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Mitigation of β_1- and/or β_2-adrenoceptor function in human heart failure

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1 Patients with congestive heart failure (CHF) have an elevated activity of the sympatho-adrenal system. We have investigated several aspects of β-adrenoceptor desensitization in such patients.
2 The positive inotropic response to isoprenaline was attenuated in CHF patients, and the pD_2-values for isoprenaline's positive inotropic effect gradually decreased in more severe forms of the disease. Stimulation of adenylate cyclase by isoprenaline was also mitigated in cardiac membranes from patients with CHF.
3 We then studied the density of cardiac β_1- and β_2-adrenoceptors in order to understand the mechanism of β-adrenoceptor desensitization in these patients. Our data show that cardiac β_1-adrenoceptors are down-regulated in all forms of severe CHF, but that cardiac β_2-adrenoceptor density decreases only in some forms of CHF including ischaemic cardiomyopathy and mitral valve disease.
4 In circulating mononuclear leucocytes (MNL) obtained from CHF patients at rest, isoprenaline- and prostaglandin E_1-stimulated cAMP generation as well as cholera toxin and pertussis toxin catalyzed ADP ribosylation were similar to those in MNL from control patients. However, pretreatment of intact MNL with pertussis toxin enhanced cAMP generation in CHF patients but not in healthy control subjects, suggesting a tonic inhibitory effect of G_i in such patients.
5 We conclude that alterations of adrenoceptors and of their signal transduction might contribute to the desensitization of β-adrenergic responses in CHF.

Keywords heart failure β_1-adrenoceptor β_2-adrenoceptor

Introduction

Congestive heart failure (CHF) is a disease state which is characterized by chronic activation of the sympatho-adrenal system (Packer, 1988). Prolonged exposure to agonists can desensitize the β-adrenergic response (Harden, 1983) in many tissues. Such desensitization can occur at various levels, most importantly involving receptor down-regulation and alterations of G protein function. As β-adrenoceptors mediate the positive inotropic effects of catecholamines in the heart via generation of cAMP (Ikezono *et al.*, 1987), it seems important to obtain detailed knowledge on possible alterations of β-adrenoceptor function in patients with CHF.

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Desensitization of β-adrenoceptor responses in failing human hearts

Bristow *et al.* (1982) first demonstrated that the number and responsiveness of β-adrenoceptors is decreased in failing human hearts. These original studies were performed in explanted hearts from patients with end-stage cardiomyopathy undergoing cardiac transplantation. As this represents a very late form of the disease state, these data do not necessarily allow conclusions about the role of β-adrenoceptor desensitization during the development of CHF. Therefore, we have studied patients with heart failure due to aortic and mitral valve disease (NYHA class II–IV) undergoing corrective valve surgery. Right atrial β-adrenoceptor density was studied in 22 patients with aortic valve disease. In these patients the β-adrenoceptor density decreased in proportion to the severity of the heart failure and was lowest in the most severely ill patients. We have investigated left ventricular β-adrenoceptor density in the papillary muscle of 24 patients with CHF due to mitral valve disease (Brodde *et al.*, 1989b). In those patients we also observed a gradual decrease in β-adrenoceptor density with the lowest values in NYHA class IV patients.

In order to assess the functional role of the decreased β-adrenoceptor density, we determined the potency (pD_2-value) of the β-adrenergic agonist isoprenaline for eliciting increases in force of contraction *in vitro* in some patients. In right atrial preparations from patients with aortic valve disease, the pD_2-value for isoprenaline in class II patients was similar to that in control hearts. The pD_2-value decreased gradually and was lowest in the patients with the most severe heart failure. We performed similar experiments with left ventricular papillary muscle from patients with heart failure due to mitral valve disease (Brodde *et al.*, 1989b). In these preparations the pD_2-value for isoprenaline also decreased with increasing severity of the disease. Thus, our data demonstrate that heart failure is associated with a gradual loss of β-adrenoceptor density and mitigation of β-adrenoceptor-mediated positive inotropic effects.

