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*Short communication*

# Distinct down-regulation of cardiac $\beta_1$- and $\beta_2$-adrenoceptors in different human heart diseases

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**Summary.** Cardiac $\beta$-adrenoceptor density and $\beta_1$- and $\beta_2$-subtype distribution were examined in human left ventricular myocardium from transplant donors serving as controls and from patients with mitral valve stenosis, aortic valve stenosis, idiopathic dilated cardiomyopathy, and ischaemic cardiomyopathy respectively. The total $\beta$-adrenoceptor density was similar in transplant donors and patients with moderate heart failure (NYHA II–III) due to mitral valve stenosis, but was markedly reduced in all forms of severe heart failure (NYHA III–IV) studied. A reduction of both $\beta_1$- and $\beta_2$-adrenoceptors was found in patients with severe heart failure due to mitral valve stenosis or ischaemic cardiomyopathy. In contrast, a selective down-regulation of $\beta_1$-adrenoceptors with unchanged $\beta_2$-adrenoceptors and hence a relative increase in the latter was observed in idiopathic dilated cardiomyopathy and aortic valve stenosis. It is concluded that the extent of total $\beta$-adrenoceptor down-regulation is related to the degree of heart failure. Selective loss of $\beta_1$-adrenoceptors is not specific for idiopathic dilated cardiomyopathy but also occurs in aortic valve stenosis. Changes in $\beta_1$- and $\beta_2$-subtype distribution are rather related to the aetiology than to the clinical degree of heart failure.

**Key words** Beta-adrenoceptor down-regulation – Cardiac $\beta$-adrenoceptor subtypes – Human myocardium – Heart diseases

## Introduction

Heart failure is associated with a decrease in $\beta$-adrenoceptor density in human left ventricular myocardium (Bristow et al. 1982, 1990; Böhm et al. 1989; Brodde et al. 1989a) which in part may explain the reduced positive inotropic efficacy of $\beta$-adrenoceptor agonists in severe heart failure (Bristow et al. 1985; Schmitz et al. 1987; Danielsen et al. 1989). It has been suggested that the reduction of $\beta$-adrenoceptors is related to an increased activity of the sympathetic nervous system (Cohn et al. 1984; Francis and Cohn 1986; Brodde et al. 1989b). The human heart contains a heterogeneous population of $\beta_1$- and $\beta_2$-adrenoceptors in atria as well as in ventricles, and both $\beta$-adrenoceptor subtypes couple to the adenylate cyclase system and mediate positive inotropic and chronotropic effects of catecholamines (Brodde 1987; Buxton et al. 1987; Lemoine et al. 1988; Bristow et al. 1990). In patients with end-stage idiopathic dilated cardiomyopathy, the $\beta_1$-adrenoceptor density is selectively reduced, whereas the $\beta_2$-adrenoceptor population remains unchanged (Bristow et al. 1986; Brodde et al. 1986; Böhm et al. 1989). The aim of the present study was to investigate whether this selective down-regulation of $\beta_1$-adrenoceptors is a general phenomenon in chronic heart failure or is specific for idiopathic dilated cardiomyopathy. Cardiac $\beta$-adrenoceptor density and subtype distribution were characterized by (−)-[$^{125}$I]-iodocyanopindolol binding in human left ventricular myocardium from patients with mitral valve or aortic valve stenosis as well as idiopathic dilated cardiomyopathy and ischaemic cardiomyopathy.

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## Materials and methods

*Patients.* Four nonfailing hearts were obtained from transplant donors [1 female, 3 male; mean age: 33.7, range 27 to 44 yrs; death due to cerebral haemorrhage] whose hearts could not be used for surgical reasons or immunological incompatibility. Aortic and pulmonary valves were excised from these hearts and used for valve replacement operations. Portions of the left ventricular myocardium were used for determination of $\beta$-adrenoceptors. Nine failing hearts were obtained from patients with end-stage heart failure undergoing orthotopic heart transplantation. Five of these patients had idiopathic dilated cardiomyopathy [NYHA IV; 2 female, 3 male; mean age: 53.2, range 46 to 61 yrs; left ventricular ejection fraction

