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Rat cardiac hypertrophy

Altered sodium-calcium exchange activity in sarcolemmal vesicles

Remy Hanf, Isabelle Drubaix, Françoise Marotte and Lionel G. Lelievre

INSERM U127, Université Paris 7, Hôpital Lariboisière, 41 Bd. de la Chapelle, 75010 Paris, France

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The sodium-calcium exchange activity has been studied in sarcolemmal vesicles isolated from rat ventricles hypertrophied by pressure overload. 4 weeks after aortic stenosis the degree of hypertrophy varied from 30 to 70%. The Na^+-dependent $^{45}Ca^{2+}$ influx and efflux were up to 50% decreased and the sensitivity to Ca^{2+} was 13-fold lower in vesicles from hypertrophied heart as compared to those from normal heart. However, the Na^+,K^+-ATPase activity, the orientation of the vesicles and the passive Ca^{2+} permeability were found to be similar in the two heart groups. These results indicate that the sarcolemmal Na^+/Ca^{2+} exchange activity could be qualitatively and/or quantitatively changed in hypertrophied rat heart.

Sarcolemma; Na^+/Ca^{2+} exchange; Hypertrophy; (Rat myocardium)

1. INTRODUCTION

Rat cardiac muscle subjected to pressure overload undergoes adaptational processes that involve qualitative and quantitative modifications in ionic transports in relation to changes in sarcolemmal activities [1-5].

In hypertrophied myocardium, both a significant lengthening of the intracellular Ca^{2+} transient increase and a change in the excitation-contraction coupling associated with lengthening during the action potential have been reported [6,7]. These features might be related to changes in the Ca^{2+} transport mechanisms across the sarcolemma such as slow Ca^{2+} channels, Na^+/Ca^{2+} exchange and/or Ca^{2+}-ATPase.

In our laboratory, it has recently been shown that the density of slow Ca^{2+} channels could not account for these changes in the rat heart hypertrophied by pressure overload [8]. Our objective was to study the Na^+/Ca^{2+} exchange activity in sarcolemmal vesicles isolated from the same experimental model.

Indeed, in addition to its role in calcium metabolism, the Na^+/Ca^{2+} exchanger is electrogenic [9-12]. Thus, it might be a crucial point for changes in the Ca^{2+} pathway across the sarcolemma and/or in the excitation-contraction coupling in hypertrophied heart.

Using the methodology developed by Reeves et al. [12] for measuring Na^+/Ca^{2+} exchange activity with isolated vesicles, our results show a decrease in Na^+/Ca^{2+} exchange activity in sarcolemmal vesicles of homogeneous orientation isolated from hypertrophied rat heart.

Correspondence address: L.G. Lelievre, INSERM U 127, Université Paris 7, Hôpital Lariboisière, 41 Bd. de la Chapelle, 75010 Paris, France

2. MATERIALS AND METHODS

2.1. *Aortic stenosis*

Male Wistar rats weighing 180-200 g were operated on and paired to sham-operated animals of the same weight [5,13]. The rats were killed 4 weeks after the surgery, hearts rapidly excised and perfused with a solution containing: 25 mM KCl, 2 mM $CaCl_2$, 39 mM sodium tetraborate/HCl (pH 7.0). Animals with a rather high degree of hypertrophy (30-70%) have been selected on the basis of a ventricular weight to body weight ratio (mg/g) greater than 3 [5].

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2.2. *Sarcolemma isolation procedure*

The same procedure, derived from that originally described by Pitts [9], was used for both hypertrophied and normal ventricles. Briefly, 4 g of ventricles from 3–5 rats were pooled, finely minced and homogenized in 15 ml of buffer containing 0.6 M sucrose and 10 mM imidazole/HCl (pH 7.0). The homogenization consisted of 2 bursts of 4 s each at half-maximum speed with a Polytron PT 20. The crude homogenate was centrifuged at 500 × *g* for 5 min. The supernatant (S1) was centrifuged at 12000 × *g* for 30 min in a Sorvall SS 34 rotor. The 12000 × *g* pellet (P2) was suspended in 15 ml of buffer A (mM): 100 NaCl, 54 LiCl, 6 KCl, 20 3-(*N*-morpholino)propanesulfonic acid (Mops)/Tris (pH 7.4) and assayed for Na^+/Ca^{2+} exchange activity. The 12000 × *g* supernatant (S2) was diluted with 1.5 vols of 160 mM NaCl, 0.1 mM phenylmethane sulfonyl fluoride and 20 mM Mops/Tris (pH 7.4). This vesicle suspension was completed to 150 ml with the same solution supplemented with 0.25 M sucrose. This fraction was then centrifuged at 160000 × *g* for 90 min. The pellet (P3) resuspended in buffer A at 2–3 mg of protein/ml was frozen in liquid nitrogen and stored at −80°C. This microsomal fraction represented the sarcolemma enriched fraction. The protein content of each fraction was determined by the method of Lowry et al. [14].

2.3. *Characterization of the isolated membrane vesicles*

The Na^+,K^+-ATPase activity, a typical enzymatic marker for sarcolemma, was determined using a coupling assay method as previously described [15].

