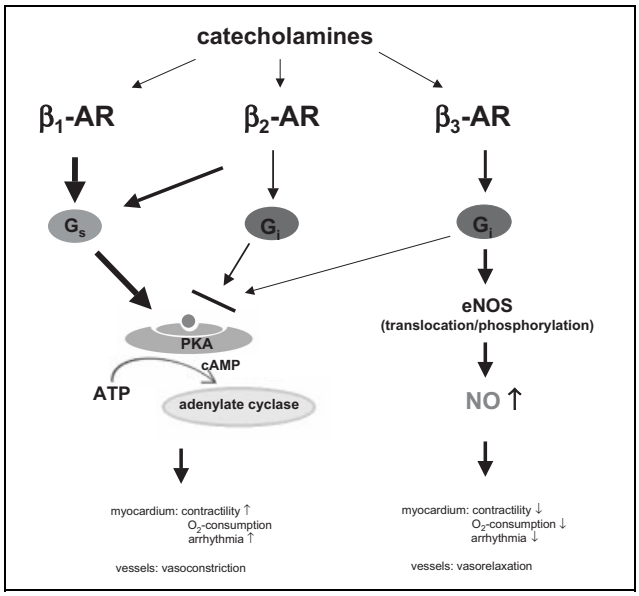

Fig. 1: Schematic diagram of the myocardial β-adrenergic signaling cascades. While β_1-adrenoceptors are coupled to G_s-proteins, there is evidence that β_2-receptors act via both G_i and G_s proteins. The stimulation of β_1-adrenoceptors leads to a release of cAMP and a subsequent activation of adenylate cyclase resulting in an increase in contractility. Presumably, β_3-adrenoceptors are exclusively coupled to G_i-proteins and reduce cardiac contractility via an activation of eNOS and a subsequent NO release. Modified from Kaumann et al. (1997) and Balligand et al. (2000)

ture and its genetic encoding (Bylund et al. 1994). Thus, it was shown that the β_3-adrenoceptor is a seven transmembrane domain receptor (Emorine et al. 1989) which shares only 40–50% amino acid identity with the common $\beta_{1/2}$-adrenoceptor and possesses an intron whereas $\beta_{1/2}$-adrenoceptors are intronless (Granneman et al. 1993). β_3-Adrenoceptors are known to be expressed in extracardial tissues such as vascular and intestinal smooth muscle and adipose tissue (Roberts et al. 1997; Sennitt et al. 1998; Trochu et al. 1999) and have recently been reported to be expressed on the membrane of human atrial and ventricular myocytes (Chamberlain et al. 1999; Moniotte et al. 2001).

A variety of studies have aimed at clarifying the influence of β_3-adrenergic stimulation on cardiac inotropy and have partially yielded controversial results. Most recently, studies have uncovered key steps in the intracellular β_3-adrenergic pathway and have uncovered different molecular mechanisms of β_3-adrenergic activation of endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) release. In addition, evidence has been gathered for significant alterations of the β_3-adrenergic system in the failing heart.

This work aims at giving an overview on the current picture of β_3-adrenoceptor mediated inotropic effects and intracellular signaling cascade. Furthermore we discuss the physiological and pathophysiological implications of cardiovascular β_3-adrenergic stimulation. In this context, we will also speculate on the β_3-adrenoceptor as a putative target for the pharmacological therapy of heart failure.

2. Intracellular signal transduction

In 1994 Chaudhry and coworkers demonstrated that β_3-adrenoreceptors couple to inhibitory G-proteins (G_i) (Emorine et al. 1989; Chaudhry et al. 1994; Begin-Heick 1995) (Fig. 1) which are known to regulate myocardial nitric oxide (NO) metabolism (Xu et al. 2000; Dedkova et al. 2002). Thus, early on, NO has been suggested as the intracellular messenger of cardiac β_3-adrenergic signaling.

In the following, this was confirmed by the observation that β_3-adrenergic inotropic effects were blunted in the presence of the NO blocker methylene blue and the nitric oxide synthase (NOS) antagonists L-NMA and L-NAME (Gauthier et al. 1998). Consistently, synthetic β_3-adrenoceptor agonists have been shown to release myocardial NO (Gauthier et al. 1998) and to increase cyclic guanosine monophosphate (cGMP), which is the main effector molecule mediating NO-induced effects (Trochu et al. 1999).

