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β-Adrenergic Receptor Number in Surgically Denervated, Transplanted Human Hearts

To the Editor:—Cardiac β_1- and β_2-adrenoceptors mediate positive inotropic and chronotropic effects of catecholamines.[1] The transplanted human heart seems to be denervated for at least as long as 5 yr after transplantation, as evidenced by reduced tissue norepinephrine levels[2] and nerve density.[3] Recently, an increased response of the transplanted human heart to β-adrenergic stimulation has been reported.[4] Thus, it is conceivable that supersensitivity to β-adrenergic stimulation could be due to an up-regulation of β-adrenoceptor number. As yet, nothing is known about changes in total β-adrenoceptor density and β_1- and β_2-subtype distribution during long-term follow-up in these transplanted human hearts and about the relationship to the pretransplantation disease.

The present study was carried out to examine total β-adrenoceptor density and β_1- and β_2-subtype distribution in right ventricular biopsies taken from 100 patients (mean age 43.4 ± 5.8 [30 − 61] yr) 1–60 months after orthotopic heart transplantation and from eight prospective transplant donors (mean age 40.1 ± 3.8 [27–53] yr, death due to cerebral hemorrhage) who served as controls and whose hearts could not be transplanted for surgical reasons or immunologic incompatibility. Written informed consent and approval by the Ethical Committees of the University of Hamburg were obtained. Patients had received a heart transplant because of end-stage dilated cardiomyopathy (n = 65) or severe ischemic heart disease (n = 35), and they revealed normal cardiac function after transplantation, as evidenced by cardiac catheterization and echocardiogram. For rejection screening, biopsies were analyzed and classified according to the Hannover Classification.[5] Patients were clinically stable and without histologic evidence of rejection (A3/A4). Plasma catecholamine levels were within normal limits (norepinephrine < 500 ng/l) and epinephrine < 100 ng/l). Chronic immunosuppressive therapy consisted of cyclosporine A, azathioprine, and prednisone. Additional medication consisted of low-dose captopril, calcium antagonists, and diuretics.

Cardiac crude membranes were prepared as recently described by Steinfath *et al.*[6] Briefly, right ventricular endomyocardial biopsies (8–12 mg) were homogenized for 10 s and 2 × 20 s in ice-cold 1 mM $KHCO_3$ with a Polytron homogenizer (PT 10–35 Kinematica, Luzern, Switzerland). Homogenates were centrifugend at 50,000 × g for 20 min at 4° C. Pellets were resuspended in 10 mM Tris-HCl, 154 mM NaCl buffer, *p*H 7.4, containing 0.55 mM ascorbic acid and were homogenized for 10 s. For determination of the total number of β-adrenoceptors, membranes were incubated with five different concentrations of the nonselective β-adrenoceptor antagonist (−)-(125 I)-iodocyanopindolol (ICYP, specific activity 2,200 Ci/mmol, New England Nuclear, Dreieich, Germany) ranging from 8 to 200 pM for 1 h at 37° C. Nonspecific binding of ICYP was defined as binding to membranes that was not displaced by a high concentration of the nonselective β-adrenoceptor antagonist (±)-CGP 12177 (1 μm, 4-[3-tertiarybutylamino-2-hydroxypropoxy]-benzimidazole-2-on). Specific binding was defined as total binding minus nonspecific binding, which amounted to 70–80% at 100 pM of ICYP. To determine the relative amount of β_1- and β_2-adrenoceptors, membranes were incubated with ICYP (100 pM) in the presence of the highly selective β_1-adrenoceptor antagonist CGP 20712 A (300 nM; 1-[2-(3-carbamoyl-4-hydroxy)phenoxy ethylamino]-3-[4-(1-methyl-4-triflouromethyl-2-imidazolyl)phenoxy]-2-propanol metha-nesulfonate). The β_2-subtype population was calculated as total β-adrenoceptor number minus β_1-subtype population. Both CGP 12177 and CGP 20712A were gifts from Ciba Geigy, Basel, Switzerland. Protein concentrations were determined by Bio-Rad Protein Assay according to Bradford.[7] Bovine γ globulin was used as protein standard.

Data are expressed as arithmetic means ± SEM. The equilibrium dissociation constant and the maximal number of binding sites were calculated from plots according to Scatchard[8] and were compared with those calculated by the computer program GraphPAD InPlot (GraphPAD Software, San Diego, CA). The two methods yielded identical results. Significant differences between means were estimated by Student's *t* test for unpaired observations and analysis of variance, respectively. The relation between two variables was assessed by linear regression analysis. A *P* value of less than 0.05 was considered significant.

