Cardiovascular Research, 1989, **23**, 859–866

Density of β adrenoceptors in rat heart and lymphocytes 48 hours and 7 days after acute myocardial infarction

W VLEEMING, P A VAN DER WOUW, J D TE BIESEBEEK, H H VAN ROOIJ, J WEMER, A J PORSIUS

From the Department of Pharmacotherapy, Faculty of Pharmacy, University of Utrecht, The Netherlands

ABSTRACT Down regulation of the β adrenoceptor is thought to play an important role in the diminished response to catecholamines in heart failure. β Adrenoceptor densities were measured on membrane homogenates of rat right ventricle and lymphocytes 48 h or 7 d after experimental myocardial infarction, and in rats exposed to a continuous infusion of isoprenaline (400 $\mu g \cdot kg^{-1} \cdot h^1$). The performance of the rat hearts was also evaluated 48 h post infarction in an isolated retrograde perfused heart preparation. In contrast to a 60% down regulation in right ventricle and a 20% down regulation in lymphocyte membranes after isoprenaline infusion, there was no change in right ventricle and lymphocyte β adrenoceptor densities after myocardial infarction. Left ventricular contractile performance was significantly depressed 48 h after myocardial infarction. Mean basal left ventricular pressure decreased from 108(SEM 3) to 63(4) mm Hg while the maximal response to dobutamine was decreased from 204(4) to 105(12) mm Hg (n=8). No correlation was found between the receptor densities of right ventricular and lymphocyte membranes.

We conclude that diminished response to β sympathomimetics after myocardial infarction cannot be attributed to a loss of surface β adrenoceptors, and that the lymphocyte β adrenoceptor does not provide an adequate system to monitor small receptor changes on the myocardium.

The monitoring of β adrenoceptor changes in less accessible tissue via the β adrenoceptor on circulating lymphocytes has attracted much interest.[1–7] A variety of physiological and pathological conditions, as well as β adrenergic drug treatment, influence the density of β adrenoceptors on various cells. In addition a change in response to catecholamines is observed. This can have important therapeutic implications, since the knowledge of both initial and post treatment receptor densities can lead to a more rational therapy in hypertension,[3] heart failure,[8] and the prophylaxis and treatment of heart failure after acute myocardial infarction.[9 10] The lymphocyte β_2 adrenoceptor density has been shown to have some correlation both with myocardial β_1 and β_2 adrenoceptor densities and with myocardial response.[2 4]

Desensitisation, the diminished response to catecholamines, is thought to begin with an uncoupling of the β adrenoceptor from the adenylate cyclase system, followed by the disappearance of the β adrenoceptors from the cell surface membrane.[11 12] However in the first few hours after myocardial infarction there is an up regulation in β adrenoceptors that might play a role in the vulnerability to arrhythmias.[13]

Cohn *et al*[14] showed that high plasma levels of catecholamines are the single most important predictor of prognosis in patients with heart failure. As high circulating catecholamines induce down regulation of the β adrenoceptors, it is not surprising that chronic heart failure is accompanied by β adrenoceptor down regulation.[8 15] Since the β adrenoceptor is an important mediator of positive inotropic response,

Address for correspondence and reprints: W Vleeming Department of Pharmacotherapy, Faculty of Pharmacy, University of Utrecht, Croesestraat 79, 3522 AD Utrecht, The Netherlands

Key words: experimental myocardial infarction; myocardial β adrenoceptors; lymphocyte β adrenoceptors; rats

Submitted 6 June 1988
Accepted 27 April 1989

down regulation further compromises the heart in heart failure.[8]

Knowledge of the extent of down regulation might influence therapy, since rational choices can be made between treatment with catecholamines or β blockers,[16] or by bypassing the β adrenoceptor with phosphodiesterase inhibitors.

Baumann *et al*[17] found a decreased density of β adrenoceptors in the uninvolved right ventricle 3 d after infarction in guinea pigs which was increased again 6 d after infarction.