Down-regulation of β_1- and/or β_2-adrenoceptors is disease-specific

The human heart contains both β_1- and β_2-adrenoceptors, and both receptor subtypes can mediate activation of adenylate cyclase and positive inotropic effects *in vitro* (Brodde, 1987). Similar to other species the ratio of β_2-/β_1-adrenoceptors is greater in the atria than in the ventricles, and the *in vitro* inotropic effects of β_2-receptor stimulation are also more prominent in the atria than in the ventricles (Brodde, 1987). The physiological role of cardiac β_2-adrenoceptors *in vivo* is still uncertain, but some data suggest a function in the regulation of heart rate in response to non-selective β-adrenoceptor agonists like isoprenaline or adrenaline (Brodde *et al.*, 1988; McDevitt, 1989). In the present studies we were interested to know whether the desensitization of cardiac β-adrenoceptors in failing hearts is specific for one of the β-adrenoceptor subtypes or involves both subtypes.

Previous studies from our and other laboratories demonstrate that explanted hearts from patients undergoing cardiac transplantation due to severe idiopathic heart failure have a reduced density of β_1-adrenoceptors but a normal density of β_2-adrenoceptors; moreover, the stimulation of adenylate cyclase and the inotropic effects in response to β_2-selective agonists appear to be unaffected or only mildly attenuated in those hearts, whereas those in response to the non-selective agonist isoprenaline are decreased (Bristow *et al.*, 1986; Brodde *et al.*, 1986). We have now extended these observations by studying hearts from patients with aortic valve disease, mitral valve disease (Brodde *et al.*, 1989b), ischaemic cardiomyopathy (Brodde *et al.*, 1989a), and tetralogy of Fallot (Brodde *et al.*, 1989a).

We studied total, β_1- and β_2-adrenoceptor density in right atrial preparations of 22 patients with aortic valve disease. The stage-dependent reduction of total β-adrenoceptors in these patients was predominantly due to a loss of β_1-adrenoceptor density, as we did detect only slight and statistically not significant decreases of β_2-adrenoceptor density (Figure 1).

A different picture, however, was obtained in 35 patients with mitral valve disease (classes III–IV) (Brodde *et al.*, 1989b). As described above, total β-adrenoceptor density stage-dependently decreased in right and left atrium, as well as in left ventricle of these patients. A subtype-analysis showed that this reduction could be attributed to similar decreases in β_1- and β_2-adrenoceptors in all three tissues. We confirmed these biochemical observations by studying the potency of the non-selective isoprenaline, the β_1-selective noradrenaline, and the β_2-selective procaterol for stimulating increases in force of contraction in right atrium. Compared with the values in non-failing atria, the potencies of isoprenaline (pD_2 6.63 *vs* 7.73), noradrenaline (pD_2 5.91 *vs* 6.80), and of procaterol (pD_2 7.42 *vs* 8.03) were reduced in the right atria of patients with mitral valve disease.

