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A post-receptor defect of adenylyl cyclase in severely failing myocardium from children with congenital heart disease

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Abstract

The aim of this study was to determine whether a defect at the post-receptor level of adenylyl cyclase may also contribute to the decreased effectiveness of cAMP-increasing agents in severely failing patients with congenital heart disease. The severity of congestive heart failure in 31 patients with congenital heart disease was graded by a scoring system which included a description of historical and clinical variables. Patients were divided into a group with no or mild heart failure (score ≤ 6) and a group with severe heart failure (score > 6). β-Adrenoceptor-stimulated adenylyl cyclase activity was significantly decreased by 65% in patients with severe heart failure in comparison to the group of patients with no or mild heart failure. In addition, receptor-independent adenylyl cyclase stimulation by forskolin was reduced by 52% in patients with score > 6 compared to patients with score ≤ 6. This post-receptor defect of adenylyl cyclase was apparently due to a decrease in the activity of catalytic subunit of adenylyl cyclase as adenylyl cyclase stimulation by forskolin in the presence of Mn^{2+} which uncouples catalytic subunit from the G proteins, G_s and G_i, was also significantly diminished in the patients with severe heart failure. In contrast, the level of inhibitory G protein α-subunits was apparently not different in the two groups. In summary, the data indicate that a defect at the catalytic subunit of adenylyl cyclase apparently contributes to the decreased effectiveness of cAMP-increasing agents in severely failing patients with congenital heart disease. © 1997 Elsevier Science B.V.

Keywords: Adenylyl cyclase; β-Adrenoceptor; Catalytic subunit; Congenital heart disease

1. Introduction

In the failing human heart numerous abnormalities in the β-adrenergic neuroeffector system have been described including down-regulation of β_1-adrenoceptors, increased level and activity of the inhibitory guanine nucleotide binding protein (G_i) and neurotransmitter depletion (Bristow et al., 1992). In infants and children with severe acyanotic or cyanotic congenital heart disease undergoing cardiac surgery a decrease in the number of β-adrenoceptors in comparison to children with acyanotic shunt lesions of moderate severity was found (Kozlik et al., 1991; Kozlik-Feldmann et al., 1993). Plasma noradrenaline levels were reported to be significantly higher in children with congestive heart failure than in those without heart failure (Ross et al., 1987). In addition to the decrease in the number of β-adrenoceptors and to uncoupling of β_1-adrenoceptors and β_2-adrenoceptors from adenylyl cyclase, alterations at the post-receptor level of cardiac adenylyl cyclase have also been shown to contribute to adenylyl cyclase desensitization in patients with severe heart failure. An increase in the level and activity of inhibitory G protein α-subunits ($G_{i\alpha}$) has been found in left ventricular preparations of patients with dilated and ischemic cardiomyopathy (Feldman et al., 1988; Böhm et al., 1994). Similar to the down-regulation of β-adrenoceptors the increase in the expression of $G_{i\alpha}$ proteins is apparently due to the exposure of the myocardium to increased levels of circulating catecholamines (Eschenhagen et al., 1992; Reithmann et al., 1989). As another post-receptor defect of adenylyl cyclase, a decrease in the activity of the catalytic

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subunit of adenylyl cyclase was found in right ventricular preparations of patients with dilated cardiomyopathy and primary pulmonary hypertension, an abnormality which was apparently not mediated by increased sympathetic tone but by the pressure overload of failing right ventricles (Bristow et al., 1992).

The aim of the present study was to determine whether – in addition to the down-regulation of β-adrenoceptors – an alteration at the post-receptor level of adenylyl cyclase may also occur in infants and children with severe congenital heart disease and whether this alteration may be located at the level of the G proteins or at the level of catalytic subunit of adenylyl cyclase.