In this study we investigated the relation between rat myocardial β_1 and β_2 adrenoceptor regulation and lymphocyte β_2 adrenoceptor regulation 48 h and 7 d after experimental acute myocardial infarction, the period in which the infarct is totally evolved and scar tissue is developed.[18 19] An isolated retrograde perfused heart preparation was used to assess the performance of rat hearts 48 h after myocardial infarction.

Methods

EXPERIMENTAL MYOCARDIAL INFARCTION

Experimental acute myocardial infarction was induced in male Wistar rats weighing 200–250 g by the method of Selye *et al*.[20] A left thoracotomy was performed under ether anaesthesia and the heart manually expressed from the thoracic cavity. The left coronary artery was ligated 2 mm after its origin. The heart was replaced in the thoracic cavity and the wound closed. This whole procedure was completed within 2 min. A sham operated group of animals had the same procedure without ligation of the coronary artery. The control group consisted of unoperated rats of the same age.

Approval for the studies was obtained from the Animal Care Committee of the University of Utrecht in accordance with the Dutch Animals Experiments Act (1977).

ISOLATED RETROGRADE PERFUSED HEART PREPARATION

Forty eight hours after operation, the heparinised animals were killed by decapitation. The hearts were rapidly excised, and immediately suspended for retrograde perfusion with an oxygenated Krebs-Ringer solution at 32°C at constant pressure (80 cm water), according to the method described by Vleeming *et al*.[21] The composition of the Krebs solution (mmol·litre^{-1}) was as follows: NaCl 128, KCl 4.7, $MgCl_2$ 0.6, NaH_2PO_4 0.4, $NaHCO_3$ 27, $CaCl_2$ 1.3 and glucose 11. The hearts were paced at 5 Hz. Left ventricular pressure was measured via a water filled balloon inserted into the left ventricle and connected via a catheter with a Statham P-50 pressure transducer. Diastolic ventricular pressure was adjusted to 5 mm Hg. Coronary flow was established by measuring the volume of perfusate leaving the coronary system. Dobutamine or its vehicle was given directly into the perfusion stream as a bolus injection in a volume of 100 microlitres, just above the orifice of the coronary arteries.

ISOPRENALINE TREATMENT

Under ether anaesthesia a mini osmotic pump (Alzet) was implanted subcutaneously in the neck region of male Wistar rats of 200–250 g weight. This pump delivered isoprenaline for 48 h at an infusion rate of 400 $\mu g \cdot kg^{-1} \cdot h^{-1}$.[22] Control rats were infused with a 0.9% NaCl solution.

MYOCARDIAL MEMBRANES

Infarcted or sham operated rats were killed 48 h or 7 d after operation by cervical dislocation. Isoprenaline treated rats were killed after 48 h of continuous infusion. The hearts were rapidly excised and the infarct size macroscopically characterised. The right ventricle free wall was separated and washed in an ice cold glucose-TRIS buffer containing glucose 0.25 M, TRIS 5 mM and $MgCl_2$ 1 mM, pH 7.4. The tissue was homogenised by three 15 s bursts with an Ystral homogeniser on low, medium and maximum speed, with 30 s intervals during which the homogenate was kept on ice. The homogenate was then centrifuged for 15 min at 400 *g*. The supernatant was filtered through three layers of medical gauze and centrifuged for 15 min at 37 000 *g*. The pellet was resuspended in ice cold TRIS buffer containing TRIS 50 mM and $MgCl_2$ 5 mM, pH 7.4, and again centrifuged for 15 min at 37 000 *g*. The final pellet was resuspended in 1 ml of the TRIS buffer and frozen in liquid nitrogen.

