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Myocardial β adrenoceptors and left ventricular function in hypertrophic cardiomyopathy

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Abstract

***Objective*—To assess the relation between left ventricular function and myocardial β adrenoceptor density.**

***Methods*—17 patients with hypertrophic cardiomyopathy, six with and 11 without heart failure, were studied. Left ventricular function was assessed by echocardiography, and myocardial β adrenoceptors by positron emission tomography. Patient data were compared with those obtained in normal controls.**

***Results*—Myocardial β adrenoceptor density in the 17 patients was 7·00 (SD 1·90) pmol/g *v* 11·50 (2·18) pmol/g in normal controls ($P < 0·01$). β Adrenoceptor density in the six patients with left ventricular failure was 5·61 (0·88) pmol/g *v* 7·71 (1·86) pmol/g in the 11 patients with normal ventricular function ($P < 0·05$), and there was a significant correlation ($r = 0·52$; $P < 0·05$) between left ventricular fractional shortening and myocardial β adrenoceptor density. A positive correlation ($r = 0·51$; $P < 0·05$) was also found between myocardial β adrenoceptor density and the E/A transmitral flow ratio, an index of left ventricular diastolic function.**

***Conclusions*—There is myocardial β adrenoceptor downregulation in patients with hypertrophic cardiomyopathy with or without signs of heart failure.**

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Keywords: hypertrophic cardiomyopathy; myocardial adrenoceptors; left ventricular function; positron emission tomography

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Hypertrophic cardiomyopathy is characterised by inappropriate myocardial thickness, most often involving the interventricular septum of a non-dilated left ventricle.[1] The disease is transmitted as an autosomal dominant trait in up to 50% of patients.[2] Recently various mutations of the β myosin heavy chain gene have been described in affected families.[3–5] However, this gene has been excluded as the cause of the disease in some families[6] and analysis of other candidate genes is awaited.

It has been hypothesised that other factors could play a role in the development and natural history of this disease, including an increased sympathetic activity in the heart.[7] Accordingly, a raised myocardial noradrenaline content[8] as well as increased cardiac spillover of noradrenaline[9] have been found in patients with hypertrophic cardiomyopathy. Moreover, a downregulation of myocardial β adrenoreceptors has recently been observed in these patients by means of positron emission tomography,[10] which is presumably a result of locally increased levels of noradrenaline.[11]

Although ventricular systolic function is usually hyperdynamic in hypertrophic cardiomyopathy, a minority of patients (5–10%) develop progressive dilatation of the left ventricular chamber and systolic dysfunction later in the course of the disease.[12–15] To investigate the relation between left ventricular function and changes in neural control of the heart, we assessed left ventricular function by echocardiography and myocardial β adrenoceptor density by positron emission tomography in a group of patients with hypertrophic cardiomyopathy with and without heart failure. The results were compared with those obtained in a group of normal controls.

Methods

STUDY POPULATION

Seventeen patients with hypertrophic cardiomyopathy were studied (11 males and six females; table). Patients were excluded from the study if they were on β blocker or amiodarone treatment or if they had asthma or any other systemic disease, and women of child bearing potential were also excluded. Eleven patients (mean age 37 (SD 10) years) had no evidence of heart failure and had preserved left ventricular systolic function with normal fractional shortening. In these 11 patients the myocardial β adrenoceptor density has previously been reported.[10] Six patients (mean age 53 (10) years, $P < 0·01$ *v* patients with preserved left ventricular function) had clinical evidence of heart failure (New York Heart Association functional class III and IV), received treatment with diuretics, and had reduced fractional shortening (table). All of these six patients had a family history of hypertrophic cardiomyopathy and had documented evidence of typical disease previously. Coronary angiography was normal in these six patients.

Eight normal volunteers (mean age 28 (7) years; all males) whose myocardial β adrenoceptor density had been reported previously,[10] served as control subjects. All the normal volunteers were selected on the basis of their clinical history and physical examination, which indicated a low risk of coronary artery disease. All had normal resting electrocardiograms and negative exercise tests in response to a high workload.