2. Patients and methods

2.1. Patients

Myocardial tissue was obtained from right atria of 31 children who underwent corrective surgery for congenital heart disease. The mean age was 2.6 ± 3.7 years (4 days to 13.9 years). The patients were divided according to hemodynamic criteria and arterial oxygen saturation into three groups. 19 cases had an acyanotic congenital heart disease and 12 a cyanotic congenital heart disease (Table 1). The following main diagnoses were found: atrial septum defect (ASD, $n = 6$), ventricular septum defect (VSD, $n = 5$), transposition of the great arteries (TGA, $n = 7$), tetralogy of Fallot (TOF, $n = 4$), complete atrioventricular canal (AV-canal, $n = 3$), congenital pulmonal stenosis (PS, $n = 1$), tricuspid atresia (TA, $n = 2$) and severe combined congenital heart diseases requiring heart transplantation (pre-HTP, $n = 2$). Group I consisted of 9 acyanotic patients with left to right shunt lesions with a systolic pulmonary/aortic pressure ratio (P_{PA}/P_{Ao}) of < 0.75 and a pulmonary/systemic bloodflow ratio (Q_p/Q_s) < 2.0. Group II included 9 children with severe left to right shunt lesions with a systolic $P_{PA}/P_{Ao} > 0.75$ and a $Q_p/Q_s > 2.0$ and one children with a pulmonal stenosis (systolic gradient 90 mmHg).

Group III comprised 12 children with cyanotic congenital heart disease. The mean arterial oxygen saturation

Table 1
Patients with congenital heart disease

Name	Age	Sex	Diagnoses	Group	Score
A.S.	4.1	f	ASD 2	I	5
C.B.	13.9	f	ASD 2	I	0
M.S.	3.5	f	ASD 2	I	0
I.S.	2.3	f	ASD 1, mitral regurgitation	I	0
S.H.	5.8	f	ASD 2	I	1
R.M.	2.9	m	ASD 2	I	6
K.N.	8	f	VSD, aortic regurgitation	I	2
V.D.	1.4	f	VSD, pulmonal stenosis (mild)	I	1
T.G.	0.9	m	VSD, ASD 2, aortic coarctation	I	3
F.R.	12.3	m	ASD, partial anomalous pulmonary venous connection	II	0
E.T.	1.8	f	pre-HTP D-TGA, pulmonal stenosis, Ballon atrial septostomy, Senning operation	II	10
M.K.	0.2	m	L-TGA, functional single ventricle with double inlet, pulmonal stenosis, ballon atrial septostomy	II	12
J.Z.	1.5	f	complete AV-canal	II	8
Y.E.	2	f	complete AV-canal	II	8
M.R.	1.7	f	complete AV-canal	II	3
T.R.	0.3	f	VSD, ASD 2	II	13
M.S.	2	f	VSD	II	1
A.M.	1.2	m	TOF (‘pink Fallot’)	II	1
C.G.	0.6	m	pulmonal stenosis, ASD 2	II	2
C.C.	0.2	m	pre-HTP, hypoplastic left heart, complete AV-canal	III	15
T.D.	0.1	m	D-TGA, pulmonal stenosis, pulmonal regurgitation, ballon atrial septostomy	III	7
H.W.	0.1	f	D-TGA, ASD	III	7
T.D.	0.5	m	D-TGA, ballon atrial septostomy	III	8
A.C.	0.1	f	D-TGA, ballon atrial septostomy	III	8
F.B.	0.1	m	D-TGA, ASD, pulmonal stenosis, subvalvular pulmonal stenosis (mild)	III	15
A.E.	0.1	m	D-TGA, patent ductus arteriosus, ballon atrial septostomy	III	9
K.B.	11.1	f	Tricuspid atresia Ib, VSD, hypoplastic pulmonary arteries, modified Blalock-Taussig anastomosis	III	7
T.B.	1.3	f	Tricuspid atresia Ib, VSD (mild), pulmonal stenosis, modified Blalock-Taussig anastomosis	III	9
T.F.	0.6	m	TOF, double outlet right ventricle, patent ductus arteriosus	III	11
M.G.	0.8	m	TOF, high degree infundibular stenosis	III	10
T.W.	0.9	m	TOF, high degree infundibular stenosis	III	5

during catheterization was 69.6 ± 7.6 ($n = 9$) (children with transposition of the great arteries, severe combined congenital heart diseases requiring heart transplantation and tricuspid atresia). The three children with tetralogy of Fallot had normal or slightly decreased arterial oxygen saturation at rest but had episodes of severe cyanosis. Two patients of group III (C.C., T.W.) received dobutamine i.v., two patients of group III (M.G., T.W.) were treated with β-blocker (propranolol) p.o. before operation.