Among the different isoforms of the nitric oxide synthases, the eNOS is known to be the predominant generator of endogenous NO in myocardial and vascular tissue (Balligand et al. 1995).

It was recently demonstrated in human myocardium that the preferential β_3-adrenoceptor agonist BRL 37344 evokes a time dependent activation of eNOS and a subsequent NO-release. These effects were resistant to $\beta_{1/2}$-adrenoceptor blockade and could not be mimicked by β_1-adrenoceptor stimulation and thus can be interpreted as a genuine β_3-adrenergic mechanism (Pott et al. 2003) (Fig. 2). Thereby, β_3-adrenergic myocardial stimulation seems to act via different molecular mechanisms that have previously been described in endothelial cells (Michel et al. 1997; Dimmeler et al. 1999; Fulton et al. 1999; Bloch et al. 2001; Fleming et al. 2001; Goligorsky et al. 2002). Thus, using specific antibodies for immunohistochemical imaging, it was recently reported that an activation of the β_3-adrenoceptor in human myocardium leads to both an Akt-dependent phosphorylation of $_{Ser1177}$eNOS as well as a translocation of eNOS from the sarcolemmal membrane. Interestingly, these different mechanisms seem to occur with regional preference: Whereas β_3-adrenergic eNOS-translocation was only detected in the right atrium, eNOS-phosphorylation was detected in both right atrium and left ventricle (Brixius et al. 2004) (Fig. 3). There is also evidence that only the translocation but not the phosphorylation of eNOS is Ca^{2+} dependent (Pott et al. 2005).

Furthermore, it should be taken into consideration that G_i-proteins are also known to directly inhibit $\beta_{1/2}$-adrenergic activation of adenylate cyclase (El-Armouche et al. 2003) and thereby can attenuate β_1-adrenergic effects. The above mentioned constellation of β_3-adrenergic G_i-coupling may thus provide an opportunity for the β_3-adrenergic system to directly oppose β_1-adrenergic effects on cardiac inotropy, chronotropy and dromotropy.

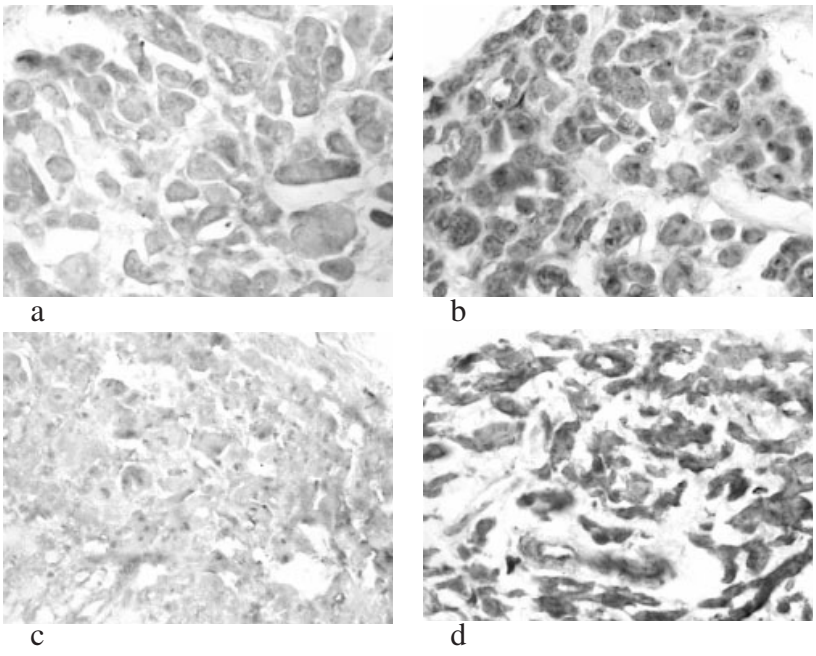

Fig. 2:
Influence of the β_3-adrenoceptor agonist BRL 37344 on eNOS activity. Translocated eNOS proteins are stained using a specific antibody in human right atrial myocardium. Cytosolic staining is observed in myocytes under basal conditions (a) and is increased after 5 min of incubation with BRL 37344 (b). Experiments were repeated in the presence of propranolol to block $\beta_{1/2}$-adrenoceptors. Staining again increased from basal (c) to pretreatment with BRL 37344 (d). Modified from Pott et al. (2003)