LYMPHOCYTE MEMBRANES

Under ether anaesthesia and just before the rats were killed, 4.5 ml blood was obtained by cardiac puncture, with a syringe containing 0.5 ml of EDTA solution 15 mg·ml^{-1}. The blood sample was then diluted with 10 ml phosphate buffered saline containing (mmol·litre^{-1}) NaCl 140, Na_2HPO_4 9, $NaH_2PO_4 \cdot H_2O$ 1·3, and the lymphocytes were separated according to the method of Böyum.[23] The lymphocytes were homogenised in ice cold TRIS buffer containing TRIS 50 mM and $Mg\ Cl_2$ 5 mM, pH 7.4, using a 10 s burst with an Ultra-Turrax homogeniser at maximum speed. The homogenate was centrifuged at 37 000 *g* for 10 min and the pellet resuspended in 1 ml of the TRIS buffer and stored in liquid nitrogen.

INFARCT SIZE DETERMINATION

Infarct size was classified as small, medium or large, meaning that <25%, 25–50% or >50% of left

ventricular free wall was macroscopically involved in the infarcted area. This correlated well with the nitro blue tetrazolium method[19] in a pilot study. We only used hearts which showed no evidence of right ventricle infarction. For receptor binding studies all infarcts in the 48 h post-infarction group were large, while in the 7 d post-infarction group three infarcts were classified large, one medium and one small. For isolated perfused heart preparations all infarcts were classified as large.

β RECEPTOR BINDING

Myocardial or lymphocyte membranes were thawed and diluted with the TRIS buffer to a protein concentration of 200–400 $\mu g \cdot ml^{-1}$. A 200 μl aliquot of this suspension was added to 300 μl of a solution containing ^{125}I-iodopindolol (final concentration in competition experiments 170 pM; in saturation experiments six concentrations were used, ranging from 15 pM to 480 pM), 20 mM HEPES, 10 mM $MgCl_2$, 120 mM NaCl, pH 7.4. In competition experiments ICI 118 551 was added in a concentration range of 5 pM to 5 μM. In saturation experiments non-specific binding was determined with 5 μM ICI 118 551. All experiments were performed in duplicate. After 30 min incubation at 37°C, the incubation was stopped by adding 4 ml ice cold HEPES buffer, and separation performed by rapid vacuum filtration through Whatman GF/B filters. The filters were counted in Scintillator 299 (Hewlett Packard) in a liquid scintillation counter (Packard 3520) at an efficiency of 76%. Both competition and saturation binding curves were fitted with non-linear regression computer programs. Competition binding curves were fitted with a non-linear least squares regression program using the simplex algorithm[24] and saturation curves with the LIGAND program,[25] extended to use non-linear regression instead of Scatchard transformation in assessing binding parameters

Protein concentrations were measured with the Biorad protein assay.[26]

DRUGS

^{125}I-iodopindolol was obtained from New England Nuclear, Boston, USA; ICI 118 551 was a gift from Imperial Chemical Industries, London; dobutamine was donated by Lilly, Indianapolis, USA. All other chemicals used were of analytical grade from standard commercial suppliers.

STATISTICS

Data are given as means (SEM). The best fit to a one receptor or a two receptor model was evaluated by the partial F test,[27] and parameters from the best fit were used in further evaluations. Linear least squares regression analysis was used to evaluate the relation between lymphocyte and cardiac β adrenoceptors. Analysis of variance (SPSSPC, SPSS Inc, Luxembourg) was used to detect differences in binding parameters among the various groups, with an α level of 0.05 being considered significant.

In the functional response study on isolated perfused hearts, the significance of differences between the group means was evaluated by Student's t test for unpaired samples (one tailed). A value of $p<0.05$ was considered significant.

TABLE 1 *^{125}I-iodopindolol dissociation constant (K_D) and β adrenoceptor density (B_{max}) from saturation experiments with lymphocyte and myocardial membranes. Data are pooled from 48 h and 7 d post-infarction experiments and control experiments. Results are means(SEM).*

	K_D (pmol·litre^{-1})	B_{max} (fmol·mg^{-1} protein)	N
	Lymphocytes		
Control	132(43)	30.9(2.0)	3
Sham	129(40)	26.5(1.0)	6
Infarction	78(13)	5.1(3.0)	6
	Right ventricle		
Control	165(50)	19.5(4.5)	3
Sham	134(18)	18.8(0.5)	6
Infarction	104(16)	17.0(2.0)	6

Results

β RECEPTOR BINDING

In the saturation experiments the dissociation constant (K_D) of ^{125}I-iodopindolol was the same in myocardial and lymphocyte membranes and did not change after myocardial infarction, as can be seen from table 1.

