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Alteration of myocardial sarcoplasmic reticulum Ca^{2+}-ATPase and Na^{+}–Ca^{2+} exchanger expression in human left ventricular volume overload ☆

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Abstract

Background: Reduced myocardial contractility is often attributed to altered Ca^{2+} transients and expression of Ca^{2+}-ATPase of the SR (SERCA) and Na+/Ca^{2+}exchanger (NCX) genes.
Aims: To assess myocardial expression of SERCA and NCX protein levels in left ventricular (LV) remodelling due to chronic severe mitral regurgitation (MR).
Methods: Myocardial expression of SERCA/NCX in biopsy specimens obtained during mitral surgery was assessed in 36 MR patients with LV remodelling and plasma neurohumoral/cytokine activation and in four non-failing hearts (NFH).
Results: Myocardial protein levels of SERCA were significantly (20%) lower in the MR group than in NFH group ($p=0.016$). No significant changes in NCX were observed. However, a lack of homogeneity with regard to SERCA/NCX proteins was observed. Moreover, SERCA was negatively correlated with BNP ($r=-0.49$, $p=0.02$), TNFα ($r=-0.68$, $p=0.0005$) and IL-6 ($r=-0.52$, $p=0.02$), whereas NCX was only negatively correlated with TNFα ($r=-0.62$, $p=0.002$).
Conclusions: MR patients showed wide variations in SERCA/NCX protein expression. Myocardial protein levels of SERCA were significantly lower in the MR population. Moreover, a correlation between BNP, cytokines (IL-6, TNFα) and the expression of SERCA/NCX proteins was observed.

Keywords: Calcium; Proteins; Myocardium; Remodelling; Heart Failure; Chronic mitral regurgitation

1. Introduction

Heart failure (HF) is a multifactorial disease, in which impaired cardiac function leads to haemodynamic instability with neurohumoral and cytokine imbalance and to cardiovascular remodelling as a result of multiple phenotype alterations. Myocytes isolated from failing hearts show impaired force development, slower relaxation, and prolongation of action potential duration, which is mainly attributed to altered expression, function and level of phosphorylation of the Ca^{2+}-regulatory proteins.

Cytoplasmic increase in Ca^{2+} activates contractile proteins and causes contraction, whereas Ca^{2+} removal from the cytosol leads to relaxation [1]. Of the numerous calcium regulatory proteins, Ca^{2+}-ATPase of the SR (SERCA) which

Abbreviations: 2D, Two-dimensional Echocardiography; 6MWT, 6-minute walking test; ANG II, angiotensin II; ALD, aldosterone; BNP, brain natriuretic peptide; CS, calsequestrin; EDVI, left ventricle end-diastolic volume index; EF, ejection fraction; ESVI, left ventricle end-systolic volume index; HF, heart failure; HS, healthy subjects; IL-6, interleukin-6; LA, left atrium; LV, left ventricle; MR, mitral valve regurgitation; NA, noradrenaline; NCX, Na^{+}/Ca^{2+} Exchanger; NFH, non-failing hearts; NYHA, New York Heart Association functional class; PRA, plasma renin activity; SE, standard error of the mean; SERCA, Ca^{2+}-ATPase of the sarcoplasmic reticulum; SR, sarcoplasmic reticulum; STNFR1, soluble TNF-α receptor R1; STNFR2, soluble TNF-α receptor R2; TNF-α, tumor necrosis factor α; SPAP, systolic pulmonary pressure.

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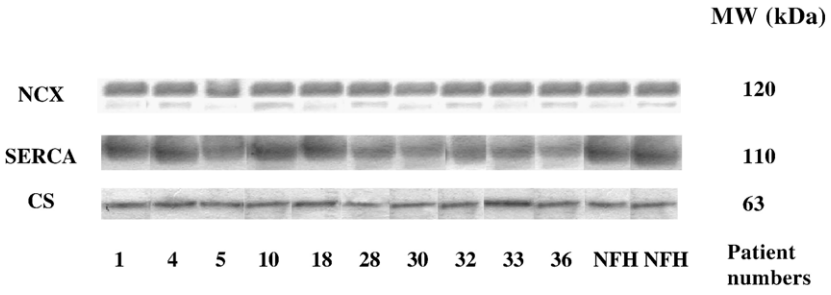

Fig. 1. Representative Western blot of samples from MR patients and NFH. Lines 1–10 — MR-group — Mitral Valve Regurgitation group (samples from patients No 1, 4, 5, etc — corresponding clinical data please see Table 1). Lines 11–12 — NFH — Non-Failing Heart. NCX–Na^{+}/Ca^{2+} Exchanger bands (120 kDa). SERCA–Ca^{+2}-ATPase bands (110 kDa). CS — casequestrin bands (63 kDa).

