Beta Adrenoceptor Desensitization in Chronic Heart Failure

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Abstract

Congestive heart failure is a clinical syndrome characterized by water and sodium retention. The chronic activation of the neuroendocrine systems leads to detrimental consequences on myocardial function. The elevation of noradrenaline leads to β-receptor desensitization, that implies a reduced sensitivity to the β-receptor agonists. In hearts with already depressed contractility this phenomenon brings about a further blunting of the inotropism. Several mechanisms have been invoked to explain this desensitization, that result in a reduced isoproterenol stimulated cAMP production, these involve decreased β-receptor density, phosphorylation of the β-receptor (operated by a specific β-adrenoreceptor kinase, βARK) with consequent uncoupling, internalization and sequestration of the adrenergic receptor. Recently modifications of the regulatory subunit of the adenylate cyclase have been described, either an increased activity of the inhibitory subunit (Gi) or a decreased activity of the stimulatory subunit of the guanin nucleotide binding protein (Gs). Whether the desensitization is due to the increased levels of circulating catecholamines or to an altered release and reuptake of the mediator at synaptic level is not clear yet.

We used the model of single cardiac atrial and ventricular myocytes isolated from experimental animal models of heart failure and from patients with the heart failure syndrome, showing that the degree of desensitization correlates with the clinical status of the patient and age. The more severe the syndrome, the more profound the degree of desensitization. Etiology of heart disease seem not to influence desensitization.

Key words: Chronic heart failure, β-adrenoceptor, isolated cardiomyocytes, catecholamines.

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Definition of cardiac failure

A number of definitions have been proposed to define heart failure. For clinical purposes, heart failure may be defined as a clinical syndrome that results from cardiac performance which is inadequate to meet the metabolic requirements of the body tissues. This includes both systolic and diastolic failure. More appropriately Harris [1] pointed out that the term "congestive heart failure" has been referred to us to describe the syndrome of oedema and engorged veins that accompanies accumulation of water. Water and sodium retention are in fact the most striking features of this syndrome. Anand *et al.* [1] found in patients with untreated congestive heart failure a total body water increase of 16% above controls whilst extracellular liquid was 33% and plasma volume 34% above controls. In the same patients total exchangeable sodium was 37% above control. The haemodynamics of the failing heart show a reduction in ejection fraction and cardiac index, a marked increase in left and right atrial pressures and an increase in pulmonary wedge pressure. End diastolic pressures and volumes are increased in the attempt to compensate, according to the Frank-Starling law, for the depressed cardiac output. In contrast to the functions of most critical organs, the heart's function is highly regulated and acutely responsive to the body's physiologic demands. This is most evident in the classic "fight or flight response", during which cardiac function must increase rapidly to provide nutrients and oxygen to the peripheral musculature. Rapid changes in cardiac function are effected through neural mechanisms, whereas slower, more sustained alterations in cardiac function are the result of humoral stimuli. In acute circumstances, the so called "stress" situations, like bleeding, strenuous exercise and myocardial infarction [26] the first goal is to maintain an

adequate aortic pressure. This is done through acute mechanisms like adrenergic and baroreceptorial regulation. But these responses cannot last for a long time. In the presence of a chronic disease, adrenergic and baroreceptorial mechanisms are activated in order to maintain an adequate blood pressure and tissue perfusion, but they fail very soon and some other, long acting, humoral mechanisms have to intervene. This is the reason why in the chronic heart failure syndrome there is a full activation of some important hormonal systems. The renin-angiotensin system is activated and leads to increased circulating levels of aldosterone. Plasma atrial natriuretic peptide, released by atrial myocytes when the atrium is stretched, vasopressin, growth hormone, and cortisol are also increased [1].

Catecholamines and heart failure

Circulating noradrenaline is extremely elevated [11, 37]. It has been also shown that the levels of circulating noradrenaline strongly correlate with the severity of the disease and are a guide to prognosis in patients with congestive failure [12]. These hormonal changes lead to water and salt retention. Fluid expansion certainly brings into play Starling's law and may be seen as a way of increasing the filling of the heart and, thereby, its output, but retention of saline is harmful. It is therefore clear that the neuroendocrine-renal response in all the cardiovascular emergencies is the same. The difference is the length of time over which the stimulus is maintained. Minutes for exercise, hours or days for shock, months or years for cardiac failure. These mechanisms are very useful and necessary for survival, but become harmful when maintained over prolonged periods because they lead to retention of saline in the body. Saline retention is harmful since the lung is very susceptible to flooding.