Clinical and echocardiographic characteristics of patients

	Sex	Age (years)	Symptoms: Dyspnoea	Symptoms: Orthopnoea	Medication	Echocardiographic findings: ivs (mm)	pw (mm)	LVEDD (mm)	LVESD (mm)	Fractional shortening (%)	LVOT gradient (mm Hg)	E:A ratio
Patients with preserved systolic function												
1	M	33	yes	no	Verapamil	21	13	51	30	41	32	1·67
2	M	33	yes	no	None	23	13	47	31	34	5	1·6
3	F	47	yes	no	Diltiazem	17	14	40	20	50	121	1·13
4	M	32	no	no	Verapamil	23	12	36	20	44	3	1·6
5	M	36	no	no	None	31	14	40	25	37	60	0·39
6	M	51	no	no	Verapamil	17	14	47	30	36	14	0·73
7	M	31	no	no	None	16	13	50	24	52	26	1·25
8	M	20	no	no	None	27	11	44	27	39	4	1·27
9	M	50	no	no	None	15	11	46	34	26	9	1·62
10	M	27	no	no	Verapamil	15	10	46	32	30	0	1·89
11	M	48	no	no	None	17	14	54	38	30	9	0·43
Mean(SD)		37(10)				19(6)	12(2)	45(5)	28(6)	38(8)	26	1·23(0·16)
Patients with systolic dysfunction												
12	M	57	yes	yes	Diuretics	13	13	62	56	10	9	0·3
13	F	68	yes	yes	Diuretics	15	15	48	38	21	9	1·52
14	F	52	yes	yes	Diuretics	12	12	47	38	19	0	2·39
15	F	39	yes	yes	Diuretics	10	10	52	42	19	0	
16	F	58	yes	no	Diuretics	8	7	48	44	8	0	1·4
17	F	47	yes	no	None	17	17	51	39	24	0	0·33
Mean(SD)		53(10)**				12(3)*	12(4)	51(6)*	43(7)**	17(6)**	3**	1·19(0·40)

pnd, paroxysmal nocturnal dyspnoea; ivs, interventricular septum; pw, posterior wall; LV, left ventricle; LVESD, LV end systolic dimension; LVEDD, LV end diastolic dimension; LVOT, left ventricular outflow tract.
*P < 0·05, **P < 0·01 *v* patients with preserved systolic function.

All subjects gave written informed consent to the protocol which was approved by the Hammersmith Hospital research ethics committee and the United Kingdom Administration of Radioactive Substances Advisory Committee.

STUDY PROTOCOL

Echocardiography

Echocardiographic studies were performed in all patients, and the left ventricular wall thickness and internal dimensions were measured at the level of the mitral valve leaflet tips.[16] The fractional shortening of the left ventricle was calculated as the percentage difference between the end systolic dimension and the end diastolic dimension normalised to the end diastolic dimension. Continuous wave Doppler velocities from the apical projection were used to calculate the peak intracardiac gradient (PG), as $PG = 4 \times V_{max}^2$, where V_{max} is the left ventricular outflow tract velocity. Transmitral early (E wave) and late (A wave) peak inflow velocities were measured by sampling with pulsed Doppler proximal to the mitral leaflet tips and recording in midexpiration; an average of three values was taken.

Positron emission tomography

The subject was positioned on the bed of an ECAT 931–08/12, 15 plane positron tomograph (Siemens/CTI) so that the left ventricle lay as close as possible to the centre of the axial and transaxial fields of view. Before performing the emission study, a circular ring source filled with about 2 mCi of ^{68}Ge was used for the blank and transmission data acquisition. Initially, a rectilinear transmission scan was recorded and used as a low resolution *x* ray film to identify the heart profile. A transmission scan was then recorded for a 20 min period in order to measure the attenuation correction coefficients to be used for each line of response of the emission sinogram.

A blood volume scan was obtained using inhaled oxygen-15 labelled carbon monoxide ($C^{15}O$). Four serial venous samples were taken during the $C^{15}O$ scan and counted in a calibrated well counter.[17]

Regional myocardial blood flow was subsequently measured using oxygen-15 labelled water ($H_2{}^{15}O$) from inhaled oxygen-15 labelled carbon dioxide ($C^{15}O_2$) as described elsewhere.[17]