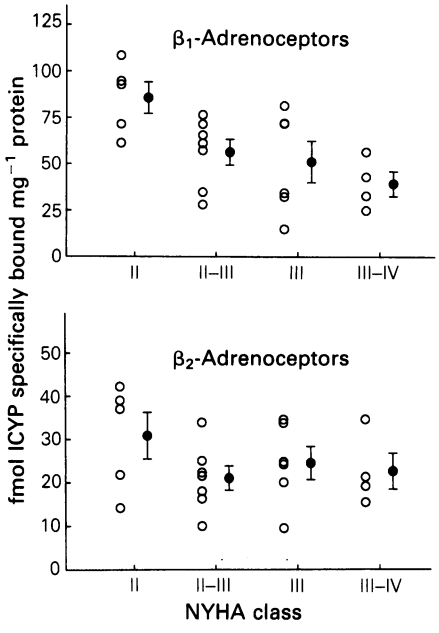

Figure 1 β_1- and β_2-adrenoceptors in right atria of 22 patients (13 male, 9 female; mean age: 57.5 ± 2.6 [range: 23–78] years) with aortic valve disease (NYHA class II–IV). None of these patients had been treated with β-adrenoceptor antagonists or had received catecholamines for at least 3 weeks before operation. However, patients had been treated with nitrates (n = 9), calcium antagonists (n = 4) and digitalis glycosides (n = 11), alone or in combination. Total β-adrenoceptor density was determined by (−)-[^{125}I]-iodocyanopindolol (ICYP) binding, and the amount of β_1- and β_2-adrenoceptors was calculated from total β-adrenoceptor density and the fraction of each subtype as determined from competition experiments with the selective β_2-adrenoceptor antagonist ICI 118,551 (for details see Brodde *et al.*, 1989b). Each open circle represents data from a single patient, the closed circles represent mean ± s.e. mean of the patients in a given stage of the disease according to the NYHA classification.

Finally, we studied a group of patients with tetralogy of Fallot (NYHA class III–IV, n = 14) undergoing corrective open heart surgery (Brodde *et al.*, 1989a). As these patients were considerably younger (age range 3 months to 23 years) than our available controls and nothing is known about cardiac β-adrenoceptors in children, we first determined the density of β-adrenoceptors in right ventricle in relation to age. We did not detect any relationship between age and right ventricular β-adrenoceptor density in these patients (Brodde *et al.*, 1989a). The total β-adrenoceptor density in right atria and right ventricles of the patients with tetralogy of Fallot was quite low compared with that of non-failing adult hearts. The ratio of β_1-/β_2-adrenoceptors, however, was similar to that of non-failing hearts. These data suggest, that tetralogy of Fallot is also associated with β-adrenoceptor down-regulation, which affects β_1- and β_2-adrenoceptors in a similar manner. Due to the lack of appropriate age-matched controls, however, this can only be a preliminary statement.

In summary, cardiac β-adrenoceptor responses are desensitized in atria and ventricles of patients with various forms of heart failure. This desensitization depends on the severity of the heart failure and is accompanied by a gradual reduction in cardiac β-adrenoceptor density. Cardiac β_1-adrenoceptor density decreases in all forms of heart failure whereas cardiac β_2-adrenoceptor density is not reduced in idiopathic cardiomyopathy and aortic valve disease but decreased in ischaemic cardiomyopathy and mitral valve disease. As noradrenaline is a rather β_1-selective agonist (Lands *et al.*, 1967) and plasma levels of the non-selective adrenaline are not elevated in most patients with heart failure (Packer, 1988) the reason for the loss of β_2-adrenoceptors in some forms of CHF is unclear. It is also not clear whether and how alterations of post-receptor components such as G proteins and the catalytic subunit of adenylate cyclase are involved in the desensitization of β-adrenergic responses in failing hearts.

Signal transduction in CHF patients

Various investigators have demonstrated alterations of the signal transducing G proteins in explanted hearts from patients with end-stage CHF mainly by use of bacterial toxin-catalyzed ADP ribosylation of cardiac membranes. Whereas some studies suggested an increased amount of the inhibitory G protein G_i with unchanged levels of the stimulatory G_s (Böhm *et al.*, 1988; Feldman *et al.*, 1988; Neumann *et al.*, 1988) others described a decreased amount of G_s (Ransnäs *et al.*, 1988). Reductions of G_s have also been shown in animal models of CHF (Hammond *et al.*, 1988; Longabaugh *et al.*, 1988). Although these data have provided valuable information regarding the role of G protein alterations in heart failure, a number questions remains open: (1) What is the functional relevance of G protein alterations in heart failure? This question appears to be particularly important as most of the current studies are based on bacterial toxin-catalyzed ADP ribosylation techniques and it is not clear whether number, function, and/or something else is assessed by this technique (Insel & Ransnäs, 1988). (2) How does the

alteration of G proteins relate to the development of heart failure? Most published studies were performed on explanted hearts from patients undergoing cardiac transplantation, and it is not clear whether alterations observed in these preparations are representative for earlier stages of the disease. (3) Are G protein alterations in patients with CHF limited to the heart or are they a generalized phenomenon which also occurs in other tissues? Therefore we attempted to study possible G protein alterations in a more accessible tissue than the heart which might then allow to address the above questions (Maisel *et al.*, 1990b).