The study protocol was approved by the local Ethical Committee.

2.2. Scoring system for grading congestive heart failure

Grading the severity of congestive heart failure in infants and children was performed using a modified scoring system described by Ross et al. (1992). The following variables were graded by a pediatric cardiologist: diaphoresis, tachypnea, episodes of hypoxemia, breathing, respiratory rate, heart rate, growth failure (weight percentile), skin perfusion, cyanosis, precordial thrill and hepatomegaly (liver edge from right costal margin) (Table 2). According to the scoring system described by Ross et al. (1992) patients with score 0–6 were classified as no or mild heart failure and with score > 6 as severe heart failure. The scores of all patients included in the study are given in Table 1.

2.3. Materials

ATP, GTP, cyclic AMP and creatine kinase were from Boehringer-Mannheim (Mannheim, Germany). Creatine phosphate, 3-isobutyl-1-methylxanthine, isoproterenol, forskolin, pertussis toxin and molecular weight markers (SDS-6H) were from Sigma (Deisenhofen, Germany). Reagents for sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were from Serva (Heidelberg, Germany). [α-^{32}P]ATP and [^{32}P]NAD were from DuPont (Dreieich, Germany).

2.4. Preparation of membranes

Right atrial tissue was immediately placed into liquid nitrogen and was stored at −80°C. For the assays, thawed tissue (about 0.03 cm^3) was minced with scissors and was homogenized in buffer A (20 mM Tris/HCl, pH 8.0, 1 M EDTA, 1 mM dithiothreitol) (6 ml) with an Ultra-Turrax at $300 \times g$ for 5 min at 4°C. The homogenates were then centrifuged at $30\,000 \times g$ for 20 min at 4°C. The pellets were resuspended with thin needles in buffer A and were diluted to a concentration of about 0.5–1 mg protein/ml. Membranes (100–200 μg protein) were then snap frozen in liquid nitrogen and stored at −80°C. Membrane protein was determined according to Lowry et al. (1951).

2.5. Adenylyl cyclase assay

Adenylyl cyclase activity of cardiac membranes was determined in a reaction mixture containing 50 μM [α-^{32}P]ATP (0.2 μCi/tube), 5 mM $MgCl_2$, 0.1 mM EGTA, 1 mM dithiothreitol, 0.1 mM cyclic AMP, 1 mM 3-isobutyl-1-methylxanthine, 5 mM creatine phosphate, 0.4 mg/ml creatine kinase and the additions indicated in 50 mM triethanolamine/HCl, pH 7.4 at 30°C, in a total volume of

Table 2
Scoring system for grading CHF in infants and children

Score		0	1	2
History				
Diaphoresis		Only head	Head and body at exercise	Marked and frequent
Tachypnea		Rare	Several times	Frequent
Episodes of hypoxemia		Absent		Present
Physical exam				
Breathing		Normal	Retractions	Dyspnoe
Respiratory rate	(infants)	< 50	50–60	> 60
	(1–6 years)	< 35	35–45	> 45
	(7–10 years)	< 25	25–35	> 35
	(11–14 years)	< 18	18–28	> 28
Heart rate	(infants)	< 160	160–170	> 170
	(1–6 years)	< 105	105–115	> 115
	(7–10 years)	< 90	90–100	> 100
	(11–14 years)	< 80	80–90	> 90
Weight percentile		> 10	3–10	< 3
Skin perfusion		Normal	Decreased (marble)	Markedly decreased (cool, grey)
Cyanosis		Absent	Peripheral	Present
Precordial thrill		Absent	Present	
Hepatomegaly (liver edge from right costal margin)		< 2 cm	2–3 cm	> 3 cm

100 μl. The reaction was started by addition of the membranes (100–200 μg/tube) and was performed for 10–20 min at 30°C. Termination of the reaction and isolation of cyclic AMP formed were carried out as described (Jakobs et al., 1976).