transports Ca^{2+} into the sarcoplasmic reticulum (SR), together with the Na^{+}/Ca^{2+} exchanger (NCX) which extrudes Ca^{2+} outside the cytosol, both contribute in an important manner to diastolic Ca^{2+} lowering and myocardial relaxation. Moreover, the amount of Ca^{2+} released by the SR depends on its Ca^{2+} load and the Ca^{2+} gradient between the SR and the cytosol, thus there is a general agreement that SERCA determines not only the extent and rate of relaxation, but also the rate and amplitude of contraction [2,3].

Although several studies have been conducted, results obtained at the protein levels of SERCA and NCX expression/function have been controversial [2,4,5]. It has been shown that the reduction in the SERCA expression may sometimes be accompanied by NCX over expression, which compensates for the impaired diastolic Ca^{2+} removal [6]. However, the functional importance of this finding in the human model of heart failure remains controversial.

Thus, the present investigation was performed to assess the interplay between phenotypic changes in SERCA and NCX protein expression in the failing left ventricle (LV) due to mitral valve regurgitation (MR).

2. Methods

2.1. Study population

The study group (MR-patients) was composed of 36 consecutive patients who presented with overt HF secondary to pure, severe, chronic, non-ischaemic MR. All patients were excluded from mitral valve repair, during cardiac surgery for technical reasons. Patients with coronary artery disease or other significant valve diseases apart from tricuspid regurgitation were excluded from the study group.

Two separate control groups were used. Twenty (12 males, 8 females) age-matched healthy subjects (HS), mean age (58± 2.5 years) with no abnormalities on physical examination, medical history, resting ECG or echocardiography, were used as a reference for the assessment of neurohumoral/cytokine activation. Four myocardial biopsies of LV papillary muscle, taken from the non-failing hearts (NFH), of four males aged 22–58 years who died from head trauma and which were rejected for transplantation for technical reasons, were used as a reference for Western Blot analysis.

2.2. Study protocol

The investigation conforms with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all patients participating in the study according to the protocol approved by the Local Ethics Committee.

All patients were referred for mitral valve surgery due to the severity of MR. Before surgery all patients underwent a clinical assessment that included rest ECG, echocardiography, angiography and a 6-minute walking test (6MWT). All patients were stratified by NYHA classification. Venous blood samples were obtained from each subject.

2.3. Rest echocardiography

Two-dimensional (2D), M-mode and colour Doppler transthoracic echocardiography was performed at rest in the left lateral position using commercially available equipment (Vingmed V) and standard views. 2D parasternal long/short axis and apical views were used for assessment of mitral anterior/posterior leaflets. LV volume indexes-diastolic (EDVI) and systolic (ESVI), together with ejection fraction (EF) and left atrial size (LA) were measured/calculated according to the recommendations of the American Society of Echocardiography. Colour Doppler was used for MR measurement by a semiquantitative method (severe MR = if maximal flow disturbance produced by the MR jet/LA area × 100% > 50%). Systolic pulmonary artery pressure (SPAP) was estimated from systolic regurgitant tricuspid flow velocity.

2.4. Plasma neurohumoral and cytokine activation

Neurohumoral assays for plasma renin activity (PRA, $n=34$), angiotensin II (ANG II, $n=34$), aldosterone (ALD, $n=34$), noradrenaline (NA, $n=34$), and BNP ($n=28$), together with the cytokines: interleukin-6 (IL-6, $n=27$), tumour necrosis alpha (TNF-α, $n=29$), soluble TNF-α receptor R1/R2 (sTNFR1/sTNFR2, $n=29$) were performed