Adrenoreceptors in the heart and their function

All the neural and humoral systems have a common final pathway which is the transmembrane signal transduction located within the myocardial cell surface. These systems perceive, interpret, and amplify neurohumoral signals and ultimately induce a change in the biochemical effectors responsible for regulating cardiac contractility. One of the primary signaling systems in the heart is the receptor G-protein-adenylate cyclase complex. This system is critical in the regulation of cardiac contractility. It is composed of three parts: membrane receptor, G proteins (Guanine nucleotide binding regulatory proteins) which transduce stimulatory (Gs) and inhibitory (Gi) signals, and the effector enzyme adenylate cyclase, which converts ATP to cAMP, the intracellular second messenger. In the presence of a receptor agonist, adenylate cyclase is activated and the intracellular concentration of cAMP is markedly enhanced. This in turn results in the activation of specific protein kinases that phosphorylate several membrane and cytoplasmic proteins [17, 30]. Contraction and relaxation are regulated by these phosphorylation reactions which increase sarcolemmal calcium transport, enhance calcium uptake by the sarcoplasmic reticulum, and alter the sensitivity of the contractile myofilament to calcium. Noradrenaline is the neurotrasmitter released from neurosecretory granules in the sympathetic nerve ending in humans and other animals [29]. Once released it occupies both post-synaptic α- and β-adrenergic receptors. As much as 75% of noradrenaline is actively reaccumulated in the nerve terminal, and the remainder is metabolized enzymatically in either the general circulation or within the neural junction. By virtue of its higher affinity for β1-receptors, noradrenaline is a β1 selective agonist [28]. Adrenaline is a catecholamine hormone with both β and α adrenergic effects that activates both β1 and β2 receptors [28]. To summarize, noradrenaline is the sympathetic neurotransmitter of the heart, adrenaline is the catecholamine hormone and acetylcholine the parasympathetic neurotransmitter.

β-receptors in cardiac failure

Agents which activate the β-adrenergic pathway are the most potent stimuli of cardiac contractility. In the human heart, as opposed to other mammalian heart which possesses almost exclusively β1-receptors [30], the presence of both β1- and β2- receptors has been described [7]. Although agonist stimulation of each subtype activates adenylate cyclase, the subtypes can be distinguished by their different affinities for the agonist adrenaline and noradrenaline. The β1 receptor binds adrenaline and noradrenaline with approximatively equal affinities, whilst the human β2 has a 30-fold greater affinity for adrenaline [7]. In the last few years a reduced sensitivity to β-adrenoceptor stimulation in patients with cardiac failure has been reported. In 1971 Goldstein *et al.* [19] observed that in the papillary muscle of patients with chronic cardiac failure, glucagon was unable to enhance contractility and the adenylate cyclase activity. In 1981 Baumann *et al.* [2] described an impaired β adrenergic stimulation in the ventricle of guinea pigs post acute myocardial infarction. Ventricular dP/dT and the dose-response curve to isoprenaline, as well as the isoprenaline-stimulated adenylate cyclase activity was depressed. The β-receptor count showed a 74% loss of receptors. Metoprolol pre-treatment was able to prevent these phenomena, which were also shown to be reversible. The Authors concluded that the reported observations were the result of a specific, reversible damage of sarcolemmal β-receptors, due to excessive levels of circulating catecholamines. Later on Ginsburg *et al.* [18] and Bristow *et al.* [5] showed that the failing human heart had a decreased catecholamine sensitivity. Ginsburg *et al.* [18] observed in fact that in papillary muscles of patients with end-stage heart failure, isoprenaline produced a smaller increase in peak force development and dF/dt. The ED_{50} for isoprenaline was also five fold higher. In severely failing human hearts a 54 to 73% reduction in maximal isoprenaline-stimulated muscle contraction has been described, which was paralleled by a 50 to 56% reduction in β-receptor density, and by a 45% reduction in maximal isoprenaline-mediated adenylate cyclase stimu-