2.6. Pertussis toxin-catalyzed ADP-ribosylation

Pertussis toxin was preactivated in 100 mM Tris/HCl, pH 8.0, with 50 mM dithiothreitol for 1 h at room temperature. The activated toxin at a final concentration of 29 μg/ml was then added to a reaction mixture (final volume 50 μl) that contained 100–200 μg of crude cardiomyocyte membranes, 100 mM Tris/HCl, pH 8.0, 25 mM dithiothreitol, 2 mM ATP and 1 μCi/tube [^{32}P]NAD. The reaction mixture was incubated for 1 h at 37°C and the reaction was terminated by addition of SDS sample buffer (Laemmli, 1970) and subsequent heating for 3 min at 95°C. SDS-PAGE and autoradiography were performed as described (Gierschik et al., 1987). [^{32}P]ADP-ribose incorporation was determined after SDS-PAGE by localizing the labelled bands on autoradiograms, cutting the bands out from the dried gel, soaking in 1 ml of 30% hydrogen peroxide overnight, adding 20 ml of a scintillation cocktail and subsequent counting in a liquid scintillation spectrometer.

2.7. Statistical methods

In most experiments means and the individual experimental error are given (means ± S.D.). Differences between the groups were assessed by Mann-Whitney-test. For testing $P < 0.05$ was taken as statistically significant.

3. Results

3.1. Influence of hemodynamic grading on adenylyl cyclase activity and inhibitory G protein α-subunits in right atrial tissue

Myocardial responsiveness to β-adrenoceptor agonists was measured by determination of adenylyl cyclase activity in the presence of the β-adrenoceptor agonist isoproterenol (100 μM) and GTP (100 μM). β-Adrenoceptor-mediated adenylyl cyclase activity in congenital heart disease according to the various (main) diagnoses is shown in Fig. 1. Isoproterenol-stimulated adenylyl cyclase activity was significantly decreased in cyanotic congenital heart disease (group III) in comparison to moderate acyanotic congenital heart disease (group I) ($P < 0.05$) but it was not significantly altered in moderate acyanotic congenital heart disease (group I) versus severe acyanotic congenital heart disease (group II) (Table 3).

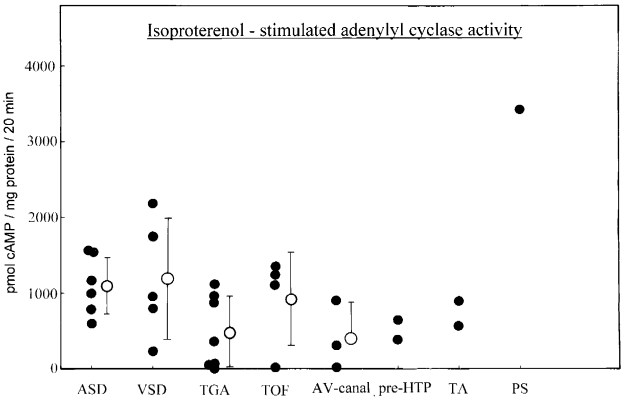

Fig. 1. Adenylyl cyclase activities in right atrial preparations determined in the presence of 100 μM isoproterenol and 100 μM GTP. Values are given according to the respective (main) diagnoses. Means ± S.D. (open circles) are indicated. Abbreviations: ASD, atrial septum defect; VSD, ventricular septum defect; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; AV-canal, complete atrioventricular canal; pre-HTP, severe combined congenital heart diseases requiring heart transplantation; TA, trikuspid atresia; PS, congenital pulmonal stenosis.

To study whether a post-receptor alteration of adenylyl cyclase may occur in myocardium of children with congenital heart disease, receptor-independent stimulation of adenylyl cyclase by direct activation of its catalytic subunit with forskolin was determined. Receptor-independent stimulation of adenylyl cyclase by forskolin (100 μM) in the presence of GTP (100 μM) is suggested to reflect activation of the whole G-protein-catalytic subunit holoenzyme complex. Forskolin-stimulated adenylyl cyclase activity in preparations from right atrial tissue according to the main diagnoses is indicated in Fig. 2. It was not significantly altered in group III (cyanotic congenital heart disease) vs. group I (moderate acyanotic congenital heart disease) nor in group II (severe acyanotic congenital heart disease) vs. group I (moderate acyanotic congenital heart disease) (Table 3).