Raw data from a competition experiment are shown in fig 1. In the competition curves no differences in pK_i

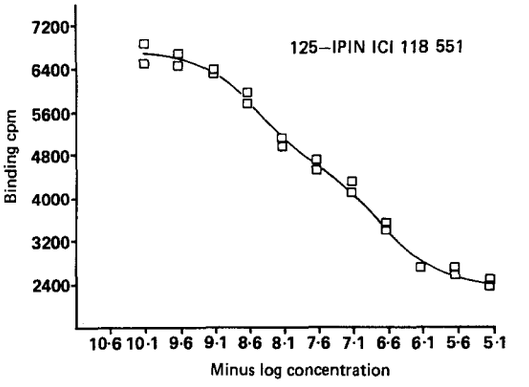

FIG 1 *Raw receptor binding data, obtained by competition experiments in rat right ventricle membranes and fitted to a two site receptor model. ^{125}I-iodopindolol is the radioligand and ICI 118 551 is the displacing agent.*

TABLE 2 *Beta-adrenoceptor density (B_{max}) and −log inhibition constant of ICI 118 551 (pK_I) from competition experiments with lymphocyte membranes 48 h and 7 d post-myocardial infarction. Results are means(SEM).*

	B_{max} (fmol·mg^{-1} protein)	pK_I	N
	Lymphocytes 48 h		
Control	40.0(4.3)	8.94(0.08)	4
Sham	30.1(2.3)	8.90(0.02)	6
Infarction	27.7(2.8)	8.92(0.04)	6
	Lymphocytes 7 d		
Control	29.0(4.8)	8.75(0.09)	4
Sham	28.7(1.6)	8.77(0.06)	4
Infarction	26.4(5.2)	8.78(0.07)	5

of ICI 118 551 before or after operation were seen. A small but significant difference was seen between the 48 h and 7 d groups. This difference could be explained by the ligand batches which were used for these experiments. In 28 out of the 30 competition experiments in right ventricular tissue the two receptor model gave a significantly better fit compared with the one receptor model. In lymphocyte membranes we found a homogeneous population of receptors with high affinity for ICI 118 551, a β_2 selective antagonist (table 2). The β_2 fraction in the myocardial membranes was 53 (SEM 11)%, and was not changed 48 h or 7 d after myocardial infarction (table 3). Analysis of variance showed no differences in β adrenoceptor density after myocardial infarction, and no differences between the 48 h post-infarction and the 7 d post-infarction groups (table 2).

The overall β adrenoceptor density was 17.6 (SEM 3.8) fmol·mg^{-1} protein in myocardial membranes and 28.3(8.7) fmol·mg^{-1} protein in lymphocyte membranes. There was no correlation between lymphocyte β_2 and myocardial β_2 receptor densities, as can be seen in fig 2 (r=0.25, p=0.18, n=29). In addition, there was no correlation between total β adrenoceptor density in right ventricle membrane homogenates and β adrenoceptor density in

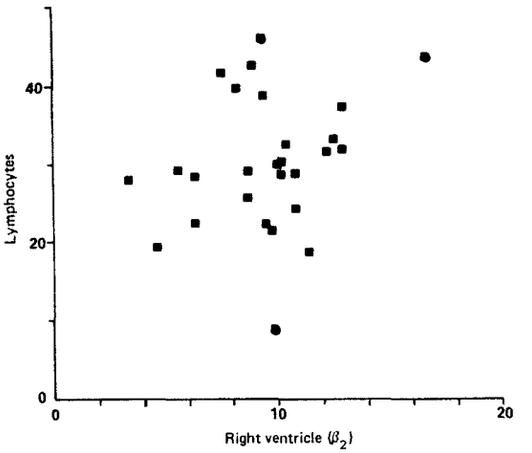

FIG 2 *Correlation between cardiac β_2 adrenoceptor density in right ventricle membrane homogenates and β_2 adrenoceptor density in lymphocyte membrane homogenates: r=0.25, p=0.18, n=29.*

