Altered Sarcoplasmic Reticulum Ca^{2+}-ATPase Gene Expression in the Human Ventricle during End-Stage Heart Failure

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Abstract

A decrease in the myocardial level of the mRNA encoding the Ca^{2+}-ATPase of the sarcoplasmic reticulum (SR) has been recently reported during experimental cardiac hypertrophy and failure. To determine if such a deficit occurs in human end-stage heart failure, we compared the SR Ca^{2+}-ATPase mRNA levels in left (LV) and right ventricular (RV) specimens from 13 patients undergoing cardiac transplantation (6 idiopathic dilated cardiomyopathies; 4 coronary artery diseases with myocardial infarctions; 3 diverse etiologies) with control heart samples using a rat cardiac SR Ca^{2+}-ATPase cDNA probe. We observed a marked decrease in the mRNA for the Ca^{2+}-ATPase relative to both the 18S ribosomal RNA and the myosin heavy chain mRNA in LV specimens of patients with heart failure compared to controls (-48%, $P < 0.01$ and -47%, $P < 0.05$, respectively). The LV ratio of Ca^{2+}-ATPase mRNA to 18S RNA positively correlated with cardiac index ($P < 0.02$). The RV ratio correlated negatively with systolic, diastolic and mean pulmonary arterial pressures ($P < 0.02$, $P < 0.02$, and $P < 0.01$, respectively). We suggest that a decrease of the SR Ca^{2+}-ATPase mRNA in the myocardium plays an important role in alterations of Ca^{2+} movements and myocardial relaxation reported during human end-stage heart failure. (*J. Clin. Invest.* 1990. 85:305–309.) gene expression • heart failure • human left ventricle • myosin heavy chain mRNA • sarcoplasmic reticulum Ca^{2+}-ATPase mRNA

Introduction

A number of studies have emphasized the importance of alterations in myocardial relaxation that occur during cardiac hypertrophy and failure. Such alterations have been reported in several animal models, and in man they are associated with the pathophysiology of most cardiac diseases such as ischemic heart disease and idiopathic dilated or hypertrophic cardiomyopathies (reviewed in 1). Myocardial relaxation is governed by multiple factors but defects in calcium (Ca^{2+}) movements within the cardiac myocyte seem to play a central role in its alterations. In the failing human myocardium, as in hypertrophied ferret ventricles, prolonged relaxation appears to correlate with changes in intracellular Ca^{2+} handling (2, 3). Furthermore in hypertrophied rabbit ventricles, the tension independent heat of the contraction-relaxation cycle, which has been attributed to the energy cost of Ca^{2+} movements, is depressed (4). Ca^{2+} movements during relaxation include dissociation from troponin C, extracellular extrusion through the sarcolemmal Ca^{2+} pump and sodium/calcium exchange, but is dominated by ATP dependent uptake by the sarcoplasmic reticulum (SR).[1] Most authors agree that the velocity of SR Ca^{2+} uptake is decreased during stable hypertrophy (reviewed in 5) and is further depressed during cardiac failure (6). We and others have recently shown that in rat and rabbit, ventricular hypertrophy is associated with a decreased myocardial level of SR Ca^{2+}-ATPase mRNA and an associated decrease in protein concentration (7–9). This, at least in part, could be responsible for the slower velocity of Ca^{2+} uptake by the SR of these hearts and could account for a delayed rate of myocardial relaxation.

The purpose of the present study was to determine if there were similar changes associated with human heart failure. To answer this question, we have compared the relative levels of SR Ca^{2+}-ATPase mRNA in left and right ventricular specimens from patients (NYHA class IV) undergoing cardiac transplantation with samples from control hearts obtained at the time of organ harvest.

Methods

Patients. Hearts from 6 patients without heart failure and 13 patients undergoing cardiac transplantation were investigated. The six patients without heart failure were accident victims maintained under intensive care as heart donor candidates but whose hearts were ultimately rejected for transplantation, for reasons other than depressed cardiac function. Main clinical characteristics of these six patients are summarized in Table I. Muscle specimens from this group are categorized in this report as controls (C). 13 patients underwent cardiac transplant surgery for chronic end-stage heart failure with the following diagnoses: six had idiopathic dilated cardiomyopathy (D), four had chronic coronary artery disease with at least one myocardial infarction (i), and the remaining three consisted of one partial atrioventricular canal (AVC), one mitral (MR) and one aortic regurgitation (AR). Relevant clinical, catheterization, and echocardiographic data from these pa-

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1. *Abbreviations used in this paper:* AR, aortic regurgitation; AVC, atrioventricular canal; C, control; D, dilated cardiomyopathy; HF, failing hearts; Li, liver; MHC, myosin heavy chain; MR, mitral regurgitation; SR, sarcoplasmic reticulum.