(LVEF): 16.9 ± 1.5%; cardiac index (CI): 1.8 ± 0.2 l/min · m$^2$], and four had ischaemic cardiomyopathy [NYHA III–IV; 1 female, 3 male; mean age: 50.8, range 48 to 51 yrs; LVEF: 18.7 ± 0.9%, CI: 1.9 ± 0.2 l/min · m$^2$]. During the operating procedure biopsies were taken from patients undergoing mitral valve replacement due to mitral valve stenosis with moderate heart failure [NYHA II–III; 1 female, 2 male; mean age: 52.3, range 48 to 59 yrs; pulmonary artery pressure (systolic/diastolic/mean; PAP): 60 ± 5.8/25.3 ± 4.9/39.3 ± 5.5 mm Hg; mean diastolic gradient (MDG): 16 ± 1.4 mm Hg; CI: 2.8 ± 0.2 l/min · m$^2$] or with severe heart failure [NYHA III–IV; 4 female; mean age: 60.3, range 51 to 69 yrs; PAP l82.3 ± 9.2/39.3 ± 7.3/55.3 ± 6.3 mm Hg; MDG: 20 ± 1.8 mm Hg; CI: 1.8 ± 0.2 l/min · m$^2$]. Furthermore, biopsies were taken from patients undergoing aortic valve replacement due to aortic valve stenosis with severe heart failure [NYHA III–IV; 2 female, 3 male; mean age: 59, range 51 to 67 yrs; left ventricular pressure (systolic/diastolic): 213 ± 28.7/14 ± 1.8 mm Hg; pressure in the ascending aorta (systolic/diastolic/mean): 140.3 ± 15/76.3 ± 8.2/101.5 ± 12.1 mm Hg; PAP: 34.6 ± 8.1/13 ± 3.6/20.7 ± 4.8 mm Hg; mean systolic gradient: 85 ± 4.2 mm Hg; CI: 3.2 ± 3 l/min · m$^2$]. Data for LVEF in patients with mitral valve stenosis or aortic valve stenosis were not available. Previous recurrent embolic events and/or pulmonary hypertension were indications for mitral valve replacement in patients with moderate heart failure (NYHA-stage II–III). None of the patients was treated with β-adrenoceptor antagonists or received catecholamines during the last three weeks before operation. Patients were treated with nitrates, diuretics, calcium antagonists and digitalis glycosides alone or in combination. Written informed consent was obtained from the families of all donors of nonfailing hearts and from patients undergoing mitral valve or aortic valve replacement or cardiac transplantation.