Table 3
Adenylyl cyclase activities and $G_{i\alpha}$ proteins in right atrial tissue

	Group I	Group II	Group III
Adenylyl cyclase activity (pmol cAMP/mg protein per 20 min)			
Isoprenaline (100 μM)	1166 ± 403 ($n = 8$)	948 ± 1092 ($n = 10$)	653 ± 474 [a] ($n = 12$)
Forskolin (100 μM)	2180 ± 882 ($n = 9$)	1970 ± 2220 ($n = 9$)	1663 ± 501 ($n = 12$)
Pertussis toxin-catalyzed ADP-ribosylation (cpm/lane)	1786 ± 244 ($n = 9$)	1981 ± 493 ($n = 7$)	1566 ± 471 ($n = 5$)

Values are given as means ± S.D.
[a] Significantly different from group I, $P > 0.05$. Statistical determination was performed acording to Mann-Whitney test.

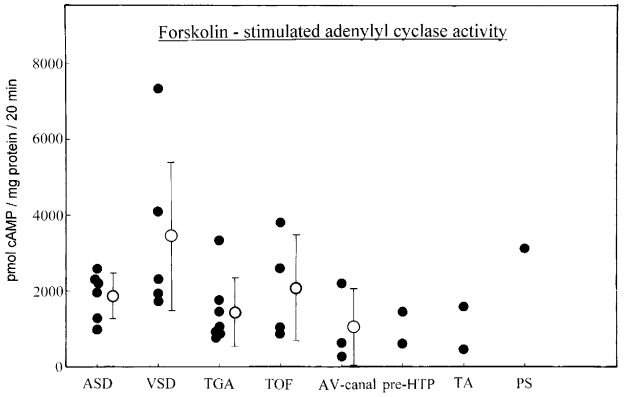

Fig. 2. Adenylyl cyclase activities in right atrial preparations determined in the presence of 100 μM forskolin and 100 μM GTP. Values are given according to the respective main diagnoses. Means ± S.D. (open circles) are indicated. Abbreviations: ASD, atrial septum defect; VSD, ventricular septum defect; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; AV-canal, complete atrioventricular canal; pre-HTP, severe combined congenital heart diseases requiring heart transplantation; TA, trikuspid atresia; PS, congenital pulmonal stenosis.

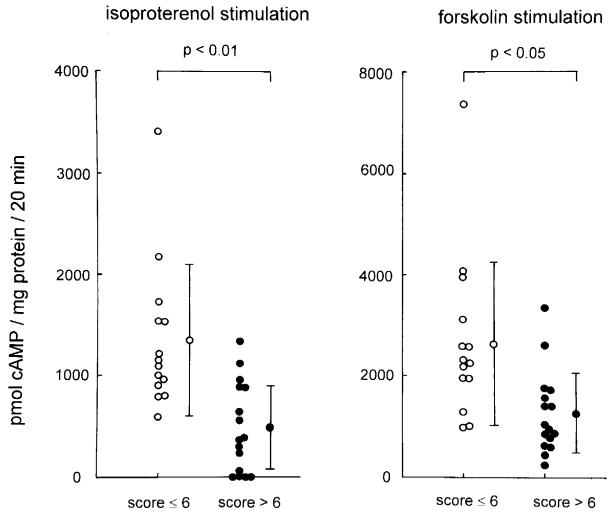

Fig. 3. Influence of the severity of heart failure on adenylyl cyclase activities in right atrial preparations. Adenylyl cyclase activities were determined in the presence of 100 μM isoproterenol and 100 μM GTP (isoproterenol) and in the presence of 100 μM forskolin and 100 mM GTP (forskolin). Means ± S.D. are also given. Significant differences are indicated. Statistical determination was performed according to Mann-Whitney test.