Table 1
Clinical and echocardiographic characteristics of MR patients

No	Sex	Age	YIN	AET	NYHA	6MWT	EDVI	ESVI	EF	LA	SPAP	Treatment
01	M	54	15	MD	2	430	93	34	63	51	31	ACEI; DL; βBL;
02	M	61	20	MD	3	300	107	50	53	58	50	ACEI; DL; S; DIG
03	F	69	15	RD	3	350	92	34	52	54	43	ACEI; DL; βBL;
04	F	63	36	MD	3	290	148	38	66	99	36	ACEI; DL; S; βBL;
05	M	62	13	MD	3	340	144	51	52	73	36	ACEI; DL; S; βBL;
06	M	78	37	MD	3	380	121	44	65	60	ND	ACEI; DL; DIG;
07	M	57	20	MD	2	440	109	36	62	55	43	ACEI; DL; βBL; DIG
08	M	71	20	MD	2	450	131	48	63	51	ND	ACEI; DL; βBL;
09	F	56	33	RD	3	200	134	52	60	73	98	ACEI; DL; S; DIG;
10	F	51	40	MD	3	420	134	40	70	60	31	ACEI; DL; βBL; DIG;
11	M	70	4	MD	3	420	115	34	62	55	61	ACEI; DL; S; βBL; DIG;
12	F	40	10	MD	3	405	95	40	57	49	36	ACEI; DL; S; βBL;
13	M	69	28	RD	3	440	98	33	53	65	44	ACEI; DL; S; βBL;
14	F	63	30	MD	2	460	90	31	67	59	36	ACEI; DL; βBL;
15	M	62	2	MD	3	420	106	25	64	52	38	ACEI; DL; βBL;
16	F	62	10	MD	3	ND	120	53	51	54	ND	ACEI; DL; S; βBL;
17	M	59	20	MD	2	420	110	27	70	51	ND	ACEI; DL; DIG
18	M	60	15	RD	3	420	106	43	61	64	ND	ACEI; DL; βBL;
19	M	61	20	MD	2	440	99	34	60	55	59	ACEI; DL; βBL;
29	M	62	10	MD	2	440	99	25	75	47	44	ACEI; DL; DIG
21	F	59	9	MD	3	415	126	51	50	54	ND	ACEI; DL; S; βBL;
22	F	62	22	MD	3	410	125	20	84	78	46	ACEI; DL; S; βBL;
23	M	70	25	RD	3	410	100	32	64	50	ND	ACEI; DL; S; βBL;
24	F	73	30	MD	3	ND	103	36	65	64	ND	ACEI; DL; βBL; DIG
25	M	79	25	MD	3	ND	130	35	66	43	46	ACEI; DL; S; βBL;
26	M	43	31	MD	2	269	131	61	65	70	50	ACEI; DL; βBL; DIG
27	M	73	69	MD	3	213	134	62	53	75	ND	ACEI; DL; DIG; N
28	F	68	20	RD	4	374	279	197	19	62	75	ACEI; DL; S; βBL; DIG; N
29	M	41	20	MD	3	280	164	57	64	52	21	ACEI; DL; S; βBL; DIG
30	F	56	15	MD	3	224	160	81	49	63	ND	ACEI; DL; S; βBL;
31	M	63	19	RD	3	272	156	71	53	61	54	ACEI; DL; S; βBL;
32	F	50	20	MD	3	264	156	87	37	57	ND	ACEI; DL; S; DIG
33	M	51	10	MD	3	239	127	72	45	45	ND	ACEI; DL; S; βBL; N
34	M	60	10	RD	3	272	144	74	36	78	ND	ACEI; DL; βBL;
35	F	67	19	MD	3	160	100	58	49	56	ND	ACEI; DL; βBL;
36	M	70	15	MD	4	208	106	69	35	55	49	ACEI; DL; S; βBL; DIG; N
Mean±SE	M-22 F-14	61±1.5	21±2	MD-28 RD-8	8/26/2 II/III/IV	382±2	124±5	51±5	57±2	60±2	46±3	ACEI-36; DL-36; S-19; βBL-29; DIG - 15; N-4pts

Clinical and echocardiographic characteristics of MR patients.
No — patients number; Sex: M/F — Male/Female; Age (years); YIN — years from initial diagnosis; AET — aetiology; MD — myxomatous degeneration; RD — rheumatic disease; NYHA — NYHA functional class; 6MWT — distance of 6-minute walking test (meters); EDVI — end diastolic volume index (ml/m^2); ESVI — end systolic volume index (ml/m^2); EF — ejection fraction (%); LA — left atrium (mm); SPAP — systolic pulmonary artery pressure (mm Hg).
Treatment: ACEI — ACE Inhibitor; DL Loop Diuretic; S — spironolactone; βBL — β-blocker; D — Digitalis; N — nitrates; pts — no of patients.
ND — not done.

for both MR-patients and HS. For technical reasons neurohumoral/cytokine measurements could not be obtained in all patients.

Neurohumoral assays were performed using commercially available kits: PRA and ALD by radioimmunoassay (DiaSorin s.r.l., Italy), ANG II by RIA (Buhlmann Laboratories AG, Switzerland), after previous reverse-phase extraction of the plasma samples with phenylsilysilca columns, NA by HPLC (BioRad, USA), and BNP by immunoradiometric assay (CIS Bio International, France).