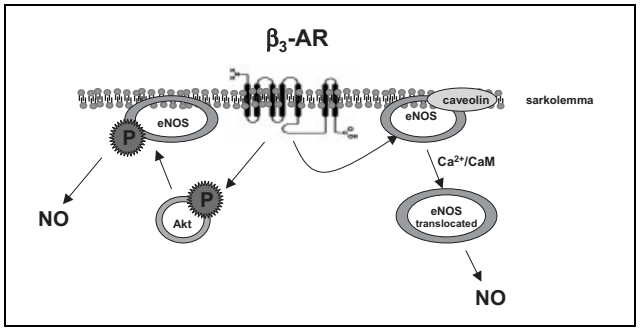

Fig. 3: Two ways of β_3-adrenergic myocardial eNOS-activation have been recognized with regional preference. In both human atrium and ventricle β_3-adrenergic stimulation leads to an Akt dependent phosphorylation of $_{Ser1177}$eNOS (left). Only in human atrium, eNOS is additionally activated by a translocation of the enzyme from the sarcolemmal membrane (right). Both mechanisms independently lead to a myocardial NO-release. Modified from Brixius et al. (2004)

In summary, cardiac β_3-adrenergic stimulation leads to an activation of eNOS via both, a phosphorylation and a translocation of the enzyme and to a subsequent release of NO within the myocyte. A direct antagonism of β_1-adrenergic effects via G_i proteins could potentially present an alternative mechanism of β_3-adrenergic signal transduction.

3. Inotropic effects

NO is reported to have negative inotropic effects (Brady et al. 1999) and it would thus appear likely that also β_3-adrenergic stimulation would lower contractility in the human heart. The experimental investigation of inotropic properties of the β_3-adrenoceptor has however proven to be difficult, which is mainly due to a lack in selectivity of most of the β_3-adrenoceptor agonists that are currently in experimental use.

Thus, several studies using human atrial myocardium observed a positive inotropic effect that was however abolished in the presence of $\beta_{1/2}$-adrenoceptor blockade (Arch et al. 1993; Wheeldon et al. 1994; Sennitt et al. 1998; Pott et al. 2003). Accordingly, we recently reported a distinct affinity of the widely used β_3-adrenoceptor agonist BRL 37344 towards $\beta_{1/2}$-adrenoceptors in membrane preparations from human right atrial myocardium (Pott et al. 2003).

This coagonism of β_3-adrenoceptor agonists however seems to be less pronounced in ventricular myocardium. A number of studies using ventricular myocardial biopsies from human non-failing and failing hearts, found β_3-adrenoceptor agonists to evoke a negative inotropic effect that was resistant to inhibition of $\beta_{1/2}$-adrenoceptors and can thus be considered to be a genuine β_3-adrenergic mechanism (Gauthier et al. 1996, 1998; Moniotte et al. 2001). These findings have also been reproduced in an animal model (Kitamura et al. 2000), as well as in a transgenic mouse model overexpressing the β_3-adrenoceptor (Tavernier et al. 2003).

The reasons for these differences between atrium and ventricle are not fully understood yet, but a different density of the β_3-adrenoceptor population could be causal. Thus, we could demonstrate that in human atrium, the β_3-adrenoceptor is expressed in lower numbers as compared to ventricular myocardium, which would promote β_3-adrenergic effects in the ventricle more than in atrium (unpublished results, Fig. 4). A further explanation could be found in a different G-protein coupling of the β_3-adrenoceptor or in the above mentioned regional differences in the molecular mechanisms of β_3-adrenergic eNOS-activation (Brixius et al. 2004).

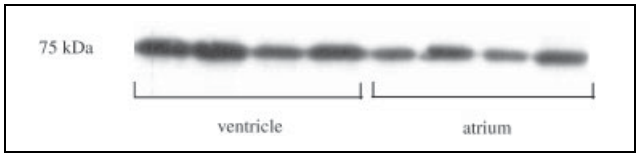

Fig. 4: Protein expression of the β_3-adrenoceptor in human right atrial and left ventricular myocardium. A significantly higher expression level of the β_3-adrenoceptor population is observed in the left ventricle (unpublished results)

Besides direct inotropic effects however, β_3-adrenergic stimulation also seems to have modulative effects on the positive inotropy induced by catecholamines. Thus, it was observed that the isoprenaline dose-response curve was shifted to the right in the presence of the β_3-adrenoceptor agonist BRL 37344 (Pott et al. 2003). This observation is consistent with reports on similar attenuative effects of NO on catecholaminergic stimulation (Brady et al. 1993; Bylund et al. 1994; Keaney et al. 1996).

