Myocardial $\beta$-adrenoeceptor density and the distribution of $\beta_1$- and $\beta_2$-adrenoeceptor subpopulations in children with congenital heart disease


Abstract. Twenty-six infants and children with congenital heart disease (CHD) undergoing cardiac surgery were investigated for alterations in myocardial $\beta$-adrenoeceptor density. The patients were divided into three groups according to type and severity of CHD: group I consisted of 6 patients with a cyanotic shunt lesion of moderate severity; group II comprised 13 children with severe cyanotic shunt and valve lesions and group III included 7 children with cyanotic CHD. The myocardial $\beta$-adrenoeceptor density was determined using $(-)^3$Iodo-cyanopindolol ($[\text{I}^3\text{I}]\text{ICYP}$) and was reduced by approximately 50% in severe acyanotic CHD (33.6 fmol/mg protein) and cyanotic CHD (35.3 fmol/mg protein) in comparison with the group with less severe acyanotic shunt defects (64.4 fmol/mg protein). The affinity dissociation constant ($K_d$, $\text{ICYP}$) did not differ statistically between the groups. The proportion of $\beta_1$- and $\beta_2$-subpopulations was evaluated by ICI 118,551-$[\text{I}^2\text{I}]\text{ICYP}$ competition studies. In group II (61.5%) and group III (69.1%) significant lower portions of $\beta_1$-adrenoeceptors were found compared with group I (78.2%). This shift of subpopulations was due to a decreased $\beta_1$-receptor density while $\beta_2$-receptor density was unchanged in all groups. While the plasma noradrenaline levels of group I were similar to those of a control group of 13 healthy children, respective values of group II and III were significantly elevated. A significant negative correlation was found between plasma noradrenaline levels and myocardial $\beta$-adrenoeceptor density. It is concluded that exposure of these receptors to increased circulating catecholamines, due to an enhanced sympathetic tone, leads to a reduction of their density. Noradrenaline, a preferential agonist of $\beta_1$-adrenoeceptors, is most probably responsible for the shift of the $\beta$-adrenoeceptor subpopulations from the $\beta_1$- to $\beta_2$-subtype, depending on severity and type of cardiac disease.

Key words: $\beta$-adrenoeceptors – Down-regulation – Catecholamines – Congenital heart disease

Introduction

Human myocardium contains a relatively high proportion of $\beta_2$-adrenergic receptors [10]. Both $\beta_1$- and $\beta_2$-adrenoeceptors appear to be coupled to adenylate cyclase stimulation [9, 12]: endogenous catecholamines and $\beta$-sympathomimetic drugs change the conformation of the $\beta$-adrenoeceptor and lead to an interaction with the stimulatory guanosine triphosphate regulation protein ($G_s$). The $G_s$ protein increases the activity of adenylate cyclase which induces the conversion of ATP to cAMP, the intracellular second messenger of $\beta$-adrenoeceptor stimulation. [24, 42, 45]. However, in non-failing myocardium $\beta_1$-receptors are coupled markedly more efficiently to adenylate cyclase than $\beta_2$-receptors [9]. The number of $\beta$-adrenoeceptors on the cell surface, the proportion of $\beta_1$- to $\beta_2$-subtypes and the signal transmission from the receptor to the adenylate cyclase by the $G_s$ protein are influenced by a number of drugs, hormones and various physiological and pathological conditions [1, 5, 7, 9, 23, 43, 49]. Long-term exposure of myocardial $\beta$-adrenoeceptors to circulating endogenous or exogenous catecholamines leads to receptor desensitization. This important physiological regulatory process is mediated by an un-
coupling of the receptor-effector system and by a decrease in receptor density [38, 41, 45]. Patients with congestive heart failure have significantly higher plasma levels of catecholamines than healthy individuals [7, 17, 26, 34, 46-48]. The increase most probably results from reflex-activation of the adrenergic system to maintain an adequate cardiac output [10]. Ventricular papillary muscles resected during cardiac surgery in adult patients with heart failure showed a decreased contractility. Stimulation of β-adrenoceptors by isoprenaline caused a significantly lower inotropic effect in patients with cardiac insufficiency than in those without [4, 5, 8, 15, 19, 25] and, consistent with a reduction of β-adrenoceptors, the effectiveness of agonists was reduced [4, 5, 25].

In infants with congenital heart disease (CHD) with heart failure [30] or cyanosis [22] catecholamine metabolism and excretion was increased. Recently, Ross et al. [39] found raised noradrenaline levels with significant relationship to the size of the left to right shunt and degree of pulmonary hypertension. However, studies investigating the density and affinity of cardiac β1- and β2-receptors and the plasma concentration of catecholamines in children with CHD have not yet been carried out. Therefore, we investigated these subjects in children with cyanotic and acyanotic CHD undergoing cardiac surgery whereby a small amount of myocardium from the right atrial appendage could be obtained.

**Subjects and methods**

**Subjects**

Myocardial tissue was obtained from right atrial appendages of 26 children who underwent corrective surgery for CHD. The mean age was 2.1 ± 3.2 years (5 days – 15.8 years). Nineteen cases had an acyanotic CHD and 7 a cyanotic CHD (Table 1). They were divided into three groups:

- **Group I** consisted of six patients with left to right shunt lesions with a systolic pulmonary/aortic pressure ratio \( \frac{P_{PA}}{P_{AO}} \) of < 0.75 and a pulmonary/systemic bloodflow ratio \( \frac{Q_p}{Q_s} \) of < 2.0.