Measurement of myocardial β adrenoceptor density was performed according to a modification of the double injection method of Delforge *et al.*[18] The carbon-11 labelled S-enantiomer of the non-selective, hydrophilic β blocker CGP 12177 (S-[^{11}C]CGP 12177) was prepared as previously described[19] and used as a high affinity radioligand to quantify myocardial β adrenoceptor density. A first dose of S-[^{11}C]CGP 12177 with high specific activity (7·2 (1·9) μg S-[^{11}C]CGP 12177; 155 (33) MBq; specific activity 6·4 (1·9) GBq/μmol) was infused intravenously over 2 min, followed 30 min later by a second dose with low specific activity (34·5 (4·5) μg S-[^{11}C]CGP 12177; 324 (148) MBq; specific activity 2·4 (0·7) GBq/μmol) infused intravenously over 2 min. A 45 frame dynamic emission scan was used to define the temporal and spatial distribution of the tracer in vivo. A single 30 s background frame was acquired before the intravenous infusion of the first dose of S-[^{11}C]CGP 12177. During the 30 min period following the start of the first infusion of S-[^{11}C]CGP 12177, 22 time frames (8 × 15 s, 4 × 30 s, 2 × 60 s, 2 × 120 s, 4 × 150 s, and 2 × 300 s) were acquired. The second infusion of S-[^{11}C]CGP 12177 was then given and scan data were acquired according to an identical sequence of time frames. Nineteen venous blood samples were collected during the S-[^{11}C]CGP 12177 scan for later correction for vascular activity of the tracer.

Sinograms were normalised, corrected for attenuation, and then reconstructed to provide images with a transaxial spatial resolution of 8·4 mm full width at half maximum (FWHM) and a slice thickness of 6·6 mm FWHM. Data

collection and initial processing were performed using dedicated array processors on a Micro Vax 2 computer (Digital Equipment Corporation). The final images were transferred to SUN 3/60 workstations (Sun MicroSystems) for further analysis by use of Analyze image analysis (Mayo Foundation)[20] and Pro-Matlab (MathWorks, Inc) mathematical software packages.

Regional values of blood volume (VB, ml blood/ml region of interest (ROI)) were obtained by relating equilibrium images of the $C^{15}O$ distribution to the radioactive concentrations of venous blood samples during the scan. Corrections were made for radioactive decay and blood density (1·06 g/ml). In each of the five adjacent scan planes being analysed, which encompassed most of the left ventricular myocardium, four different ROI corresponding to anterior, lateral, infero-posterior, and septal myocardium were selected. The mean left ventricular myocardial blood flow was obtained by defining a further ROI which encompassed the whole left ventricle within the five adjacent scan planes. Myocardial blood flow (ml/min/g) for each ROI was calculated by fitting the arterial input (obtained from a left atrial ROI) and tissue time-activity curves from the blood flow scan to a single tissue compartment tracer kinetic model, as previously described.[17] This model includes corrections for the underestimation of tissue activity due to the partial volume effect and the spillover of activity from the left ventricular chamber into the myocardial ROI.

The same ROI which had been used to calculate myocardial blood flow were then applied to the S-[^{11}C]CGP 12177 scan. The myocardial tracer time-activity curves were corrected for the radioactive decay and for the vascular activity using the regional values of blood volume and the radioactive concentrations of blood samples taken throughout the dynamic scan. The sections of the curve corresponding to the two slow phases, which represent S-[^{11}C]CGP 12177 bound to β adrenoceptors, were exponentially extrapolated on the y axis back to the start of the infusions. The β adrenoceptor density was derived from the maximum number of available specific S-[^{11}]CGP 12177 binding sites per g of tissue (B_{max}) in the ROI. B_{max} values were calculated using a modification of the equation derived by Delforge *et al*[18] to take account of the molar content of S-CGP 12177 in both injections.[10] In addition, the values of B_{max} were corrected for the partial volume effect by normalising to the extravascular tissue volume (ml tissue per ml of ROI), obtained by subtracting vascular density image from the normalised transmission scan.[17] To convert the myocardial blood flow and β adrenoceptor density values from units per ml of tissue to units per gram tissue, all final values were divided by 1·04 (myocardial tissue density).