3.2. Influence of clinical grading (severity of heart failure) on adenylyl cyclase activity in right atrial tissue

The severity of heart failure in children and infants with congenital heart disease was graded by a modified scoring system based on the scoring system described by Ross et al. (1992). Children with score 0–6 were classified as no or mild congestive heart failure and children with score > 6 were classified as severe heart failure. The following diagnoses were found in the group with no or mild heart failure (score ≤ 6): atrial septum defect ($n = 7$), ventricular septum defect ($n = 4$), complete atrioventricular canal ($n = 1$), congenital pulmonal stenosis ($n = 1$), tetralogy of Fallot ($n = 1$), transposition of the great arteries ($n = 1$). In the group of children with severe heart failure (score > 6) the following diagnoses were found: severe combined congenital heart disease before heart transplantation ($n = 2$), complete atrioventricular canal ($n = 2$), ventricular septum defect ($n = 1$), transposition of the great arteries ($n = 7$), tricuspid atresia ($n = 2$), tetralogy of Fallot ($n = 2$). Mean age was 4.1 (± 4.2) years ($n = 15$) in the group with score ≤ 6 and 1.3 (± 2.7) years in the group with score > 6 ($P < 0.01$).

β-Adrenoceptor-stimulated adenylyl cyclase activity was significantly decreased by 65 ± 30% (mean ± S.D.) in the patients with severe heart failure (score > 6) in comparison to the group with no or mild heart failure (score ≤ 6) ($P < 0.01$) (Fig. 3). In addition, β-adrenoceptor-independent adenylyl cyclase stimulation by forskolin was also significantly decreased by 52 ± 31% in the patients with score > 6 compared to the patients with score ≤ 6 ($P < 0.05$) (Fig. 3).

To study the mechanism of this post-receptor alteration of adenylyl cyclase in severe congenital heart disease, the level of $G_{i\alpha}$ proteins was determined (Fig. 4). Pertussis toxin-catalyzed ADP-ribosylation of 40 kDa protein (pertussis toxin substrates) showed virtually no difference in the level of $G_{i\alpha}$ proteins in the group of patients with score ≤ 6 vs. patients with score > 6 (Fig. 4).

Stimulation of adenylyl cyclase with forskolin in the presence of Mn^{2+} (10 mM) which uncouples the catalytic subunit from the G proteins (G_s and G_i) apparently reflects selective activation of the catalytic subunit. Forskolin + Mn^{2+}-stimulated adenylyl cyclase activity was significantly decreased by 66 ± 24% in the patients with severe

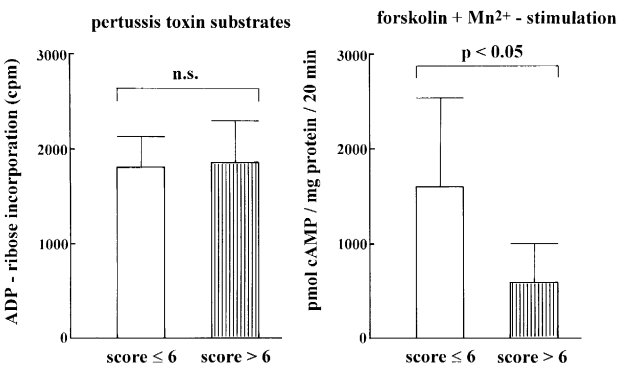

Fig. 4. Influence of the severity of heart failure on forskolin + Mn^{2+}-stimulated adenylyl cyclase activity and pertussis toxin substrates in right atrial preparations. The level of $G_{i\alpha}$ proteins was determined by quantitation of pertussis toxin-catalyzed ADP-ribosylation of 40 kDa proteins (pertussis toxin substrates). The activity of the catalytic subunit of adenylyl cyclase was determined in the presence of 100 μM forskolin and 10 mM Mn^{2+} (and absence of Mg^{2+} and GTP). Values are given as means ± S.D. Significant differences are indicated. Statistical determination was determined according to Mann-Whitney test. n.s., not significant.

heart failure (score > 6) in comparison to patients with no or mild heart failure (score ≤ 6) ($P < 0.05$) (Fig. 4). These results suggest that the post-receptor alteration of adenylyl cyclase in severely failing patients with congenital heart disease is probably located at the catalytic subunit of adenylyl cyclase.