In summary, β_3-adrenergic stimulation exerts a negative inotropic influence in ventricular myocardium, which however seems to be overshadowed by nonselective $\beta_{1/2}$-adrenergic effects in atrial myocardium. In addition, β_3-adrenoceptor agonists attenuate the positive inotropic response to catecholamines in human myocardium.

4. Functional implication for the non-failing heart

As mentioned above, the selective stimulation of cardiac β_3-adrenoceptors leads to a release of NO which at least in ventricular myocardium directly causes a negative inotropic effect. Besides, β_3-adrenergic stimulation evidently attenuates the inotropic action of catecholamines. These findings are by all means consistent with the effects of NO on cardiac inotropy as previously reported (Brady et al. 1993; Balligand et al. 1995; Keaney et al. 1996; Chesnais et al. 1999) and even though the existence and function of β_3-adrenoceptors in the sinuatrial node has not yet been investigated, one could readily expect a negative chronotropic influence when considering the attenuating effects of NO and cGMP on cardiac frequency (Massion et al. 2003).

Considering these issues, the function of β_3-adrenergic cardiac stimulation in the non-failing heart may be to provide a negative feedback system against the classical β_1-adrenergic stimulation. In situations of high physical and mental stress, the β_1-adrenergic system mediates positive chronotropic, inotropic and dromotropic influences thereby enhancing cardiac performance but also increasing oxygen consumption and promoting cardiac arrhythmia.

In case of excessive catecholamine release, the β_3-adrenoceptor could mitigate the effects of these so called "stress hormones" and thus protect the myocardium from possible myocytal damage induced by exuberant β_1-adrenergic stimulation (Fig. 1) (Balligand 2000; Gauthier et al. 2000).

In this context, extracardial β_3-adrenergic effects could be supportive: β_3-adrenoceptor mediated vascular relaxation (Trochu et al. 1999) would lower the total peripheral resistance and thus decrease cardiac afterload and oxygen consumption.

In addition to this "rescue" function, one could also interpret the circumstance that cardiodepressant β_3-adrenoceptors as well as positive inotropic β_1-adrenoceptors are ex-

pressed within the same myocyte as a physiological mechanism to fine-tune adrenoceptor mediated regulation of cardiac contraction.

5. Putative role of the β_3-adrenoceptor in the failing heart

Alterations of the β-adrenergic system are extensively described to occur during heart failure. One of the prominent characteristics is a chronic increase in the plasma catecholamine concentration (Gaffney et al. 1963; Chidsey et al. 1965). In the first instance, this has to be viewed as an adaptive mechanism that in the short term can uphold cardiac output via an increase in contractility and cardiac frequency. In the long term however, the chronic exposition of the heart to raised catecholamine levels reverses into a maladaptive mechanism. Thus, despite a decrease in expression and a desenzitation of β_1-adrenoceptors (Bristow et al. 1982; Schwinger et al. 1990), the increased plasma concentration of epinephrine and norepinephrine finally leads to a decrement of cardiac performance which is predominantly caused by an increase in cardiac frequency, oxygen consumption and aptitude towards arrhythmic events as well a direct toxicity of catecholamines on cardiomyocytes (Bristow 2000).

Much less is known about the role of the β_3-adrenergic system in human heart failure. Interestingly, other than β_1-adrenoceptors, the β_3-adrenoceptor expression is not decreased but rather upregulated in the human failing heart (Moniotte et al. 2001). In line with this, the β_3-adrenoceptor protein is known to lack recognition sites for kinases involved in β-adrenoceptor desensitisation and downregulation (Ligett 1993; Strosberg 1993). Consequently, it has been observed that the negative inotropic response toward β_3-receptor agonists was widely preserved in human failing hearts whereas the β_1-adrenergic agonist isoproterenol lost 75% of its positive inotropic effect (Moniotte et al. 2001).