Table I. Main Clinical Characteristics and Ca^{2+}-ATPase Relative mRNA Levels of Six Patients without Heart Failure

Patient No.	Age (yr) and sex	Cause of coma	Cardiovascular abnormalities	Life support duration	Ca^{2+}-ATPase/18S		Ca^{2+}-ATPase/MHC	
					LV	RV	LV	RV
				h				
C_1	31 F	Multiple trauma	No	26	2.27	—	1.82	—
C_2	27 M	Head trauma	No	72	1.17	—	—	—
C_3	58 M	Suicide	Hypertension	48	1.05	—	—	—
C_4	23 M	Head trauma	RBBB + ST depression in V1-V2	72	0.95	—	0.59	—
C_5	40 M	Suicide by gun	No	24	0.90	1.31	0.95	0.73
C_6	17 F	Head trauma	Vegetation on AV valve from IV catheter	96	0.74	—	0.80	—

C, control; LV, left venticle; RV, right ventricle; RBBB, right bundle branch block; AV, atrioventricular; IV, intravenous; ST, segment ST on the electrocardiogram; V1 and V2, corresponding precordial leads on the electrocardiogram.

tients are listed in Table II. Tissue specimens were obtained as approved by the committees for the protection of human subjects at the respective institutions.

Tissue preparation. Hearts were placed in ice-cooled saline immediately after removal and myocardial specimens gathered < 1 h after explantation. A transmural specimen of ~ 500 mg was taken from the medial part of the left ventricular free wall when possible, and midway between the apex and base, at least 2 cm away from the scar in patients with myocardial infarctions. In 1 control and 11 transplanted hearts, a right ventricular sample was also taken from the medial part of the right ventricular free wall. Immediately after sampling, myocardial specimens were blotted dry, frozen in liquid nitrogen, and stored at −80°C until used.

RNA gel analysis. Total cellular RNA was purified using the method of Chirgwin et al. (10). The presence of SR Ca^{2+}-ATPase mRNA was tested with a cDNA probe complementary to the 5′ end (nucleotides +260 to +1446) of the rat cardiac SR Ca^{2+}-ATPase mRNA (11). Conditions for Northern blot analysis, hybridization, and washing were identical to those used by de la Bastie et al. (12), except that the final wash was 0.5× SSC (1× SSC = 0.15 M sodium chloride and 0.015 M sodium citrate), 50°C. Since in rat ventricular hypertrophy, total RNA and mRNA vary in parallel (13), we used a 24 mer synthetic oligonucleotide specific for the rat 18S ribosomal RNA (nucleotides +1046 to +1070) as a control for the quantity of RNA bound to the filters (14). Filters were dehybridized and rehybridized with the 18S probe labeled at the 5′ end with [γ-^{32}P]ATP (Amersham Corp., Arlington Heights, IL) by T4 polynucleotide kinase (Boehringer-Mannheim, Inc., Indianapolis, IN) and diluted with cold oligonucleotide (1/400) to a specific activity of 1–2 × 10^6 dpm/μg. Hybridization conditions were the same as those used for the SR Ca^{2+}-ATPase except that formamide was omitted and replaced by water. Washing conditions were 3× SSC at room temperature. To indicate the relative amount of contractile element mRNA present in total RNA, filters were dehybridized and rehybridized to a myosin heavy chain (MHC) cDNA probe (pMHC 26) (15). Hybridization and washing conditions were those used for SR Ca^{2+}-ATPase, except that the final wash was 0.1× SSC, 50°C. Autoradiography was always performed using Cronex 4 film (Du Pont Co., Wilmington, DE).