IL-6 and TNF-α were measured by immunoradiometric assay (Biosource Europe S.A) and sTNFR1/sTNFR2 were measured using ELISA (R and D Systems, USA).

2.5. Preparation of cardiac tissue

During mitral valve replacement, samples of LV anterior papillary muscle (avoiding connective tissue, endocardium and vessels) weighing 100–150 mg were obtained in 27 patients. The biopsies were rinsed immediately, blotted dry, frozen in liquid nitrogen and kept at −80 °C until use.

The protein studies were performed on crude homogenates according to Rannou et al. [7]. Protein concentration was determined in triplicate by the method of Lowry et al. [8] The yield of protein per gram of wet weight was 115±8 and 125±5 mg/g for the MR and NFH groups respectively, with no significant differences between groups.

2.6. Western Blot analysis

Equal amounts of protein from all samples were subjected to SDS-PAGE according to the Laemmli method [9] and blotted to PVDF membrane [10]. Membranes were saturated in 5% fat-free milk, and incubated for 2 h with either: monoclonal antibody anti-SERCA 2 (ABR Inc.) diluted in 1:2500 in TBS containing 0.1% Tween 20 or with monoclonal antibody anti-NCX (ABR Inc) diluted in 1:1000 or monoclonal antibody anti-calsequestrin (1:2500). Then, the membranes were incubated for 1 h with a peroxidase-conjugate antibody. The specific bands were revealed by ECL technique on Kodak X-ray films. The linearity of different amounts of each protein to densitometric analysis was confirmed.

Band density quantifications were performed using Molecular Analyst (BioRad) software. Specific bands were seen at 110 kDa with the SERCA antibody, 120 kDa with the NCX antibody and 63 kDa with the calsequestrin (CS) antibody used as an internal standard to normalize the specific signal in each lane (Fig. 1). Each individual value represents the mean of two independent measurements.

2.7. Statistical analysis

The results are expressed as mean±standard error of the mean (SE). For technical reasons protein and neurohumoral/cytokine measurements could not be obtained in all patients. Therefore the number of patients included in each investigation is represented by *n*. Test for normality of each analyzed parameter was performed by Shapiro–Wilk test. Comparisons between groups (MR vs. HS and MR vs. NFH) were performed with chi-square, *t*-Student or Wilcoxon tests. Pearson–Spearman correlation matrices were used to establish univariate correlations between plasma neurohumoral/cytokine activation, echocardiographic data and protein levels.

3. Results

3.1. Study group

The MR group consisted of 36 consecutive patients (22 males, 14 females), mean age 61.5 years (SE±1.5) with severe, chronic non-ischaemic MR due to myxomatous