lation [15]. The histamine stimulated adenylate cyclase activity was found to be normal. In 1983 Ginsburg *et al.* [18] found in the heart of a patient with end-stage heart failure a strongly depressed isoprenaline contractile response and a nearly total loss of β-adrenoceptors, whilst the contractile response to calcium was normal. At the same time Newman *et al.* [33] in dogs with congestive heart failure found a reduced inotropic response to catecholamines. Since they also demonstrated an increased adenosine release in the same hearts, they attributed the blunted inotropic responsiveness to catecholamines of the failing heart to this phenomenon. Brodde *et al.* [8] found both in atria and ventricles of patients with congestive cardiomyopathy a selective reduction of the β1-receptors, whereas the β2 were not at all affected. Bristow *et al.* [6] showed similarly that in the heart of patients with severe cardiac failure a relatively higher proportion of β2-receptors was present. He also concluded that this was due to a selective down-regulation of the β1-receptor component. Fowler *et al.* [16] with a radioligand technique developed a method for identifying β-receptors in endomyocardial biopsies of patients with different degrees of cardiac failure. He suggested that there is a progressive receptor down-regulation and subsensitivity to agonist response. This down-regulation begins with mild-moderate ventricular dysfunction and correlates with the degree of cardiac failure (ejection fraction). Brodde and co-workers [9] in patients with mitral valve disease and different degree of cardiac failure found that there is a reduction in β-adrenergic receptor function, the extent of which correlates with the severity of the disease. However, both in atria and papillary muscles, the density of β1 and β2-receptors declined gradually and in parallel. In a turkey model of congestive cardiomyopathy Staley *et al.* [36] found a 30% reduction in NaF-stimulated adenylate cyclase activity even when ventricular function and isoprenaline-stimulated adenylate cyclase activity were still normal. Later on, when cardiac dilatation appears, also the isoprenaline-stimulated adenylate cyclase and the adenylate-stimulated contractile response become compromised.

Feldman *et al.* [15] studied the effects of different classes of inotropic drugs on human failing hearts *in vitro*. They observed that, although peak isometric force generated in response to increased extracellular calcium reached control levels in muscles from patients with heart failure, the time course of contraction and the rate of relaxation were greatly prolonged. The inotropic response to several phosphodiesterase inhibitors and isoprenaline was markedly reduced in the failing muscles. In contrast, the response to forskolin, an activator of adenylate cyclase was preserved. They therefore suggested that an abnormality in cyclic AMP production may be a fundamental defect present in patients with end-stage heart failure. Brown *et al.* [10] studying the effect of calcium and several other inotropic agents on strips of myocardium of patients with chronic heart failure, suggested that a more generalized defect than a decreased number of receptors occurs. This defect leads to a decreased inotropic effect of all the agents acting through cyclic adenosine monophosphate. Colucci *et al.* [13] infusing coronary dobutamine in patients with heart failure observed that the response to dobutamine was inversely correlated with the plasma levels of noradrenaline. He therefore put forward the hypothesis that patients with severe congestive heart failure and elevated resting plasma noradrenaline concentration have a reduced myocardial inotropic response to β-adrenergic receptor stimulation. He also suggested that the degree of sympathetic nervous system activation could be one factor involved in determining the level of myocardial β-adrenergic receptor sensitivity. On the contrary Pouler *et al.* [35] in two groups of patients with ischaemic heart disease and mild to moderate heart failure and severe heart failure respectively, showed that even though the isovolumic indexes of inotropic status were depressed, there was no shift to the right of the dose/response curve to xamoterol, a partial β1 agonist. The absence of a left to right shift of the dose/response curve for a β1-agonist, made them think that the alteration of the myocardial performance is not primarily caused by a decrease in β-adrenoceptor sensitivity. Bohm *et al.* [3] studying papillary muscles of patients with different degree of cardiac failure, and their response to different inotropic agents acting at various cellular level, some adenylate cyclase-dependent like dopamine, dobutamine, histamine and forskolin, others adenylate-cyclase independent like isobutylmethylxanthine, dibutyryl cAMP, digoxin, and calcium, confirmed that the number of cardiac β receptors is diminished according to the severity of heart failure. They also showed that the receptor coupling of histamine (H_2) receptors to adenylate cyclase may be impaired, but only in severe cardiac failure. The response to forskolin, db-cAMP and digoxin were not impaired in any of the degree of cardiac failure, suggesting that the defect is before the cAMP step. Horn *et al.* [27] in lymphocytes from patients with congestive heart failure, not only confirmed that the number of β-receptors is diminished, but they also found a 80% decrease in levels of Gs compared with age- and sex- matched normal volunteers. Treatment with ACE-inhibitors restored both the normal β-receptors and Gs pattern. Almost at the same time Neumann *et al.* [34] published the observation that in hearts from patients with end-stage heart failure due to dilated cardiomyopathy, the myocardial content of Gi protein was increased. Feldman *et al.* [14] reported an increase of the 40 kDa pertussis toxin substrate, presumably the β-subunit of the guanine-nucleotide binding protein (Gi-α) in myocardial membranes of patients with severe heart failure. No changes in the Gs-α subunit were observed. A similar observation has been published by Bohm *et al.* [4] who described in patients with dilated cardiomyopathy and end-stage heart failure an increased level of Gi-α. Patients with ischaemic heart disease did not show the same modification. The theory that specific alterations in Gs and Gi subunits could be due to the underlying disease seems therefore tenable. If there are some evidence that the reduction in β-adreno-