Quantification of SR Ca^{2+}-ATPase mRNA. This was performed by dot blot hybridization analysis. The RNA samples were denatured by

Table II. Main Clinical Characteristics and Ca^{2+}-ATPase Relative mRNA Levels of 13 Patients with Heart Failure

Patient No.	Age (yr) and sex	Catheterization			Echography				Ca^{2+}-ATPase/18S		Ca^{2+}-ATPase/MHC	
		PAP	PCWP	CI	LA	LV	Δ%	HW	LV	RV	LV	RV
		mmHg	*mmHg*	*l/m²*	*mm*	*mm*		*g*				
D_1	16 M	30/10/--	15	1.40	40	83/77	7	—	—	—	—	—
D_2	50 F	73/26/49	39	2.90	50	81/69	15	410	0.85	0.76	1.06	0.50
D_3	49 M	38/15/21	14	2.25	44	85/75	12	410	0.70	1.17	0.67	—
D_4	43 M	55/33/41	26	1.90	54	76/65	28	435	0.64	0.74	1.07	0.55
D_5	41 M	44/20/29	19	3.26	50	75/68	10	475	0.62	0.61	0.45	0.52
D_6	40 M	60/30/44	23	2.69	50	68/61	10	360	0.41	0.72	0.51	0.57
I_1	48 M	17/10/12	11	3.43	48	70/60	14	310	0.88	1.59	0.46	0.56
I_2	48 M	90/46/61	35	1.45	50	75/68	9	550	0.42	0.29	0.38	—
I_3	31 M	50/22/32	22	1.90	52	68/58	14	320	0.38	0.70	0.37	0.50
I_4	51 M	73/38/46	34	1.94	33	74/61	17	—	0.34	0.68	0.23	0.52
AVC	36 M	75/30/47	30	4.30	80	75/50	20	515	0.86	0.60	0.57	0.86
MR	24 F	41/20/27	13	2.68	—	64/58	9	315	0.70	0.49	0.37	0.49
AR	53 M	80/55/63	—	2.67	—	—	—	583	0.46	—	0.48	—

D, idiopathic dilated cardiomyopathy; I, ischemic heart disease; AVC, partial atrioventricular canal; MR, mitral regurgitation; AR, aortic regurgitation; PAP, pulmonary arterial pressures (systolic/diastolic/mean); PCWP, pulmonary capillary wedge pressure; CI, cardiac index; LA, left atrium; LV, left ventricle (end diastole/end systole); Δ%, percentage of fractional shortening; HW, heart weight; RV, right ventricle.

heating at 65°C in 15× SSC for 15 min and cooled on ice. Serial dilutions were spotted on nitrocellulose filters using a Schleicher and Schuell minifold apparatus (Schleicher & Schuell, Keene, NH). The filters were then successively hybridized with either the SR Ca^{2+}-ATPase probe or the MHC probe and then with the 18S probe to control for the quantity of RNA bound as described above. After each hybridization, three different exposure times were chosen to obtain densitometric scans in the linear response range of the X-ray film. The contents of SR Ca^{2+}-ATPase mRNA and MHC mRNA relative to 18S RNA were expressed as the Ca^{2+}-ATPase/18S and MHC/18S hybridization signals, respectively, from which was calculated the relative ratio of Ca^{2+}-ATPase mRNA to MHC mRNA. Reproducibility of the procedure, calculated according to Henry (16) on seven pairs of duplicates, was ±7.9% (for example, a ratio of 1.00, ± a range from 0.92 to 1.08).

Statistical analysis. Results were expressed as means ± standard error. The statistical significance of differences between the various groups was determined by one-way analysis of variance and group to group comparisons by the Scheffe F test. Correlations were tested by linear regression analyses using the least square method. The statistical significance threshold was $P < 0.05$.

Results

Specificity of the probes used. Northern blot analysis (Fig. 1 *A*) demonstrated that the probes hybridized as single sharp bands in both control (C) and failing (HF) hearts, showing that our RNAs were not degraded. The sizes of the Ca^{2+}-ATPase and MHC mRNAs (~ 4 and 6.9 kb, respectively) were similar to those previously reported in rat and rabbit (8, 9, 12, 15). Strong signals were also observed for both cardiac RNAs by dot blot analysis (Fig. 1 *B*). By contrast, the signals obtained from rat liver RNA samples (Li) were very weak, indicating that our experimental conditions allowed unambiguous identification and quantification of human cardiac Ca^{2+}-ATPase mRNA.