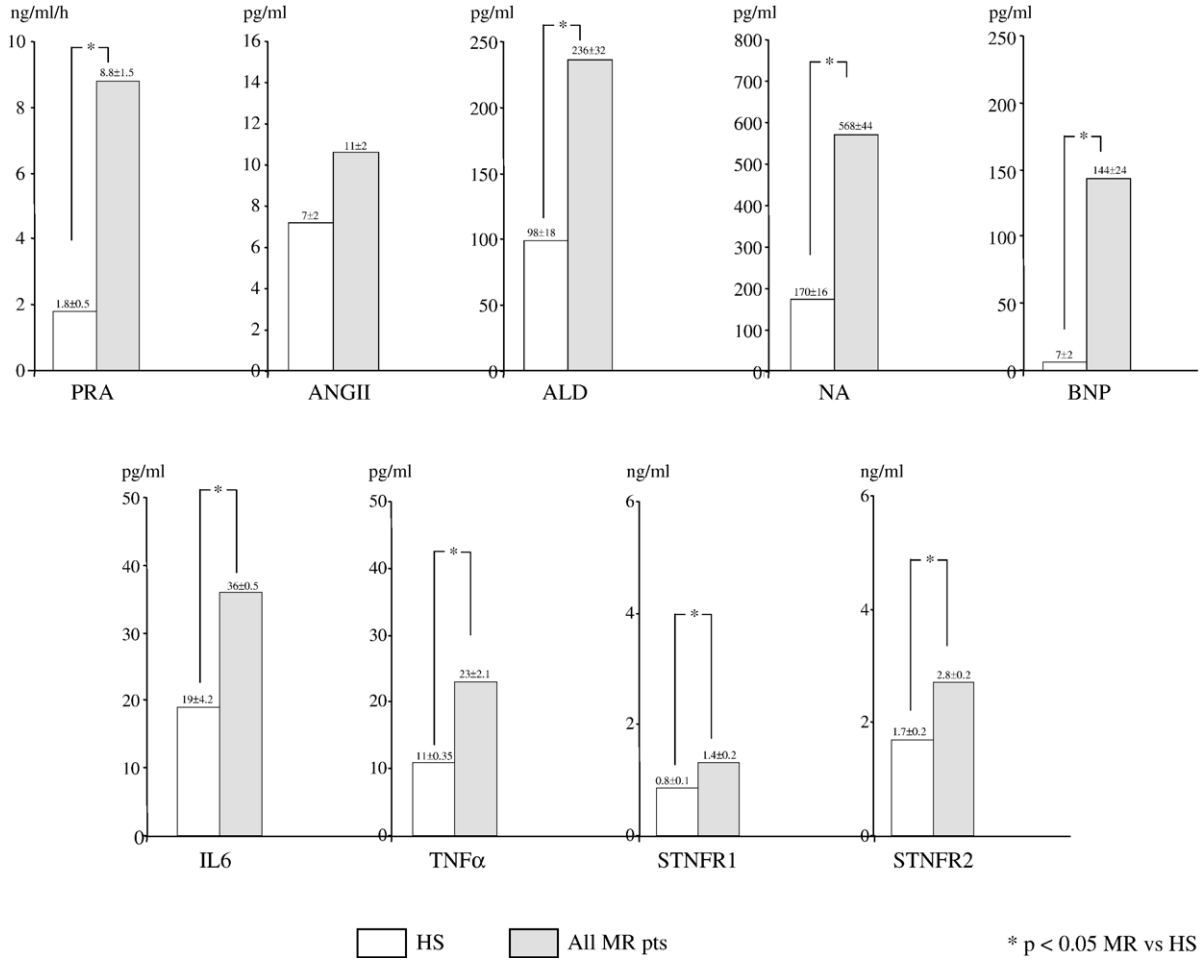

Fig. 2. Plasma neurohumoral/proinflammatory activity for MR patients. HS — healthy subjects; MR — mitral regurgitation patients; PRA — plasma renin activity; ANG II — angiotensin II; ALD — aldosterone; NA — noradrenaline; BNP — brain natriuretic peptide; IL-6 — interleukin-6; TNF-α — tumor necrosis factor α; STNFR1 — soluble TNF-α receptor R1; STNFR2 — soluble TNF-α receptor R2.

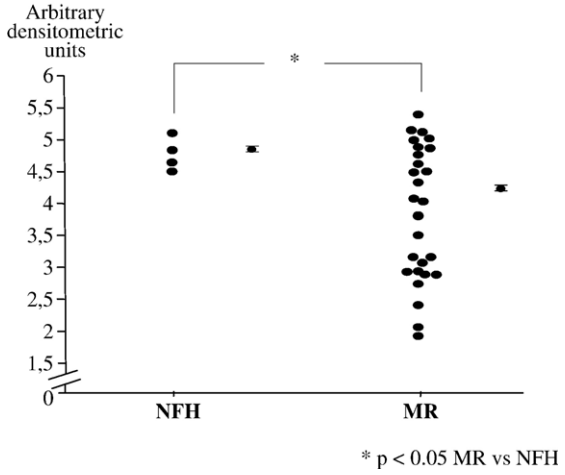

Fig. 3. Protein levels of SERCA normalized to calsequestrin protein levels NFH — non-failing hearts; MR –mitral regurgitation patients.

degeneration (78%) or rheumatic disease (22%). Presenting symptoms of HF were classified according to New York Heart Association (NYHA) functional class (class II $n=8$, class III $n=26$, class IV $n=2$) and reduced distance of 6 MWT (382±2 m). The mean period from the initial MR diagnosis to inclusion in the study was 21 years (±2). The patients had either sinus rhythm (17%) or atrial fibrillation (83%).

The study group presented with LV dilatation (EDVI 124±5 ml/m^2; ESVI 51±5 ml/m^2), together with slightly reduced contraction (EF 57±2%), left atrial enlargement (LA 60±2 mm) and pulmonary hypertension (SPAP 46±3 mm Hg) (Table 1).

In comparison to the HS, MR patients presented with significant neurohumoral activation expressed as an increase in PRA, ALD, NA and BNP; however, there was no difference in ANG II levels.

Evaluation of plasma cytokine levels revealed that MR patients had greater pro-inflammatory activation expressed as IL-6, TNFα and its soluble receptors STNFR1 and STNFR2 compared to the HS group (Fig. 2).