We prepared circulating mononuclear leucocytes (MNL) from 23 patients with CHF (NYHA class II–IV) and 19 age-matched healthy subjects. Eleven patients suffered from idiopathic and 12 from ischaemic cardiomyopathy. The amount of G_i and G_s in MNL membranes was quantified by pertussis and cholera toxin catalyzed ADP-ribosylation, respectively. Pertussis toxin catalyzed the incorporation of [^{32}P] into two major bands with apparent molecular weights of 39 and 41 kDa. The combined amount of pertussis toxin substrates in these two bands in CHF patients (5812 ± 972 fmol mg^{-1} protein, $n = 23$) did not differ significantly from that in control subjects (6100 ± 224 fmol mg^{-1} protein, $n = 19$). Cholera toxin-catalyzed ADP ribosylation yielded incorporation of [^{32}P] into a major band with an apparent molecular weight of 42 kDa. The amount of cholera toxin substrates was also similar in CHF patients (7522 ± 1405 fmol mg^{-1} protein, $n = 11$) and in control subjects (5654 ± 707 fmol mg^{-1} protein, $n = 14$). A separate analysis of patients with idiopathic *vs* ischaemic cardiomyopathy or of patients with different severities of CHF did not reveal any marked differences either. These data suggest that CHF does not lead to major quantitative alterations of the pertussis and cholera toxin substrates (G proteins) in MNL.

The function of MNL β-adrenoceptors and prostaglandin E-type receptors was also assessed. These studies were performed in MNL which were prepared from blood obtained under resting conditions. The number of MNL β-adrenoceptors was greater in patients with CHF (2561 ± 259 sites/cell, $n = 20$) than in control subjects (1867 ± 236 sites/cell, $n = 15$, $P < 0.025$) with no apparent change in the affinity of receptors for the radioligand [^{125}I]-pindolol. The cAMP generation in response to 10 μM isoprenaline was slightly but not significantly higher in CHF patients (24.5 ± 4.0 pmol/10^6 cells, $n = 13$) than in control subjects (19.2 ± 2.6 pmol/10^6 cells, $n = 10$). The cAMP accumulation after stimulation with 10 μM prostaglandin E_1 was also similar in both groups (43.5 ± 1.5 pmol/10^6 cells, $n = 13$ in CHF and 43.4 ± 5.2 pmol/10^6 cells, $n = 10$ in control patients). Thus, we could not detect a major β-adrenoceptor desensitization in MNL obtained under resting conditions from CHF patients.

We then developed a novel technique to investigate more subtle alterations of MNL signal transduction. For this purpose we incubated intact MNL with and without pertussis toxin (240–400 ng ml^{-1}) for 4 h at 37° C. This treatment was sufficient to completely ADP-ribosylate the MNL pertussis toxin substrates. Thereafter the cells were washed and agonist-stimulated cAMP accumulation was determined. Pertussis toxin treatment did not alter the cAMP generation in response to isoprenaline or prostaglandin E_1 in healthy subjects. In CHF patients, however, the cAMP generation in response to prostaglandin E_1 increased from 44.3 ± 4.4 to 64.3 ± 9.3 pmol/10^6 cells ($n = 15$, $P < 0.025$). Isoprenaline-stimulated cAMP accumulation increased only slightly and not significantly from 23.4 ± 3.5 to 25.4 ± 6.2 pmol/10^6 cells ($n = 9$).