Ventricular Ca^{2+}-ATPase mRNA levels. The ratio of Ca^{2+}-ATPase mRNA to 18S RNA was 1.18±0.22 in control left ventricles and 0.61±0.06 in failing ones, which represents a decrease of 48% ($P < 0.01$, Fig. 2). The ratio of Ca^{2+}-ATPase mRNA to MHC mRNA in the failing left ventricles was also markedly lower than that in controls (0.55±0.08 vs. 1.04±0.27, −47%, $P < 0.05$), indicating that in the failing human heart, Ca^{2+}-ATPase mRNA decreased not only relative to total cardiac RNA, but also to myocyte specific RNA. The ratios in the right ventricles of the failing hearts were not statistically different from those in the left ventricles (0.76±0.11 and 0.56±0.04 for Ca^{2+}-ATPase/18S and Ca^{2+}-ATPase/MHC, respectively).

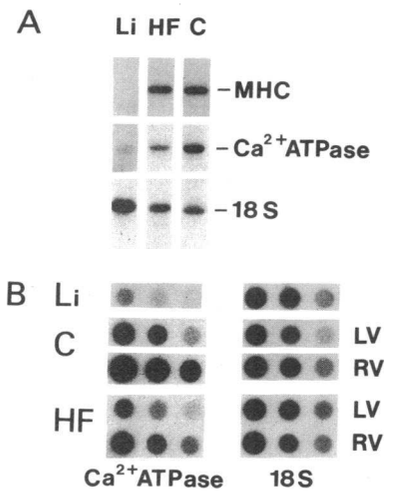

Figure 1. Northern (*A*) and dot blot (*B*) analysis of RNAs from control (*C*) and failing ventricles (HF). MHC, Ca^{2+}-ATPase and 18S indicate hybridization with their corresponding probes. (*A*) 7 μg of left ventricular and liver RNA were loaded on each lane, Li, liver; HF, D_1; C, C_1. (*B*) 5, 2.5, and 1.25 μg of RNA were spotted from left to right, Li, liver; C, C_5; HF, I_4; LV, left ventricle; RV, right ventricle. Note that the intensity of hybridization of the Ca^{2+}-ATPase probe with RNAs from failing ventricles is weaker than that obtained with controls.

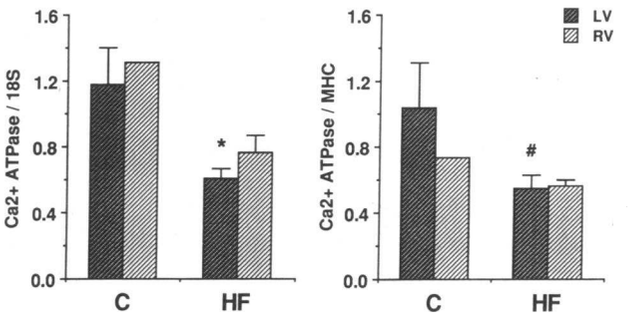

Figure 2. Mean values of the Ca^{2+}-ATPase/18S and Ca^{2+}-ATPase/MHC hybridization signals in the left ventricles (LV) of the control hearts (C) and in the left and right (RV) ventricles of the failing hearts (HF). For control hearts, the RV columns represents the values in the only control RV available. *$P < 0.01$ and #$P < 0.05$ vs. the corresponding value in C.

We found no difference between patients with idiopathic dilated cardiomyopathies and those with coronary artery disease, in either the left or right ventricular Ca^{2+}-ATPase mRNA/18S RNA. By contrast, we found significant correlations between both left or right ventricular Ca^{2+}-ATPase mRNA to 18S RNA ratios and several indices of heart function. The left ventricular ratio correlated positively with cardiac index ($r = 0.70$, $P < 0.02$). The right ventricular ratio correlated with the systolic, diastolic and mean pulmonary arterial pressures ($r = 0.74$, $P < 0.02$; $r = 0.73$, $P < 0.02$; and $r = 0.75$, $P < 0.01$, respectively). Only the right ventricular Ca^{2+}-ATPase/MHC correlated with cardiac index ($r = 0.71$, $P < 0.05$). No correlation was found between the Ca^{2+}-ATPase mRNA to 18S RNA or MHC mRNA and any of the echocardiographic parameters or heart weight.