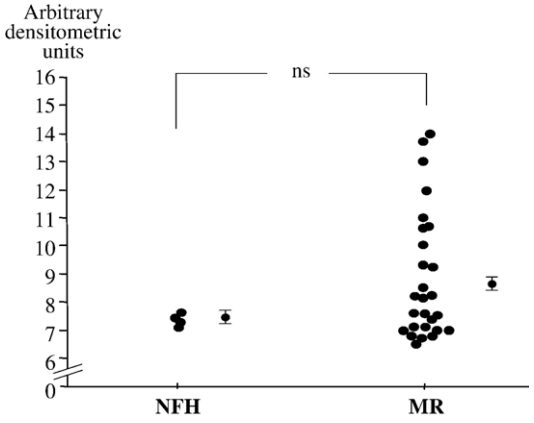

Fig. 4. Protein levels of NCX normalized to calsequestrin protein levels. NFH — non-failing hearts; MR — mitral regurgitation patients.

3.2. Myocardial protein levels in MR group

There were no differences in CS protein levels, which were used as a standard, between the MR and NFH groups (5.16±0.9 vs. 5.2±0.76, densitometric units per milligram of protein).

Myocardial protein levels of SERCA (normalized to calsequestrin) were significantly lower (20% reduction) in the MR group ($p=0.016$) than in the NFH group. However, a lack of homogeneity in the changes in SERCA protein expression was observed. Thus, the changes in SERCA protein levels ranged from normal levels to a decrease of 54% (Fig. 3).

On the other hand, myocardial NCX protein levels (normalized to calsequestrin) showed highly variable changes ranging from normal expression to an increase of 96%, with higher levels in the whole MR group of 20%, but this difference failed to reach statistical significance in comparison to NFH (Fig. 4).

Moreover, as a consequence of changes in NCX and SERCA protein levels, the ratio of NCX to SERCA was significantly increased in the whole MR group by 50% ($p=0.011$) in comparison to NFH (Fig. 5).

3.3. Correlation between echocardiographic parameters and protein levels in MR group

LV diastolic index showed a significant inverse correlation with myocardial protein levels of SERCA ($r=-0.41$, $p=0.04$). Also, significant inverse correlations between both LV diastolic ($r=-0.48$, $p=0.01$) and systolic ($r=-0.45$, $p=0.02$) indices and NCX were found.

3.4. Correlation between protein levels and plasma neurohumoral/cytokine activation in MR group

Of the several neurohumoral activity parameters measured, only BNP plasma level was negatively correlated with SERCA protein levels ($r=-0.49$, $p=0.02$).

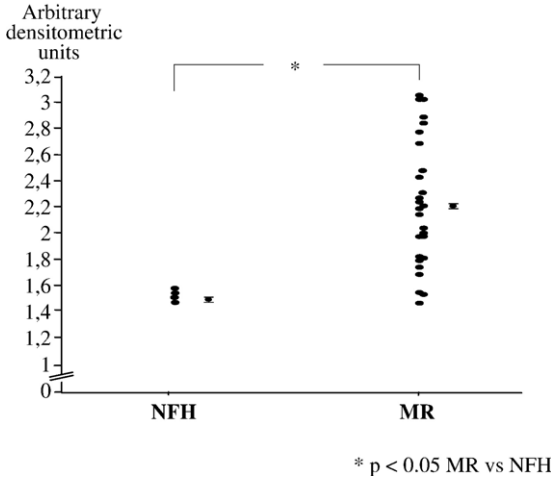

Fig. 5. Ratio of NCX to SERCA Protein Levels. NFH — non-failing hearts; MR — mitral regurgitation patients.

There was also a negative correlation between plasma levels of TNFα and protein levels of both NCX ($r=-0.62$, $p=0.002$) and SERCA ($r=-0.68$, $p=0.0005$). Moreover, plasma IL-6 was significantly inversely correlated with SERCA protein expression ($r=-0.52$, $p=0.02$), but not with NCX.

4. Discussion

4.1. Main findings

The present data confirm the hypothesis that the levels of Ca^{2+} handling proteins (SERCA and NCX) in patients with HF due to volume overload are not uniform. Major observations are as follows: (1) SERCA protein levels in MR patients were lower in comparison to NFH, regardless of the wide variation in SERCA protein expression in patients with MR. Moreover, LV diastolic size was negatively associated with protein levels of SERCA. (2) NCX protein levels in MR patients were highly variable. LV diastolic and systolic size was inversely correlated with NCX abundance. (3) The ratio of NCX to SERCA was significantly increased in MR patients compared to NFH. (4) Plasma BNP level was inversely correlated with SERCA protein levels. (5) Plasma TNFα was inversely related to both SERCA and NCX protein expression, while plasma IL-6 was negatively associated with SERCA protein levels.