4. Discussion

Recent data have shown a marked decrease in the number of β-adrenoceptors and in β-adrenoceptor-stimulated adenylyl cyclase activity in myocardial membranes from infants and children with severe acyanotic or cyanotic heart disease undergoing cardiac surgery (Kozlik et al., 1991; Kozlik-Feldmann et al., 1993). This desensitization of β-adrenoceptor stimulation was reported to be due to the increased levels of circulating catecholamines in patients with severe acyanotic and cyanotic congenital heart disease (Kozlik et al., 1991; Kozlik-Feldmann et al., 1993). The results of this study show that a defect at the post-receptor level of adenylyl cyclase also occurs in severely failing infants and children with congenital heart disease.

Due to the limited amount of myocardial tissue available it was not possible to determine the number and affinity of β-adrenoceptors by radioligand binding experiments in this study. As the activity of the catalytic subunit of adenylyl cyclase was shown to be reduced, the decrease in isoproterenol-stimulated adenylyl cyclase activity found in this study is not necessarily equivalent to a decrease in β-adrenoceptor responsiveness but may also be mediated by the apparent post-receptor defect. However, the results of our previous studies (Kozlik et al., 1991; Kozlik-Feldmann et al., 1993) suggest that the number and responsiveness of β-adrenoceptors is decreased in the group of children with severe heart failure (score > 6) in comparison to the group with no or mild heart failure (score 0–6).

In left ventricular preparations of myocardium obtained from patients with severe heart failure (ischemic or dilated cardiomyopathy) an increase in the level and activity of inhibitory G protein α-subunits was identified as the apparent post-receptor defect of adenylyl cyclase responsible for the decrease in receptor-independent adenylyl cyclase stimulation (Feldman et al., 1988; Böhm et al., 1994). In contrast, in right ventricular preparations of patients with ischaemic or dilated cardiomyopathy the amount of $G_{i\alpha}$ proteins was reported to be unchanged (Böhm et al., 1994). Bristow et al. (1992) had found a defect at the catalytic subunit of adenylyl cyclase in right ventricular myocardium from patients with dilated cardiomyopathy and patients with primary pulmonary hypertension. Similarly, we have recently found a decrease in adenylyl cyclase catalytic subunit activity in preparations of right ventricular endomyocardial biopsies obtained from severely failing patients with dilated cardiomyopathy (Reithmann et al., 1996). The results of the present study indicate that the post-receptor defect of adenylyl cyclase in right atrial membranes of severely failing infants and children with congenital heart disease is also located at the catalytic subunit of adenylyl cyclase. In contrast, the level and activity of $G_{i\alpha}$ proteins determined by pertussis toxin-catalyzed ADP-ribosylation was apparently unaltered.

The severity of heart failure in patients with congenital heart disease was estimated by a modified scoring system according to Ross et al. (1992). The scoring system by Ross et al. (1992) was described to grade the severity of heart failure in infants including an accurate description of historical and clinical variables. Ross et al. (1992) classified infants with score 0–2 as no congestive heart failure, score 3–6 as mild, 7–9 as moderate and 10–12 as severe heart failure. In the present study some parameters of this score such as 'volume consumed per feeding' and 'time taken per feeding' were replaced by parameters such as diaphoresis, tachypnoea and episodes of hypoxemia in order to grade the severity of heart failure in infants and children up to an age of 14 years. Patients with score ≤ 6 were classified as no or mild heart failure, patients with score > 6 were classified as severe heart failure. The mean age of children with severe heart failure (1.4 years) was lower than the mean age of the children with no or mild heart failure (4.2 years). It may, therefore, be discussed that the differences in adenylyl cyclase activity between the group with score ≤ 6 and the group with score > 6 are due to differences in the age of the patients rather than to the severity of heart failure. However, a significant decrease in isoprenaline-, forskolin- and Mn^{2+}-stimulated adenylyl cyclase activities with age has previously been found in right atria from patients of different ages (7 days to 83 years) without apparent heart failure (Brodde et al., 1995b). This suggests that the decrease in β-adrenoceptor-dependent and receptor-independent adenylyl cyclase activity in right atria of severely failing patients does not reflect an age-dependent reduction of adenylyl cyclase activity. Due to the negative correlation between adenylyl cyclase activity in right atria and the age of the patients as shown by Brodde et al. (1995b), the desensitization of adenylyl cyclase found in the present investigation may even be underestimated.