Discussion

The alterations of systolic myocardial function, which clinically describe cardiac failure have been extensively studied, and changes in myocardial myosin phenotype, myofibrillar ATPase activity, and myofibrillar network density have been suggested as possible mechanisms for these alterations (17, 18). The molecular basis for the abnormal myocardial relaxation has been less well documented. However, a decrease in calcium reuptake by the SR from human failing hearts (reviewed in 5) and indirect proof for this decrease based on altered intracellular calcium movements have been described (2). We now report a decrease in the SR Ca^{2+}-ATPase mRNA content as a first step in the elucidation of the molecular mechanisms of abnormal calcium handling during diastole in human heart failure.

The SR Ca^{2+}-ATPase belongs to a multigene family with a single isoform analogous to that from slow skeletal muscle which is expressed in ventricular and atrial myocardium of rabbit, rat and man (8, 9, 11, 19–22). In rabbit and rat, the

same isoform accumulates under the effects of an increased hemodynamic load (7–9). This isoform is also expressed in the aorta and at low levels in other smooth muscle and nonmuscle tissues, which contain a different isoform identical to the slow skeletal/cardiac one except for an approximately 150 nucleotide addition at the 3′ coding end and a different untranslated region (11, 12, 21, 22). The hybridization of our probe to a single 4-kb band (Fig. 1 *A*) therefore reflects the presence of both slow skeletal/cardiac muscle and smooth/nonmuscle Ca^{2+}-ATPase iso-mRNAs. However, we recently demonstrated by S1 nuclease analysis that smooth/non-muscle Ca^{2+}-ATPase iso-mRNA is barely detectable in normal and hypertrophied rat ventricles (7). Moreover the hybridization signals obtained by Northern blot analysis with RNA isolated from human endothelial cells and rat smooth muscle tissues are respectively 200 and 50 times less than those obtained with similar amounts of RNA from rat ventricles (12, 23). It therefore seems reasonable to assume that the Ca^{2+}-ATPase mRNA hybridization signals obtained from normal and failing hearts originate primarily from hybridization to the slow skeletal/cardiac isoform of the SR Ca^{2+}-ATPase.

We observed a decrease in the SR Ca^{2+}-ATPase mRNA content of failing ventricles relative to controls. This decrease is one of the first indices of human cardiac failure to correlate exactly with results from animal models of heart failure. In rat ventricular hypertrophy the decrease in SR Ca^{2+}-ATPase mRNA content parallels a decrease in Ca^{2+}-ATPase protein concentration, which suggests a decrease in SR Ca^{2+}-ATPase pump density (7, 8). One can hypothesize a similar relationship between the amount of mRNA and protein in human cardiac failure. Consequently, the low Ca^{2+}-ATPase mRNA content that we have demonstrated here should be accompanied by a decrease of the Ca^{2+}-ATPase protein concentration. Such a reduction in the number of SR Ca^{2+} pumps in failing human hearts may account for the prolonged Ca^{2+} transients and the depressed rate of Ca^{2+} uptake by SR vesicles (2, 5, 24).

The absence of any significant difference in Ca^{2+}-ATPase mRNA/18S RNA between idiopathic dilated cardiomyopathic and infarcted myocardial samples suggests that this decrease is not related to a specific etiology. Indeed, we found a positive correlation between the left ventricular ratio and the cardiac index. We also found negative correlations between the right ventricular ratio and the systolic, diastolic, and mean pulmonary arterial pressures. These pressures, which gradually increase in the failing heart first as a result of elevated left ventricular filling pressures and then because of increased pulmonary vascular resistances (25), are considered as a reflection of the severity and duration of the hemodynamic overload. The correlations that we have found suggest that the severity of myocardial hemodynamic overload is an important determinant of the Ca^{2+}-ATPase mRNA content.

Chronic congestive heart failure is associated with an inability of the ventricular myocardium to generate sufficient force to ensure normal cardiac output and with reduced diastolic relaxation, which alters ventricular filling and further compromises cardiac function. Possible alterations in SR function that may result from the changes in Ca^{2+}-ATPase mRNA accumulation reported here may be one of the pathophysiological mechanisms responsible not only for abnormal relaxation but also for altered contractility due to insufficient activation of myofibrillar proteins during systole. In this respect, the decrease in the ratio of the Ca^{2+}-ATPase to the MHC mRNAs could indicate an imbalance between the contraction and relaxation abilities of the failing myocardium. Elucidation of the mechanisms responsible for this reduction in SR Ca^{2+}-ATPase mRNA accumulation in the failing human ventricle is now required.

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