These results support the hypothesis that an impaired Ca^{2+} homeostasis of cardiac myocytes is relevant for pathophysiological mechanism responsible for the LV dysfunction.

It has been shown that alterations in myocyte Ca^{2+} regulation are centrally involved in depressed cardiac contractility and contractility reserve, and also in diminished relaxation. Previous studies on animal models of heart failure and on failing human hearts have also shown a marked variability in the abundance in SERCA and NCX proteins levels.

A decrease in SERCA protein levels has been observed in different animal models of heart failure. It has been suggested that the decrease in SERCA RNA levels may serve as a marker of the transition from compensated hypertrophy to failure; however, this theory remains the subject of debate [11].

To our knowledge, changes in the SERCA protein expression in failing human myocardium have been assessed mainly in ischaemic and dilated cardiomyopathy, and evidence in volume overload is limited [11,12].

The alterations in SERCA protein levels in human heart failure are controversial. Although several studies have indicated that mean SERCA protein level was decreased, others have been unable to demonstrate any changes in SERCA protein expression [2,4,11–13]. More recent results have confirmed that a decrease in SERCA protein in human HF is not a universal finding. Our observations are in concordance with the fact that there is a wide variation in SERCA protein levels in failing human hearts. However, in spite of this variation, we observed a reduction of SERCA protein expression in MR patients compared to NFH.

Changes in SERCA protein content have been attributed to the grade of myocardial dysfunction. In a series of studies by Hasenfuss et al., the subgroup with unchanged SERCA protein abundance in comparison to the NFH group, had preserved myocyte systolic function as assessed by force–frequency relation [2]. In addition, decreased SERCA protein levels accompanied by unchanged NCX protein expressions was found to contribute to disturbed myocyte diastolic function [6]. Our results also confirm an association between a pronounced decrease in SERCA protein levels and LV dysfunction expressed as LV diastolic dilation.

We cannot rule out the possible influence of β-blocker treatment on protein levels of SERCA, as reported by Kubo et al. [14], since almost all of our patients were receiving β-blockers.

An additional role in LV remodelling is played not only by the reduction in SERCA protein expression, but also its lower activity, relative increase in protein levels of phospholamban, especially in low phosphorylated state, together with the increased rate of Ca^{2+} leakage from the SR [15].

More recent studies using gene transfer techniques, have confirmed that SERCA gene transfer restores not only SERCA protein expression but also ATPase activity to the non-failing level, which was reflected in the restoration of systolic as well as diastolic function [16,17].

Several studies have indicated that NCX protein abundance is significantly increased in failing human hearts [3,18], few have doubted the over expression of NCX in terminal HF [19]. However, the concept of over expression of the NCX as a general feature in HF appears to be oversimplified, due to evidence of marked variability in NCX protein levels [6]. Importantly, we also found wide heart to heart heterogeneity in NCX protein expression within the study group and failed to demonstrate an increased abundance of NCX in the whole group.

Several findings support the hypothesis that reduced SERCA protein levels disrupt the balance of Ca^{2+} transport between SERCA and NCX, with increased reliance on NCX in failing human myocytes. The work on ventricular muscle strips from failing human hearts by Hasenfuss et al. suggests that in the subset of HF patients, with decreased systolic but relatively preserved diastolic function, there is a combination of a large increase in NCX and a modest decrease in SERCA expression [6]. However, other researchers have observed marked SERCA down regulation without a significant increase in NCX abundance [20]. Finally, a recent study has demonstrated that improved contractility after LV assist device implantation, reflects decrease in NCX protein levels and Ca^{2+} transportation without changes in SERCA protein expression [21].

So even if the associations between diastolic/systolic LV size and abundance of NCX were relatively weak and might be considered as accidental, our results did not confirm the beneficial influence of the increase in NCX protein levels on LV dilatation. These controversial data are difficult to reconcile, as it was also impossible to rule out the potential

influence of other components of remodelling, such as extracellular matrix, cytoskeleton, and contractile proteins, etc, on LV dilatation.

As stated previously, it was also impossible to exclude the potential influence of β-blocker treatment on NCX abundance in our population.