This long-term follow up study demonstrates that for 60 months after heart transplantation, the total β-adrenoceptor density was not significantly reduced. On the other hand, the β_1:β_2-adrenoceptor ratio was surprisingly shifted with increasing time after transplantation, from about 80:20 to 60:40%, which was due to a decrease in β_1- and an increase in β_2-adrenoceptors. Compared with controls, substantial intergroup difference in either the total β-adrenoceptor density or in the β_1- and β_2-subtype distribution was not observed between biopsies taken 48–60 months after cardiac transplantation from patients with previous end-stage dilated cardiomyopathy and previous severe ischemic heart disease (table 1). The equilibrium dissociation constant was similar in all groups investigated (12.6 ± 2.3–18.7 ± 2.8 pM).

The surgically denervated, transplanted human heart very likely does not develop supersensitivity to β-adrenergic stimulation due to an increase in total β-adrenoceptor number. Our hypothesis is that up-regulation of the β_2-adrenoceptor subtype in these hearts could be due to an increased functional importance of circulating catecholamines (epinephrine) in modulating positive inotropic and chronotropic effects. Epinephrine is known to be a nonselective β-adrenoceptor agonist with similar affinities to β_1- and β_2-adrenoceptors, whereas norepinephrine is a rather β_1-selective agonist.[9] Optimal inotropic support is of fundamental importance in the denervated human heart in which systolic function is compromised, *e.g.*, in early postoperative settings or in cases of chronic rejection (nonspecific myocardial allograft failure). The change in β_1:β_2-adrenoceptor subtype distribution could have clinical consequences because it is conceivable that with increasing time after transplantation nonselective β-agonists (epinephrine or isoproterenol) may provide better inotropic support than β_1-selective agents (nor-

TABLE 1. Total β-Adrenoceptor Density and β_1- and β_2-Subtype Distribution

Reason for Transplantation	Time after Transplantation (months)	B_{max} (fmol/mg protein)	β_1 (fmol/mg protein)	β_2 (fmol/mg protein)	β_1 (%)	n
	Control	70.8 ± 7.1	56.6 ± 4.2	14.2 ± 2.4	80	8
End-stage dilated cardiomyopathy or severe ischemic heart disease	1–3	66.6 ± 7.2	50.4 ± 5.6	16.2 ± 3.1	76	15
	4–7	72.1 ± 6.2	55.7 ± 5.1	16.4 ± 2.0	77	15
	8–11	68.2 ± 8.5	49.2 ± 3.8	19.0 ± 3.4	72	6
	12	71.1 ± 5.8	47.1 ± 4.3	24.0 ± 2.1*	66*	15
	24	65.2 ± 6.3	40.5 ± 3.6*	24.7 ± 2.9*	62*	15
	36	65.4 ± 7.0	41.7 ± 4.6*	23.7 ± 3.1*	64*	12
	48	59.3 ± 4.7	35.5 ± 3.0*	23.8 ± 2.5*	60*	12
	60	55.8 ± 4.2	33.9 ± 2.8*	21.9 ± 2.3*	61*	10
End-stage dilated cardiomyopathy	1–12	70.3 ± 4.6	52.7 ± 3.7	17.6 ± 1.8	75	28
	24–36	69.9 ± 5.0	43.4 ± 3.6*	26.5 ± 2.6*	62	20
	48–60	55.9 ± 3.9	32.6 ± 2.1*	23.7 ± 1.3*	59*	16
Severe ischemic heart disease	1–12	63.8 ± 5.1	45.7 ± 4.3	18.1 ± 1.7	71	21
	24–36	53.9 ± 9.6	34.5 ± 6.6*	19.4 ± 4.6	66*	7
	48–60	58.4 ± 6.7	37.0 ± 5.0*	21.4 ± 2.2*	65*	7

Total β-adrenoceptor density (B_{max}) and β_1- and β_2-subtype distribution in absolute values (fmol of ICYP specifically bound per mg protein) as well as β_1-subtype population expressed as percentage of total β-adrenoceptor density in right ventricular biopsies from transplant donors serving as controls and patients after heart transplantation because of end-stage dilated cardiomyopathy or severe ischemic heart disease and in relation to the pretransplantation disease.

* $P < 0.05$ *versus* control.

epinephrine) which might, due to the decreased β_1-adrenoceptor population, have reduced positive inotropic effects at doses that can be administered clinically.

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