In a more detailed analysis we compared the alteration of cAMP accumulation after pertussis toxin treatment in subgroups of CHF patients. The enhancement of the prostaglandin response was weakest in NYHA class II and strongest in class IV patients. Moreover, each of the four patients in class IV also showed an enhancement of the isoprenaline-stimulated cAMP generation. We also grouped the CHF patients according to their plasma noradrenaline levels. The enhancement of prostaglandin E_1-stimulated cAMP generation correlated significantly with plasma noradrenaline ($r = 0.798$, $n = 11$, $P < 0.01$), and isoprenaline-stimulated cAMP accumulation was also significantly enhanced in those patients with more than 700 pg ml^{-1} noradrenaline. This finding is somewhat surprising because MNL have a homogeneous population of β_2-adrenoceptors (Brodde *et al.*, 1981), which are rather insensitive towards noradrenaline. However, within the lymphoid tissues MNL get in very close contact with sympathetic nerve endings (Felten *et al.*, 1987) and therefore might be exposed to high local noradrenaline concentrations, sufficient to activate β_2-adrenoceptors. Subanalysis of patients with ischaemic *vs* those with idiopathic cardiomyopathy did not reveal significant major differences.

As hormone-stimulated cAMP generation did not appear to be desensitized in MNL from CHF patients, the relevance of the above findings is not clear. It should be noted, however, that the blood samples for the MNL preparation were

obtained under resting conditions which are not necessarily representative for everyday situations of CHF patients. Therefore, we tested the possibility that desensitization of MNL β-adrenoceptors becomes apparent following physical stress, i.e. a dynamic treadmill exercise until exhaustion. After the exercise the β-adrenoceptor number increased in MNL from CHF and control subjects to reach similar values (from 1867 ± 236 to 3110 ± 229 sites/cell in control subjects and from 2561 ± 259 to 3480 ± 313 sites/cell in CHF patients). The isoprenaline (10 μM)-stimulated cAMP accumulation increased from 36.5 ± 3.5 to 84.4 ± 15.4 pmol/10^6 cells ($P < 0.005$) in control subjects but decreased from 48.9 ± 5.9 to 38.9 ± 7.5 pmol/10^6 cells in CHF patients. Thus, MNL β-adrenoceptors obtained from CHF patients after dynamic exercise are clearly desensitized compared with those of healthy subjects. In order to exclude that this is caused by plasma catecholamines sticking to the MNL from the CHF patients, we also determined cAMP generation in response to 10 μM prostaglandin E_1 and to 20 μM forskolin. Both responses increased in control subjects (74.1 ± 12.0 pmol/10^6 cells before and 118.0 ± 11.9 pmol/10^6 cells after exercise for prostaglandin E_1 and 7.2 ± 1.6 pmol/10^6 cells before to 14.5 ± 2.3 pmol/10^6 cells after exercise for forskolin). In contrast, both responses were reduced in CHF patients following exercise (from 119.5 ± 24.8 to 65.0 ± 11.2 pmol/10^6 cells for prostaglandin E_1, and from 16.7 ± 6.7 to 6.3 ± 2.4 pmol/10^6 cells for forskolin). Thus, a considerable desensitization of cAMP generation can be observed in MNL from CHF patients under conditions of physical activity. This desensitization does not appear to be receptor dependent and might reflect the alteration of G protein function which is detected by the treatment of intact MNL with pertussis toxin.

In conclusion, the treatment of intact MNL with pertussis toxin with subsequent measurements of cAMP generation reveals a tonic inhibition of cAMP generation in CHF patients. This technique appears to be useful for the assessment of G protein function in heart failure patients in several ways: (1) It measures alterations of G protein function that are not detected by ADP-ribosylation. (2) It measures the effect on one given cell preparation and, thus, results are not obscured by possible differences of G proteins between MNL subsets. This is particularly important in CHF patients because alterations of circulating MNL subsets have been described in such patients (Maisel *et al.*, 1990a). (3) Our preliminary data in a small number of patients suggest that this technique is useful to perform longitudinal studies of G protein in CHF patients of various etiologies.

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