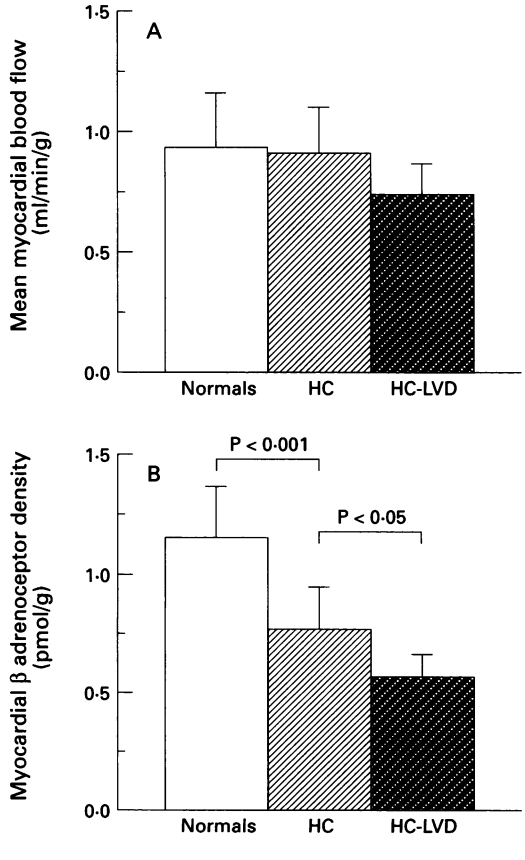

Figure 1 Mean left ventricular myocardial blood flow (panel A) and β adrenoceptor density (panel B) in the normal subjects, and in the patients with hypertrophic cardiomyopathy with preserved systolic function (HC) or with left ventricular dysfunction (HC-LVD)

STATISTICS

All values are expressed as means (SD). Two tailed unpaired Student's *t* tests were used for all between group comparisons except where not indicated. The left ventricular outflow tract gradients between the patients with preserved and impaired systolic function were compared using Wilcoxon's rank sum test. Analysis of variance (ANOVA) was used to compare regional myocardial blood flow and regional β adrenoceptor density in the four different ROI within each group, and to compare perfusable tissue index values among the two patient groups and control subjects. Linear regression analysis was used to examine the relation between myocardial β adrenoceptor density and left ventricular systolic and diastolic function in the patients with hypertrophic cardiomyopathy. A P value < 0·05 was considered statistically significant.

Results

VENTRICULAR ANATOMY AND FUNCTION

All our patients had a clinical history of hypertrophic cardiomyopathy; at the moment of the study some significant differences were evident in the echocardiographic indices between patients with and without preserved systolic function (table). Note the lower thickness of interventricular septum, the increased left ventricular diastolic diameter, and the lower fractional shortening in patients with systolic dysfunction.

MYOCARDIAL BLOOD FLOW

The myocardial blood flow was homogeneously distributed throughout the different regions of the left ventricular wall in the two groups of patients and control subjects, and the mean myocardial blood flow did not differ significantly among the three groups (fig 1A). The perfusable tissue index was 1·05 (0·08) in

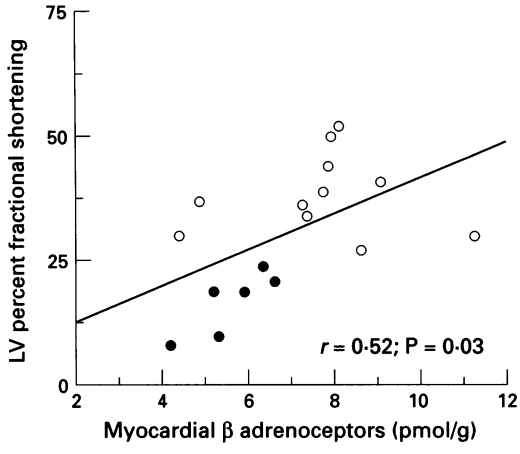

Figure 2 Linear regression analysis of the relation between myocardial β adrenoceptor density and left ventricular fractional shortening in the patients with hypertrophic cardiomyopathy. The filled symbols represent the patients with heart failure and the empty symbols represent the patients with preserved systolic function

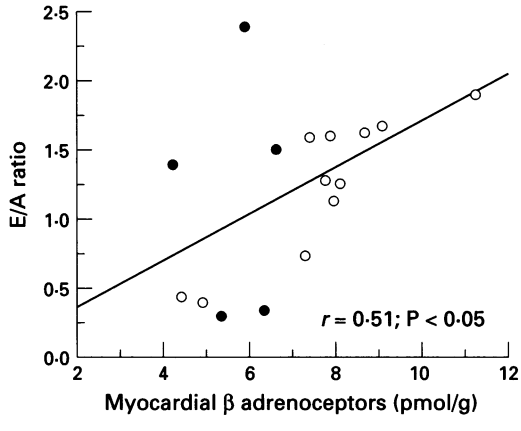

Figure 3 Linear regression analysis of the relation between myocardial β adrenoceptor density and the E/A (transmitral early and late flow velocity) ratio. The filled symbols represent the patients with β adrenoceptor density < 7·00 pmol/g

controls, 1·08 (0·05) in patients with preserved left ventricular function and 1·05 (0·06) in patients with heart failure (P = NS).