Experimental data regarding the expression of NCX is controversial. Schillinger et al. proved (healthy adult rabbit myocytes) that over-expressed NCX demonstrated systolic contractile dysfunction explained by the decrease in SR Ca^{2+} load as a result of Ca^{2+} loss to the extracellular space by increased forward-mode NCX. [22] However, other authors have shown (in myocytes from transgenic mice over-expressing NCX) a significant improvement in contraction and relaxation together with increased SR Ca^{2+} content as a result of increased Ca^{2+} influx by reverse mode NCX exchange [23]. Of note these controversial data could be related to differences between these animal models in excitation–contraction phenomenon.

It is also important to remember that even if NCX over-expression tends to compensate for impaired Ca^{2+} handling in HF myocytes, it may participate in the occurrence of arrhythmias [24–26].

In spite of the fact that there are doubts about protein levels of SERCA and NCX in human heart failure, the ratio of NCX to SERCA, as proposed by Hasenfuss et al. [6] seems to characterize patients with heart failure more uniformly, despite the heart to heart individual variation in NCX/SERCA abundance, which was also found in our volume overload model.

Regulation of the expression of calcium-regulatory proteins in heart failure is practically unknown. Although it is difficult to differentiate between cause and effect, there seems to be general agreement that mechanical wall stress together with neurohumoral and cytokine activation may contribute not only to the onset and progression of heart failure but also to altered myocardial gene expression.

LV enlargement, accompanied by increased wall stress can be considered a major determinant for reduced SERCA expression [27] and also increased BNP secretion [28]. Moreover, significant relations between ventricular levels of SERCA and BNP mRNA have been shown, [28] by some, but not all, authors. Our results are in accordance with previous studies demonstrating that there is an important negative correlation between the levels of expression of SERCA protein and plasma BNP level.

Sympathetic activation has been identified as a potential stimulus in the regulation of myocardial NCX expression in human HF [26]. However, the present study failed to demonstrate a correlation between NCX protein expression and plasma neurohumoral activity.

Of note, a potential effect on sympathetic activation and the renin-angiotensin system may be concealed by treatment with ACE inhibitors and β-blockers.

In a previous animal study, TNFα infusion was associated with LV remodelling, and, when the infusion was discontinued, there was at least partial reversal of LV remodelling. Moreover, TNFα highly expressed in the failing heart, directly or indirectly via IL-6 exerts potent negative inotropic effects through the change of expression of several genes. At the cellular level, TNFα reduces SERCA protein expression which is associated with the depression and prolongation of the Ca^{2+} transient [29]. Also, IL-6 plays a key role in the development of HF and causes decreased SERCA and increased ANP/BNP gene expression in the cardiac myocytes. The present study confirms the associations between myocardial abundance of SERCA proteins with both cytokines — TNF α and IL-6.

Besides the association between increased levels of TNFα and decreased protein levels of SERCA there was also a negative correlation with NCX levels. Since TNFα has been established as an important marker of LV remodelling, a positive correlation with NCX levels in HF would be expected. However, as stated previously, we found a significant increase in TNFα in our MR patients, but failed to document an increase in NCX protein levels. One could argue that the association between TNFα and NCX was artificial. However, it seems to be important that in our volume overloaded population, of several neurohumoral factors and cytokines assessed, only TNFα correlated with NCX whereas SERCA correlated with several others. We can also speculate that in our population, who were all receiving treatment with ACE inhibitors and mostly with β-blockers, the potential role of both the renin-angiotensin system and sympathetic activation in the regulation of Ca^{2+} cycling proteins may be masked. In this particular situation, we did not observe an increase in NCX protein levels, moreover we cannot rule out the possibility that increased levels of TNFα modified NCX protein expression, supporting the hypothesis of a casual link between myocardial pro-inflammatory cytokine expression and extent of LV remodelling [30].

It is also difficult to reconcile if the correlation between neurohumoral/cytokine activity and protein expression could be attributed to general neurohumoral and cytokine activation typical of HF or be treated as independent factors modifying gene expression.

To sum up, to our knowledge this is the first study showing the high variability in both myocardial SERCA and NCX protein levels in patients with severe chronic mitral regurgitation. Moreover, it seems to be important that different factors such as BNP, TNFα and IL-6 may also influence expression of the Ca^{2+} handling proteins.

5. Limitation

A small population of four LV biopsies from brain injured donors was used as a reference for Western Blot analysis. An additional 10 NFH donors were analysed after the termination of the experiment. This additional analysis (non-published data) confirmed that the protein levels of NCX, SERCA and CS within our small NFH population did not differ significantly from the whole control population.

In our study, the assessment of LV performance was based on echocardiography utilizing load-dependent parameters, which gives only an approximation of LV function. The use of load-independent methods such as tissue Doppler velocities or pressure/volume loops could provide improved LV assessment data and expand the relation to molecular assessment.

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