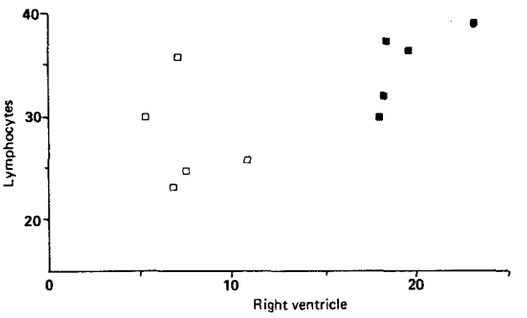

FIG 3 *Correlation between total β adrenoceptor density in right ventricle membrane homogenates and β adrenoceptor density in lymphocyte membrane homogenates from rats treated with isoprenaline (open squares) or saline (filled squares) for 48 h: r=0.67, p=0.04, n=10.*

TABLE 3 *Adrenoceptor density (B_{max}), −log inhibition constant of ICI 118 551 (pK_I) for β_1 and β_2 adrenoceptors, and β_2 receptor fraction ($f\beta_2$) from competition experiments with right ventricle membranes 48 h and 7 d post-myocardial infarction. Results are means(SEM).*

	B_{max} (fmol·mg^{-1} protein)	pK_I (β_1)	pK_I (β_2)	$f\beta_2$	N
		Right ventricle 48 h			
Control	18.2(2.8)	7.03(0.10)	8.88(0.07)	0.57(0.05)	4
Sham	18.8(0.9)	7.14(0.06)	8.96(0.06)	0.55(0.03)	6
Infarction	16.7(1.3)	7.12(0.08)	8.93(0.14)	0.54(0.06)	6
		Right ventricle 7 d			
Control	16.2(1.4)	6.96(0.15)	8.59(0.08)	0.50(0.06)	4
Sham	15.3(2.4)	7.08(0.14)	8.84(0.11)	0.50(0.05)	4
Infarction	17.4(1.4)	6.56(0.31)	8.70(0.16)	0.52(0.06)	5

TABLE 4 *β Adrenoceptor density (B_{max}) on lymphocyte and myocardial membranes after 48 h of isoprenaline or 0.9% NaCl infusion.*

	B_{max} (fmol·mg^{-1} protein)	N
	Lymphocytes	
Isoprenaline	27.9(2.2)	5*
0.9% NaCl	35.0(1.7)	5
	Right ventricle	
Isoprenaline	7.7(0.8)	6†
0.9% NaCl	19.2(0.9)	6

*p<0.05, †p<0.001 *v* saline.

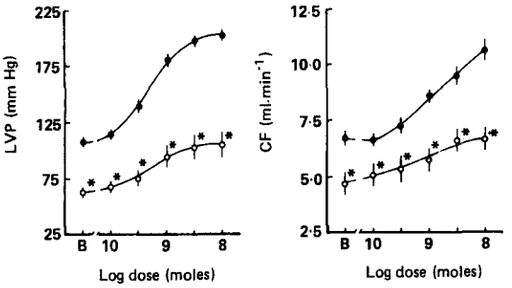

FIG 4 *Baseline values (B) and increase of ventricular pressure (left panel) and coronary flow (right panel) after dobutamine. Closed circles: sham operated animals; open circles: myocardial infarcted animals. *p<0.05 v sham operated.*

lymphocyte membrane homogenates (r=0.29, p=0.12, n=29).