MYOCARDIAL β ADRENOCEPTOR DENSITY

There was no significant difference in the myocardial β adrenoceptor densities among the four left ventricular regions within each group studied. Myocardial β adrenoceptor density in all the 17 patients with hypertrophic cardiomyopathy was 7·00 (1·90) *v* 11·50 (2·18) pmol/g in the control subjects (P < 0·01). β adrenoceptor density in the six patients with hypertrophic cardiomyopathy and left ventricular failure was 5·61 (0·88) *v* 7·71 (1·86) pmol/g in the 11 patients with preserved systolic function (P < 0·05: fig 1B). These latter values are 49% and 33% lower than in normal subjects respectively.

MYOCARDIAL β ADRENOCEPTOR DENSITY AND LEFT VENTRICULAR FUNCTION

In the patients with hypertrophic cardiomyopathy, there was a significant correlation ($r = 0·52$; P < 0·05) between left ventricular shortening and myocardial β adrenoceptor density (fig 2). In addition, a positive correlation ($r = 0·51$; P < 0·05) was detected between the β adrenoceptor density and the E/A transmitral flow ratio (fig 3), an index of left ventricular diastolic function.[21]

Discussion

These results lend further support to the hypothesis of an increased sympathetic activity in the heart of patients with hypertrophic cardiomyopathy.[7] Moreover, they underline the strict relationship between the dysfunction of the autonomic control of the heart and systolic and diastolic function of the left ventricle in these patients.

Although it is unlikely that the autonomic dysfunction is the prime mover in the pathogenesis of hypertrophic cardiomyopathy, which seems to be genetically transmitted,[2-5] it might be an important cofactor in the development of the disease. There is strong experimental evidence that myocardial hypertrophy can be induced by direct stimulation of α and β adrenergic receptors on cardiac myocytes, an action which seems to be independent of the haemodynamic load.[22] Furthermore, there is now evidence that a higher level of local myocardial catecholamines is an important determinant of the downregulation of myocardial β adrenoceptors.[11] It has recently been found that myocardial β adrenoceptor density is reduced in patients with hypertrophic cardiomyopathy who have preserved left ventricular systolic function.[10] The results of the present investigation show a more profound reduction of myocardial β adrenoceptor density in patients with hypertrophic cardiomyopathy who have developed impairment of left ventricular systolic function and clinical evidence of heart failure. It is noteworthy that the density of β adrenoceptor is correlated with the systolic and diastolic function of the left ventricle. Reductions in the myocardial β adrenoceptor density of approximately 50% have been reported in patients with heart failure of different causes,[23,24] using in vitro ligand binding to homogenised myocardial biopsy samples. In a recent study, Merlet *et al*,[25] using S-[^{11}C]CGP 12177 and positron emission tomography, reported a 53% decrease in myocardial β adrenoceptor density in patients with dilated cardiomyopathy compared to normal controls. In the latter study, the absolute value of myocardial β adrenoceptor density measured in normal controls is about 40% lower than that measured in our study. Two main technical reasons could account for this difference: (1) the data of Merlet *et al* are corrected for partial volume using echocardiography, which does not account for partial volume effect arising from movement; (2) the equation used by the French group has been modified by us[10] to take into account the amount of cold CGP 12177 which is co-injected with S-[^{11}C]CGP 12177 and results in an additional underestimation of receptor density of the order of 30%. However, several other potential problems may limit the accuracy of the measurement of myocardial β adrenoceptors with S-[^{11}C]CGP 12177 and positron emission tomography. These include the following: (1) Non-specific receptor binding. Non-specific binding of S-[^{11}C]CGP 12177 in rat myocardium is approximately 10%.[26] We have calculated non-specific binding of S-[^{11}C]CGP 12177 to human myocardium (measurements performed during control conditions and following administration of propranolol) to be in the range of 8% to 12%

(unpublished results). It is worth noting, however, that the same problem of non-specific binding would also be encountered with in vitro techniques. (2) Potential transmural differences in receptor densities which cannot be assessed because of the limited spatial resolution of the present positron cameras. (3) Enhanced tissue fibrosis in patients with cardiomyopathy, which might lead to erroneously lower receptor density compared with controls. To account for this problem, the perfusable tissue index (an index of viable tissue) was measured in the three groups (normals and patients with and without heart failure) and found to be comparable, suggesting a lack of significant interstitial fibrosis.

In conclusion, we have confirmed the presence of myocardial β adrenoceptor downregulation in patients with hypertrophic cardiomyopathy with or without signs of heart failure. This study is the first to show that, although a certain degree of β adrenoceptor downregulation can be associated with a preserved systolic function, values lower than 6·5–7·0 pmol/g are almost invariably accompanied by heart failure.

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