ceptor density is an agonist-induced phenomenon, whether this is due to elevated levels of circulating or tissue catecholamines is not clear yet. Murphree and Saffitz [32] in crude membranes obtained from different layers of failing myocardium found that a more marked degree of β-receptor down-regulation was present in the subendocardium. They suggested that the down-regulation itself does not occur uniformly and thus is not likely to be due to circulating catecholamines. In the failing human myocardium the intracellular distribution of adrenoceptors is also different. Limas *et al.* [31] in fact showed that the distribution of α1- and β-adrenoceptors differs in the failing myocardium. The membrane β2-receptor density was lower in patients with more advanced disease, whilst the vesicular fraction of β2-receptors was higher. An inverse correlation between number of membrane receptors and vesicular receptors was found only in patients with less severe ventricular dysfunction.

β-receptors desensitization: studies on isolated myocytes

The monocrotaline-treated rat model

Isolated adult cardiac myocytes provide a useful model for the study of heart muscle, since the contractile response may be observed free of neural and humoral influences.

Rats treated with the alkaloid monocrotaline develop pulmonary hypertension, with severe right ventricular hypertrophy and eventually cardiac failure. The occurrence of cardiac failure can be easily detected by means of urine volume, urinary total daily sodium excretion and increased body water content. In this animal model the urinary content of noradrenaline is significantly increased, whilst adrenaline and dopamine levels are similar to those of control rats. Results of our group indicated that in this animal model cells in either left or right ventricle do not show a depression in maximum contraction amplitude with calcium and isoproterenol. However right ventricle cells from treated rats show reduced rates of contraction in maximum isoproterenol and a significant rightward shift in PD_2, representing a two-fold increase in EC_{50} when compared to right ventricular cells from control animals. We did not find shift in EC_{50} in the left ventricle cells. The mechanisms underlying desensitization have been discussed previously and include uncoupling of receptors from adenylate cyclase activity, internalisation and loss of receptors. The role of Gs and Gi proteins have been discussed as well. The role of circulating catecholamines and local release and reuptake of noradrenaline at synaptic level in determining the receptor desensitization is still debated. In our animal model left ventricular cells from monocrotaline-treated rats were not different from control in their sensitivities to isoproterenol. This argues against a role for circulating catecholamines in desensitization, since these should affect left and right ventricles to the same degree. We therefore tend to support the hypothesis that in this model the local release and reuptake of noradrenaline play a major role in the desensitization process. Catecholamines might be raised locally in the right ventricle only, or the balance between synthesis, release and uptake of noradrenaline may be altered in the right ventricle only resulting in an increased noradrenaline concentration at the β-adrenoceptor [38].