*Radioligand binding experiments.* Tissues were rapidly frozen in liquid nitrogen and stored at −80° C. Two to three determinations were performed in each tissue of each patient. Biopsies (5–12 mg) were homogenized for 10 s and 2 × 20 s in ice-cold 1 mmol/l $KHCO_3$ with a "Polytron"-homogenizer (PT 10-35 Kinematica, Luzern, Switzerland). Homogenates were centrifuged at 50000 × *g* for 20 min at 4° C. Pellets were resuspended in 10 mmol/l Tris-HCl, 154 mmol/l NaCl buffer pH 7.4 containing 0.55 mmol/l ascorbic acid and homogenized for 10 s. For determination of the total number of β-adrenoceptors, membranes were incubated with five to six different concentrations of (−)-[$^{125}$I]-iodocyanopindolol ([$^{125}$I]-ICYP) ranging from 5 to 200 pmol/l for 1 h at 37° C. Nonspecific binding of [$^{125}$I]-ICYP was defined as binding to membranes that was not displaced by a high concentration of the nonselective β-adrenoceptor antagonist (±)-CGP 12177 (1 μmol/l; 4-[3-tertiarybutylamino-2-hydroxypropoxy]-benzimidazole-2-on). Specific binding of [$^{125}$I]-ICYP was defined as total binding minus nonspecific binding; it amounted to 70%–80% at 50 pmol/l of [$^{125}$I]-ICYP. To determine the relative amount of $\beta_1$- and $\beta_2$-adrenoceptors, membranes were incubated with [$^{125}$I]-ICYP (50 pmol/l) in the presence of the highly selective $\beta_1$-adrenoceptor antagonist (±)-CGP 20712A (300 nmol/l, 1-[2-(3-carbamoyl-4-hydroxy)phenoxyethylamino]-3-[4-(1-methyl-4-tri-fluoromethyl-2-imidazolyl) phenoxyl]-2-propanol methanesulfonate). Previous studies and own experiments (data not shown) have demonstrated that in many tissues of different species, including human left ventricle (Kaumann and Lemoine 1987), CGP 20712A has an about 6000–10000-fold higher affinity for $\beta_1$- than $\beta_2$-adrenoceptors (Dooley et al. 1986; Molenaar and Summers 1987; Böhm et al. 1989). Thus, with a $K_D$-value of approximately 1 nmol/l at $\beta_1$-adrenoceptors (Dooley et al. 1986; Kaumann and Lemoine 1987; Molenaar and Summers 1987), CGP 20712A 300 nmol/l occupies more than 99% of $\beta_1$-adrenoceptors, but less than 5% of $\beta_2$-adrenoceptors ($K_D$-value at $\beta_2$-adrenoceptors: 6–10 μmol/l; Dooley et al. 1986; Kaumann and Lemoine 1987; Molenaar and Summers 1987). Hence, $\beta_1$-adrenoceptor density was calculated as the specific binding of [$^{125}$I]-ICYP that was displaced by CGP 20712A 300 nmol/l, whereas $\beta_2$-adrenoceptor density was calculated as the specific binding of [$^{125}$I]-ICYP that persisted in the presence of CGP 20712A 300 nmol/l.

*Statistical evaluations.* The experimental data are expressed as the arithmetic mean ± SEM. The equilibrium dissociation constant ($K_D$) and the maximal number of binding sites ($B_{max}$) were calculated from plots according to Scatchard (1949) and by the computer program GraphPAD InPlot (GraphPAD Software, San Diego, California, USA). The two methods yielded identical results. Statistical significance was estimated by unpaired Student's *t*-test. The relation between two variables was assessed by linear regression analysis. A $P$ value < 0.05 was considered significant.

## Results

### *Total β-adrenoceptor density and binding affinity*

Figure 1 A shows the total β-adrenoceptor density in human left ventricular myocardium obtained from transplant donors and patients with different kinds of heart failure. In left ventricular papillary muscles from patients with mitral valve stenosis and moderate heart failure (NYHA II–III), the total β-adrenoceptor density was unchanged compared with transplant donors. In patients with severe heart failure due to mitral valve or aortic valve stenosis, idiopathic dilated cardiomyopathy or ischaemic cardiomyopathy the total β-adrenoceptor density was reduced by about 50–60%. The binding affinity was similar in all groups studied ($K_D$: 7.9 ± 1.4–12.6 ± 2.5 pmol/l).

### *$\beta_1$- and $\beta_2$-subtype distribution*

The $\beta_1$- and $\beta_2$-adrenoceptor density, expressed as % of total β-adrenoceptor density and in absolute values, is summarized in Fig. 1 B and 1 C, respectively. About 80% $\beta_1$- and 20% $\beta_2$-adrenoceptors were found in myocardium derived from transplant donors as well as from patients with ischaemic cardiomyopathy and moderate or severe heart failure due to mitral valve stenosis (Fig. 1 B). In contrast, in myocardium from patients with idiopathic dilated cardiomyopathy or aortic valve stenosis, the $\beta_1/\beta_2$-adrenoceptor ratio was markedly changed. These tissues were characterized by only about 60% $\beta_1$-adrenoceptors and about 40% $\beta_2$-adrenoceptors (Fig. 1 B). In severe heart failure due to mitral valve stenosis or ischaemic cardiomyopathy, both $\beta_1$- and $\beta_2$-adrenoceptor densities (Fig. 1 C) were reduced resulting in an unchanged $\beta_1/\beta_2$-adrenoceptor ratio. On the other hand, in patients with idiopathic dilated cardiomyopathy or aortic valve stenosis a selective loss of $\beta_1$-adrenoceptors was observed, whereas the $\beta_2$-adrenoceptor density was unchanged as compared with transplant donors (Fig. 1 C).