The β_3-adrenergic system could by all means have a beneficial influence in the failing heart. For example, the coupling of β_3-receptors to G_i-proteins (Chaudhry et al. 1994; Begin-Heick 1995) could directly antagonize the excessive activation of the β_1-adrenergic system with its consequential adverse effects (Bristow 2000). Additionally, the activation of eNOS and the subsequent NO release (Pott et al. 2003; Brixius et al. 2004) could present a further beneficial influence. Thus, in addition to a direct negative chronotropic effect (Balligand et al. 1993; Feron et al. 1998), NO is also imputed to attenuate the effects of catecholamines. Along with this, the β_3-adrenergic NO-mediated peripheral vasorelaxation could reduce cardiac afterload (Trochu et al. 1999) and improve oxygen supply via an increase in coronary blood perfusion (Chambers et al. 1996; Heymes et al. 1999). Direct influences of NO on diastolic relaxation (Heymes et al. 1999), mitochondrial respiration (Loke et al. 1999) and myocardial microcirculation (Lefer et al. 1996; Liu et al. 2002) may further contribute to increase cardiac output and to reduce oxidative stress in the failing heart.

Next to all these potentially beneficial properties of the β_3-adrenoceptor, one should however not neglect its negative inotropic effect. Especially in the terminally failing heart, a progressive downregulation of β_1-adrenoceptors along with an unchanging or even growing β_3-receptor population (Moniotte et al. 2001) may lead to an imbalance of the cardiac β-receptor populations. As a result, this could lead to an excessive promotion of β_3-adrenergic negative inotropic effects resulting in a reduction of cardiac contractility and a potentially fatal aggravation of heart failure (Massion et al. 2001).

6. Therapeutic perspectives

Against this background, the β_3-adrenoreceptor is discussed as a target for the pharmacological therapy of heart failure (Balligand 2000; Moniotte et al. 2002). In principal, both a promotion but also an inhibition of the β_3-adrenergic system could have beneficial effects in the failing heart. The strategy of pharmacological β_3-adrenoceptor stimulation would above all promise lead to an attenuation of the excessive catecholaminergic state of the failing heart along with an increase in endogenous NO production. The potential advantages of this constellation have been discussed above.

Should it however become apparent that the overexpression of the β_3-adrenoceptor population (Moniotte et al. 2001) and a resulting persistent negative inotropic influence aggravate the terminal stages of heart failure, one could also imagine the specific blockade of the β_3-adrenergic system as a therapeutic strategy (Moniotte et al. 2002).

At present however, the targeted pharmacological manipulation of the cardiac β_3-adrenergic system *in vivo* is hardly feasible due to a lack in agonists and antagonists with sufficient specificity. Thus, next to their β_3-agonistic properties a variety of synthetic β_3-agonists that are presently in experimental use show significant positive inotropic effects that are mediated via classical β_1-adrenoreceptors (Kaumann et al. 1998; Sennitt et al. 1998; Kozlovski et al. 2003; Pott et al. 2003; Tavernier et al. 2003).

Yet, a variety of new synthetic β_3-adrenoceptor agonists and -antagonists are under development and being tested for their specificity (Candelore et al. 1999; Ahmed et al. 2002, 2003; Saccomanni et al. 2003; Baker 2005), so that a more targeted pharmacological stimulation or inhibition of the cardiac β_3-adrenoceptor may be possible in the near future.

The β_3-adrenoceptor may however also be of significance in the pharmacotherapy with beta blockers. The use of β-adrenergic antagonists to counterbalance the excessive catecholaminergic stimulation of the failing heart is a long established therapy for the treatment of heart failure (Bristow 2000; Bundkirchen et al. 2004). Beta blockers presumably unfold their beneficial effects on progression and prognosis of cardiac failure via a decrease in cardiac frequency, a reduction of oxygen consumption and an attenuation of the cardiotoxic and arrhythmogenic effects of catecholamines (Bristow 1993). The β-adrenoceptor antagonists that are currently in use are subdivided according to their selectivity for either β_1- and/or β_2-adrenoceptors (Bristow 2000), however it is widely unknown whether these compounds also exert antagonistic or stimulating effects on the β_3-adrenoceptor population. Thus, only the beta blockers nebivolol and esmolol have so far been investigated for their interactions with the β_3-receptor. Interestingly, esmolol revealed significant antagonistic properties toward the β_3-adrenoceptor (Murao et al. 2002), whereas nebivolol was shown to activate the β_3-adrenergic system resulting in a release of NO (de Groot et al. 2003).

The testing of additional beta blockers on possible β_3-agonistic or -antagonistic properties could decisively contribute to clarify the partially different acting profiles of these substances. The awareness of possible β_3-adrenergic

properties of particular beta blockers may allow a more specific integration of these substances in the therapeutic regimen of cardiovascular diseases.

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