In the isoprenaline treated group there was a decrease of 60% (p<0.001) in β receptor density on myocardial membranes and a 20% (p<0.05) decrease in lymphocyte β receptor density (table 4). There was a weak correlation between myocardial and lymphocyte β adrenoceptor densities (r=0.67, p<0.05, fig 3).

FUNCTIONAL RESPONSE

In isolated perfused heart experiments (n=8), both the baseline left ventricular pressure value and the maximal obtainable pressure response to dobutamine were significantly lower for the 48 h post-infarct group (fig 4, left panel). The coronary flow showed the same pattern (fig 4, right panel). The mean $-\log EC_{50}$ values of dobutamine with respect to left ventricular pressure were 9.28(0.09) and 9.33(0.11) for the sham operated and infarcted group respectively (NS).

Discussion

Down regulation as a physiological response to continuous stimulation of receptor systems is well known, both for the β adrenoceptor system and for other receptor systems.[28] However, with respect to the regulation of β adrenoceptors in different tissues it still remains controversial whether the down regulation occurs to all receptors on all tissues in the same way and to the same extent.

A number of investigators have found that changes in lymphocyte β_2 adrenoceptors correlate with alterations in β adrenoceptor density and with β adrenergic responsiveness in less accessible target tissues.[2 4 7] However correlation coefficients are rather small, partly due to biological variation. Recently Brodde *et al* have provided evidence for a correlation between atrial β_2 adrenoceptors and lymphocyte β_2 adrenoceptors, while the atrial β_1 receptor is less well correlated with the β_2 receptor on the lymphocyte.[5] It seems that lymphocyte β_2 adrenoceptor density alone is not sufficient to predict myocardial responsiveness. Although β_1 and β_2 receptors might not be affected in the same manner,[29] this has become less important in comparing lymphocyte and myocardial β adrenoceptors, since a number of recent studies have shown not only the presence of β_2 adrenoceptors in the mammalian heart,[4 29] but also its involvement in both inotropic and chronotropic responses. The β_2 receptor mediated response might become more overt in heart failure, as the β_1 receptor is more down regulated.[29] However, the loss of β adrenoceptors is just one step in the desensitisation process, and presumably a late step. Desensitisation as a response to high levels of β adrenoceptor agonists occurs first at the level of the coupling to adenylate cyclase.

Some reports indicate that the coupling to adenylate cyclase is different for β_1 and β_2 adrenoceptors, with more β_2 adrenoceptors actually coupled to the adenylate cyclase.[30] The unusually high amount of β_2 adrenoceptors [53(11)%] found in this study in the rat ventricular muscle contrasts with other studies.

Several β_1/β_2 ratios have been reported for different species and for ventricular tissue and whole heart, for example 83:17 in rat, whole heart;[31] 100:0 in rat, whole heart;[32] 57:43 in rat, ventricles;[33] 74:26 in rat, left ventricle;[34] 69:31, in human left ventricular papillary muscle;[34] 43:57, in human ventricular muscle;[35] 55:45, in rat ventricles.[36] In addition, the proportion of β_1 and β_2 adrenoceptors in rat atria has been reported as 67:33.[37]

An insufficient definition of the non-specific binding, using ICI 118 551, is not an explanation for this discrepancy, as can be concluded from fig 1, in which the raw data of our competition experiment are shown. However the use of iodopindolol, which is about three times more selective for β_2 receptors than for β_1 receptors,[38] as a radioligand might have distorted the measurement of the density of the β_1 receptor population by a factor of about 1.10-1.15.[39]

Another, although speculative, factor influencing the β_1/β_2 ratio might be the presence of endothelial plasma membranes from myocardial tissue, which contain substantial amounts of β_2 adrenoceptors.[36] However, in our opinion the exact ratio of the β_1 and β_2 receptor densities does not influence our conclusion that no changes occur in the β receptor population after myocardial infarction in the rat.