## Discussion

The most striking finding of the present study was that differential down-regulation of β-adrenoceptor subtypes might be related to the etiology of heart failure. Not only in idiopathic dilated cardiomyopathy but also in aortic valve stenosis $\beta_1$-adrenoceptors were selectively down-

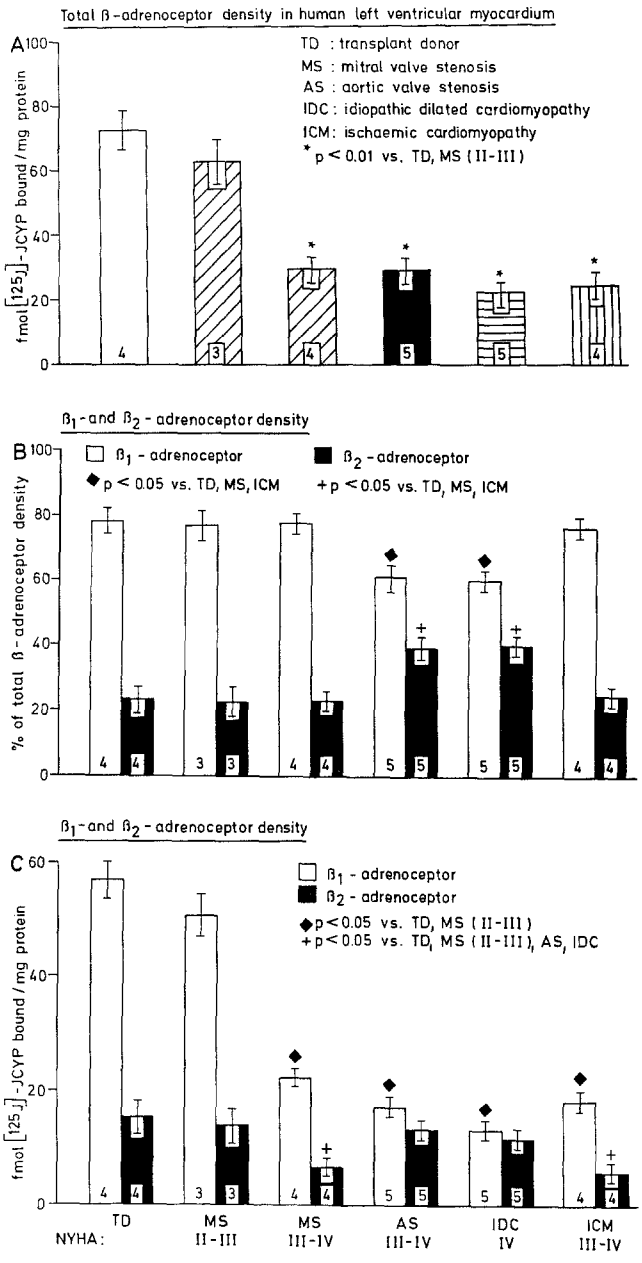


**Fig. 1A–C.** Total $\beta$-adrenoceptor density (**A**), $\beta_1$- and $\beta_2$-adrenoceptor density, expressed as % of total $\beta$-adrenoceptor density (**B**), and $\beta_1$- and $\beta_2$-adrenoceptor density (**C**) in membranes from transplant donors serving as controls and patients with moderate (NYHA II–III) or severe (NYHA III–IV) heart failiure due to different heart diseases. Ordinates: $\beta$-adrenoceptor density in fmol [$^{125}$I]-ICYP bound/mg protein mean (**A**), $\beta_1$- and $\beta_2$-adrenoceptor density as % of total $\beta$-adrenoceptor density (**B**), $\beta_1$- and $\beta_2$-adrenoceptor density in fmol [$^{125}$I]-ICYP bound/mg protein mean; numbers of experiments are given in the columns