- **Group II** included 13 children with left to right shunt lesions with a systolic \( \frac{P_{PA}}{P_{AO}} \) > 0.75 and a \( \frac{Q_p}{Q_s} > 2.0 \). Three infants with aortic stenosis (systolic pressure gradient = 72 ± 20 mmHg), left ventricular end diastolic pressure = 11 ± 1 mmHg) and 2 children with severe mitral insufficiency were added to this group.

- **Group III** comprised seven children with cyanotic CHD. The mean arterial oxygen saturation during catheterization was 71% ± 4.5%. None of the children were treated with adrenoceptor agonists or antagonists, but 16 of them received digoxin and 11 furosemide. Blood for plasma catecholamine determination was obtained while resting in all but 6 patients and was drawn from a central venous catheter in young children or, in older children, from a peripheral venous catheter. The control group consisted of 13 children (mean age 5.8 ± 4.1 years, 3 days – 11.6 years) without heart disease or other diseases, which might influence the sympathetic nervous system.

**Methods**

**Membrane preparation.** The blood cells of myocardial tissue were rinsed with modified ice-cold Krebs-Henseleit solution (89.1 mM NaCl, 3.6 mM CaCl\(_2\), 5 mM KCl, 0.49 mM MgSO\(_4\), 1 mM NaH\(_2\)PO\(_4\), 29.1 mM NaHCO\(_3\), 1.2 mM glucose and 0.3 mM EDTA; equilibrated with a mixture of 95% O\(_2\) and 5% CO\(_2\) and immediately placed into liquid nitrogen. The tissues were then stored at –80°C.

Myocardial membranes were prepared as described by Kauermann [27] and Brodde [12]: the thawed tissues were minced in 10 ml of 1 mM KHCO\(_3\) and homogenized with an Ultra-Turrax (Jahnke & Kunkel, Stauffen, FRG). The homogenate was centrifuged at 100g for 20 min at 4°C. The supernatants were filtered through four layers of gauze, made up to 20 ml with KHCO\(_3\), centrifuged at 50000g for 20 min at 4°C and then discarded. The pellets were resuspended in the assay buffer solution (10 mM Tris-HCl, 154 mM NaCl, 0.55 mM ascorbate, pH 7.4).

**Binding assays.** For determination of total β-adrenoceptor density, the myocardial membranes were radiolabelled with \( [\text{3}^\text{H}] \)-iodocyanopindolol (\( [\text{3}^\text{H}] \)ICYP); Amersham, Braunschweig, FRG), a non-selective, highly specific β-receptor ligand [11, 18]. The incubation lasted 60 min at 37°C and each assay was performed in triplicate. Membranes were trapped by vacuum filtration (Whatman GF/C Maidstone, GB) glass fibre filters), and radioactive decay was counted on a gamma radiation counter (Gamma 5500; Beckman, München, FRG). Saturation assays were performed with six increasing concentrations of \( [\text{12}^\text{5I}] \)ICYP (20-310 pmol/l) in the presence and absence of high concentrations of \( (+) \)CGP 12177 (1 nmol/l, 4-[3-tertiarybutylamino-2-hydroxypropoxy]benzimidazole-2-on; Ciba Geigy, Wcr, FRG), a non-selective, highly specific β-receptor ligand. Non-specific \( [\text{12}^\text{5I}] \)ICYP-binding was defined as binding with and total \( [\text{12}^\text{5I}] \)ICYP-binding as binding without \( (+) \)CGP 12177. Specific binding of \( [\text{12}^\text{5I}] \)ICYP to β-adrenoceptors was obtained by subtraction of non-specific from total binding and amounted to 60%-80% at 160 pmol/l \( [\text{12}^\text{5I}] \)ICYP. Binding data were related to myocardial membrane protein; the protein concentration was measured as described by Lowry et al. [31] using bovine serum albumin as standard.

The \( \beta_1- \) and \( \beta_2- \)adrenergic receptor subtypes were identified by ICI 118,551-[\( ^{125} \text{I} \)]ICYP competition studies performed in each preparation. Right atrial membranes in presence of 50 pmol/l \( [\text{12}^\text{5I}] \)ICYP were then incubated with 21 increasing concentrations of the selective \( \beta_2- \)ligand ICI 118,551 (erythro-\( \pm \)-1-(7-methylindan-4-yl oxy)-3-isopropylamino-butan-2-ol; ICI Pharma, Plankstadt, FRG) [3, 36]. Specific binding was determined as described above.

**Plasma catecholamines.** Blood for plasma catecholamine determination was collected in precooled tubes containing 1.9 mg ethylene glycol tetra-acetic acid, and 1.2 mg gluthathione/ml blood. Centrifugation at 4°C (2300 g for 5 min) was carried out within 20 min. The plasma samples were stored at –80°C and analysis of plasma catecholamines was performed within 2 months by HPLC, with a model 510 solvent delivery system, M 460 electrochemical detector with a C18 HPLC-column and a M 740 data module (Waters, Eschborn, FRG). Plasma standards were used to control the quality of the analysis.

**Data analysis.** The saturation isotherm data were analysed according to Scatchard [40], using linear regression analysis of bound to