Bristow et al. (1992) had suggested that the decrease of adenylyl cyclase catalytic subunit activity in patients with dilated cardiomyopathy and primary pulmonary hypertension is the result of pressure overload of the failing right ventricles. To study the role of pressure and volume overload and of cyanosis on adenylyl cyclase activity in right atria, the 31 patients were divided into a group with moderate pressure and volume overload without cyanosis (group I), a group with severe pressure and volume overload without cyanosis (group II) and a group with cyanosis (group III). The results demonstrate that the higher P_{PA}/P_{Ao} and Q_p/Q_s ratios in group II vs. group I were not accompanied by significant changes in forskolin-

stimulated adenylyl cyclase activities. Therefore, in patients with congenital heart disease the development of a post-receptor defect of adenylyl cyclase seems not to be closely correlated with the degree of pressure and volume overload of the right ventricle. In patients with cyanotic congenital heart disease (group III) forskolin-stimulated adenylyl cyclase activity was reduced by 24% in comparison to the patients with moderate acyanotic congenital heart disease (group I) but the difference was not significant.

The mechanism of the defective catalytic subunit in congenital heart disease is at present unknown. The results of this study indicate that the activity of catalytic subunit of adenylyl cyclase in myocardium of children with congenital heart disease apparently depends on the severity of heart failure. It may, thus, be speculated that the decrease in the activity of catalytic subunit of adenylyl cyclase is mediated by systemic mechanisms rather than by the pressure and volume overload of the right ventricle.

In different forms of heart failure the activity of the catalytic subunit of adenylyl cyclase was shown to be unchanged in human left ventricular myocardium (Brodde, 1991). In contrast to the down-regulation of β-adrenoceptors and the up-regulation of $G_{i\alpha}$ proteins the reduction of the activity of the catalytic subunit found in failing right ventricles is apparently not a β-adrenoceptor agonist-induced phenomenon as in different experimental models chronic β-adrenoceptor agonist exposure did not induce an alteration of Mn^{2+}-stimulated adenylyl cyclase activity (Eschenhagen et al., 1992, Reithmann et al., 1989). Interestingly, a decrease in adenylyl cyclase catalytic subunit activity has been found in experimental models of rapid pacing-induced heart failure: in experimental canine congestive heart failure produced by rapid ventricular pacing the ability of Mn^{2+} and purified G_s to stimulate adenylyl cyclase was decreased suggesting a defect at the catalytic subunit (Marzo et al., 1991). In dogs rapid-pacing induced heart failure caused a reduction in steady-state mRNA-levels of type V and VI adenylyl cyclase (Ishikawa et al., 1994). At present at least eight isoforms of membrane-bound adenylyl cyclase have been cloned, among these the heart seems to contain mainly type V and VI (Brodde et al., 1995a). As a possible mechanism of the reduction of catalytic subunit activity an alteration of the ratio of the isoforms of the enzyme has been discussed (Brodde et al., 1995a).

In summary, the results suggest that the defect at the catalytic subunit of adenylyl cyclase is not strongly correlated to the pressure and volume overload of the right ventricle but it may be mediated by systemic mechanisms in severe congenital heart disease. As a functional consequence, the decrease in adenylyl cyclase catalytic subunit activity apparently contributes to the decreased effectiveness of β-adrenoceptor agonists. Furthermore – in addition to the decreased effectiveness of β-adrenoceptor agonists – the effect of β-adrenoceptor-independent cAMP-increasing positive inotropic agents such as phosphodiesterase inhibitors may also be decreased in severely failing patients with congenital heart disease.

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