Isolated ventricular myocytes from failing and non-failing human hearts

In single human ventricular myocytes isolated from surgical biosies of patients undergoing cardiac surgery we related age and clinical status of patients to isoproterenol response. The study on isolated cells enabled us to avoid problems with whole muscle and multicellular preparations. For instance the effect on contractility due to fibrosis, scar tissue, accumulation of cardioactive substances within the tissue, such as adenosine, the antiadrenergic effect of which is well known, the orientation of fibres etc. We measured contraction amplitude, time-to-peak shortening and time to 50% and 90% relaxation. The effects of increasing concentrations of isoproterenol and calcium were investigated by using cumulative concentration/response curves. Maximum contraction amplitude in high calcium or velocities of contraction and relaxation were not impaired in cells from failing hearts. β-adrenoceptor function in a single cell was assessed by the maximum contraction amplitude in the presence of isoproterenol relative to that with high calcium in the same cell (isoproterenol/calcium ratio). This index in left ventricular myocytes decreases with increasing severity of the disease, correlating with failure as defined by the New York Heart Association class, left ventricle ejection fraction, left ventricular end-diastolic pressure and amount of diuretics prescribed. In right ventricular myocytes only increasing NYHA class correlated with decreasing isoproterenol/calcium ratios. There was a correlation of the isoproterenol/calcium ratio between right and left ventricular cells from patients with ischaemic heart disease. β-adrenoceptor subsensitivity occurred in mitral valve disease, congenital heart disease, and congestive cardiomyopathy, but not in the right ventricle of patients with myocarditis. We also found a negative correlation between isoproterenol sensitivity and age. Multiple regression indicated that the maximum contraction amplitudes in either high isoproterenol or high calcium declined significantly with age only, but that both age and severity of the disease contributed to the decrease of isoproterenol/calcium ratio. Time to peak tension in isoproterenol, as well as relaxation times in high calcium also decreased with the age of the patient. We concluded that β-receptor desensitization may be detected in myocytes from failing human hearts, and this relates more to the severity of the disease and the age of the patient rather than the etiology of heart failure. A decline in absolute contractility of muscle cells with age was detected [22]. Similar results were obtained by our group on single atrial myocytes isolated from patients with different degree of heart failure and undergoing cardiac surgery [23]. In this case the pattern of desensitization seems to be different from that of the monocrotaline-induced heart failure. In fact the general pattern of desensiti-

zation both in ventricular and atrial myocytes is consistent with a change secondary to increased catecholamines which are raised in response to severity of heart disease [25, 38].

Similarly we have demonstrated in isolated ventricular cells from man with non-failing hearts that forskolin, isoprenaline, dibutyryl cAMP and Ca^{2+} produce similar changes in contraction amplitude [20, 24]. The ability of isoprenaline, forskolin and dibutyryl cAMP to increase the contraction amplitude of ventricular myocytes from failing human heart decreases with increasing severity of the disease, while the effect of Ca^{2+} is unchanged. In severely failing hearts the increase in contraction amplitude obtained with forskolin and dibutyryl cAMP was, in the same cell, no larger than that with isoprenaline in cases where arrhythmic effects of cAMP are limiting. Where arrhythmias are not limiting, forskolin and dibutyryl cAMP produced significantly greater increases than isoprenaline, but considerably lower than the effect of high Ca^{2+} in the same cell. Pertussis toxin can reverse this behaviour thus suggesting a role played by Gi in the desensitization process [21]. On the whole we can suggest that there is a lesion in the failing heart distal to the β-adrenoceptor, and that changes in the response to cyclic AMP may contribute to the subsensitivity to β-adrenoceptor agonists.

Conclusions

The raised level of catecholamines in the heart failure syndrome produces a reduced sensitivity to the β-adrenoceptor stimulation. The direct effect of this is a reduced isoproterenol-stimulated cyclic AMP production. This change in sensitivity may be an important factor contributing to the reduced contractile reserve in patient with heart failure.

The mechanisms of desensitization are not completely clear yet. A reduced β-adrenoceptor density has been described, either due to receptor internalization, sequestration or destruction. A functional desensitization with β-receptor uncoupling due to phosphorylation (operated by a specific β-adrenoreceptor kinase, βARK) might occur. In patients with heart failure there are reports suggesting of alterations of the α-subunit of the guanine-nucleotide binding protein, which is part of the regulatory unit of the adenylate cyclase. Increased activity of the Gi subunit and/or decreased activity of the Gs have been reported in different heart diseases. It is not completely clear yet whether the desensitization is due to high levels of circulating noradrenaline or to alterations in the release and reuptake of the mediator at synaptic level.

Studies carried out from our group on single atrial and ventricular myocytes from animals with cardiac failure and patients undergoing cardiac surgery show that desensitization can be detected in isolated cells, that the degree of desensitization correlates with the clinical status of the patient (severity of the disease) and age and not with etiology.

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