An interesting question relates to the meaning of changes in affinity to antagonist ligands accompanying the decrease or increase in β adrenoceptors as reported by some authors.[1 17 40] Molinoff attributes the observed affinity change to propranolol retained in the lymphocyte membrane.[1] In other studies, including the present one, no affinity change has been found.[4 13 33 40] As the process of desensitisation is very complex, involving uncoupling of the adenylate cyclase system, agonist affinity shifts, internalisation and phosphorylation, all having their own time course,[12] it is possible that an affinity change for antagonist ligands occurs during this sequence of events, although there is not much evidence for such an affinity change. Heart failure has been shown to be accompanied by down regulation of β adrenoceptors in human hearts.[8 29] Pfeffer *et al* show that heart failure occurs in rat hearts with myocardial infarction with loss of more than 45% of ventricular tissue.[41] Drexler *et al*[42] found a depressed cardiac performance in the rat and an infarct size of about 39% 42 d after myocardial infarction.

In guinea pig right ventricles, down regulation of β adrenoceptors ranged from 58 to 90 percent 3 d post-infarction.[17] This correlated well with infarct sizes ranging from 15% to more than 35%. Six days post-infarction β adrenoceptor number and the response to isoprenaline were almost back to normal.

In our experiments at 48 h and 7 d, infarct sizes were in the same range as in experimental models which produced heart failure at 3 weeks and 42 d after myocardial infarction.[41 42] In addition, using isolated perfused heart preparations we found that the basal values for both left ventricular pressure and coronary flow were significantly decreased at 48 h (fig 4) and also at 7 d (data not shown) post-infarction. Furthermore, in the infarcted group, the maximal positive inotropic effect of dobutamine was reduced by 50%, suggesting a depressed cardiac performance, without a change in the potency (EC_{50} value) of dobutamine.

However no down regulation of β_2 adrenoceptors was detectable on myocardial or lymphocyte membranes 2 d or 7 d after myocardial infarction. Our data in the isoprenaline treated rats showed that down regulation readily occurs within 48 h in both tissues when circulating agonist levels are high. However De Blasi *et al* found decreased β receptor density on lymphocytes in 3 month old rats after 30 min of stress.[6] This shows that down regulation under physiological conditions occurs in vivo in rats. We studied the β adrenoceptor density in the right ventricle, and not in the infarcted left ventricle, based on the hypothesis that circulating catecholamines would affect the β adrenoceptors in both ventricles in the same way.

There is evidence that β receptor densities on both ventricles can be regulated in an independent manner, since Bristow *et al* found no down regulation in the non-failing left ventricle in patients with severe isolated right ventricular failure and down regulated β adrenoceptors in the right ventricle.[29] However, they did not find an isolated down regulation in the left ventricle, since severe left ventricular failure is accompanied by right ventricular failure. β Adrenoceptor down regulation in the right ventricle has been shown to occur after left myocardial infarction in guinea pigs.[17] Although in our study both right ventricular and lymphocyte β adrenoceptors were not down regulated after myocardial infarction, we cannot be assured that no down regulation occurred in the part of the left ventricle that was not involved in the infarction area. However, it is doubtful if down regulation of β adrenoceptors in the setting of massive infarction is an important factor contributing to the failing heart. In moderate heart failure the reduction in inotropic response to the β_1 agonist xamoterol could not be attributed to a decreased β receptor responsiveness.[43] The loss of contractile tissue therefore seems to be the most important factor contributing to the failing heart. Due to the large biological variation, also reported by other authors,[44 45] small variations in mean β adrenoceptor density cannot be detected in small samples. Furthermore, β adrenoceptor density on the heart could not be predicted from lymphocyte β adrenoceptor density, especially as there was no down regulation of β adrenoceptors after myocardial infarction. This makes it difficult to use β adrenoceptor measurements on lymphocytes as a simple tool to predict β adrenoceptor density in heart tissue of patients.

The present study begs the question as to the stage during development of heart failure when the levels of circulating catecholamines increase sufficiently to cause a down regulation of β adrenoceptors.

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