regulated with unchanged $\beta_2$-adrenoceptors and hence a relative increase in the latter. In contrast, in mitral valve stenosis and ischaemic cardiomyopathy both $\beta_1$- and $\beta_2$-adrenoceptors were diminished so that the $\beta_1/\beta_2$-adrenoceptor ratio remained unchanged. In addition, the present results confirm that the extent of *total* $\beta$-adrenoceptor down-regulation is related to the clinical degree of heart failure (Fowler et al. 1986; Brodde et al. 1989b). In patients with mitral valve stenosis and moderate heart failure (NYHA II–III), the total $\beta$-adrenoceptor density was similar to that of transplant donors, while in all forms of severe heart failure (NYHA III–IV) the total $\beta$-adrenoceptor density was markedly reduced, irrespective of the underlying heart disease.

It is well known that the human heart contains both $\beta_1$- and $\beta_2$-adrenoceptors (Brodde 1987; Buxton et al. 1987; Lemoine et al. 1988). From data hitherto available it has been suggested that selective down-regulation of $\beta_1$-adrenoceptors is a unique phenomenon in heart failure due to idiopathic dilated cardiomyopathy (Bristow et al. 1986), while in all forms of mitral valve disease, tetralogy of Fallot, and ischaemic cardiomyopathy a reduction of both $\beta_1$- and $\beta_2$-adrenoceptors has been observed (Brodde et al. 1989a, b). The present results confirm these observation; they show in addition, however, that a selective loss of $\beta_1$-adrenoceptors also occurs in myocardium obtained from patients with severe heart failure due to aortic valve stenosis, and that a selective down-regulation of $\beta_1$-adrenoceptors probably is not specific for idiopathic dilated cardiomyopathy. These findings strengthen the hypothesis that changes in $\beta_1$- and $\beta_2$-subtype distribution might rather be related to the aetiology than to the clinical degree of heart failure. During the time this manuscript was in press Michel et al. 1990 have published that in patients with aortic valve stenosis the $\beta_1$-adrenoceptor population is selectively reduced in human right atria, a cardiac chamber which is primarily not involved in aortic valve diseases.

The pathophysiological mechanism underlying this differential regulation of cardiac $\beta_1$- and $\beta_2$-adrenoceptors in different kinds of heart failure is not known at present. It has been shown that plasma noradrenaline levels are markedly increased in patients with idiopathic dilated cardiomyopathy (Cohn et al. 1984; Francis and Cohn 1986), whereas plasma adrenaline levels are normal (Francis 1985). Noradrenaline is a rather selective $\beta_1$-adrenoceptor agonist, and adrenaline is known to be a nonselective $\beta$-adrenoceptor agonist with similar affinities to $\beta_1$- and $\beta_2$-adrenoceptors (Lands et al. 1967). Thus, noradrenaline might be responsible for the selective down-regulation of $\beta_1$-adrenoceptors in idiopathic dilated cardiomyopathy (Bristow et al. 1986; Böhm et al. 1989). Similar data in patients with aortic valve stenosis and selective loss of $\beta_1$-adrenoceptors are not available. On the other hand, it has been reported that in patients suffering from mitral valve disease both endogenous catecholamines, adrenaline *and* noradrenaline, are elevated and this could be responsible for a concomitant reduction of $\beta_1$- and $\beta_2$-adrenoceptors (Brodde et al. 1989b).

The left ventricular $\beta$-adrenoceptor density has not yet been determined in patients with aortic valve stenosis. Besides the "catecholamine hypothesis" it is interesting to note that hypertrophy and/or dilation of the left ventricular myocardium is a common feature of idiopathic dilated cardiomyopathy and aortic valve stenosis. Thus, one might speculate that selective down-regulation of $\beta_1$-adrenoceptors is the consequence of an as yet unknown

compensatory mechanism of the compromized myocardial cell.

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