The orientation of the vesicles was assessed assuming that the ouabain-sensitive Na^+,K^+-ATPase activity observed in the absence of detergent pretreatment was due to 'leaky' vesicles in which both membrane sides were freely accessible to ATP or ouabain. The digitoxigenin-sensitive activity observed in the absence of detergent pretreatment was due to both leaky and inside-out vesicles. Indeed, right-side-out vesicles could not exhibit ATPase activity, since their ATP-binding sites face the interior of the vesicles. A pretreatment with detergent revealed the latent Na^+,K^+-ATPase activity due to impermeable right-side-out vesicles. The pretreatment of the vesicle suspension was performed at 0.05 mg SDS per mg of protein at 20°C for 30 min.

Azide-sensitive ATPase activity reflecting the presence of mitochondrial subparticles was assayed in the presence of 2 mM sodium azide.

2.4. *Measurements of Ca^{2+} uptake*

2.4.1. Total Ca^{2+} uptake.

This was measured at a well defined membrane potential (85 mV) as previously described by Reeves et al. [12]. Vesicle suspensions (P2 or P3) were thawed at 37°C, incubated for 30 min at 37°C and were sodium loaded by passive diffusion with their suspension medium, i.e. buffer A. For each experimental value, 4 μl of the vesicle suspension were placed as a bead on the side of a polystyrene hemolyse tube containing 96 μl of: 160 mM KCl, 0.25 mM EGTA, 2 μM valinomycin (Sigma), 20 mM Mops/Tris, pH 7.4 (K-reaction medium). The free Ca^{2+} concentration was adjusted by adding appropriate amounts of $^{45}CaCl_2$ (Amersham, 100 cpm/pmol). At zero time, the vesicles were mixed with the K-reaction medium. The Ca^{2+} influx was stopped by diluting the reaction mixture after appropriate intervals with 5 ml of ice-cold termination medium: 160 mM KCl, 1 mM $LaCl_3$, 20 mM Mops/Tris (pH 7.4) and measured by harvesting the vesicles on Millipore HAWP 0.45 μm filters. The filters were washed once by an additional 5 ml aliquot of the termination medium. The remaining radioactivity on the filters was counted.

The imposed potential value was calculated as follows [12]: $\Delta\psi = -60 \log [K^+]_i/[K^+]_o$ where $[K^+]_i$ is the K^+ concentration of the equilibration medium (6 mM) and $[K^+]_o$ the final concentration in the reaction medium (160 mM).

2.4.2. Unspecific Ca^{2+} uptake

Except for the reaction medium, the experimental conditions were those described for the total Ca^{2+} uptake. The reaction medium was buffer A + 0.25 mM EGTA + 0.3 mM $^{45}CaCl_2$ + 2 μM valinomycin. No Na^+ gradient across the membrane could train the Na^+/Ca^{2+} exchanger.

2.4.3. Na^+-dependent Ca^{2+} uptake

This uptake due to Na^+/Ca^{2+} exchange activity was the difference between total and unspecific Ca^{2+} uptakes.

2.5. *Measurements of Ca^{2+} efflux*

2.5.1. Total Ca^{2+} efflux

Total Ca^{2+} efflux from passively $^{45}Ca^{2+}$-loaded vesicles was measured after an incubation period of 30 min at 37°C with a medium containing: 160 mM KCl, 1 mM $^{45}CaCl_2$ (100 cpm/pmol) and 20 mM Mops/Tris (pH 7.4). Calcium efflux was initiated by addition of 20 μl of Ca^{2+}-loaded vesicles (2–3 mg/ml) to 480 μl of buffer A. Calcium ions leaving the vesicles were chelated by EGTA. At time points indicated, 50 μl aliquots were filtered and the reaction was stopped as described for the uptake procedure.

2.5.2. Passive Ca^{2+} efflux

This was followed as described above except that KCl replaced NaCl in the reaction medium.

2.5.3. Na^+-dependent Ca^{2+} efflux

Na^+-dependent Ca^{2+} efflux was the difference between total and passive Ca^{2+} efflux.

3. RESULTS AND DISCUSSION

Inasmuch as the sarcolemmal Na^+/Ca^{2+} exchange was electrogenic (more than 2 Na^+ were transported per Ca^{2+}) [9–12], our measurements were carried out at an imposed membrane potential of 85 mV in order to have the same potential difference across the two types of cardiac sarcolemmal vesicles. At this potential, the Na^+-dependent Ca^{2+} uptake in microsomes was maximal and was linear versus time from 0 to 2 s (Hanf, R., unpublished).

The initial velocities of the total Ca^{2+} uptake were found to be 3.25 ± 0.48 and 4.32 ± 0.14

nmol/mg per 2 s ($p \leq 0.01$) in hypertrophied and normal cardiac microsomes, respectively. The unspecific Ca^{2+} level (1.57 ± 0.27 nmol/mg per 2 s) was similar in both groups. As a result, the Na^+-dependent Ca^{2+} uptake in preparations from hypertrophied hearts (1.67 ± 0.48 nmol/mg per 2 s) represented only 60 ± 13% of that in normal hearts (table 1).

The decreased activity observed in microsomes would not be due to a redistribution of membrane-bound Na^+/Ca^{2+} exchange in the different fractions along with the isolation procedure. Indeed, no Na^+-dependent Ca^{2+} uptake could be detected in P2 fractions from either normal or hypertrophied hearts. Furthermore, the same type of results have been observed with microsomes isolated by the unmodified procedure published by Pitts [9]. This method yielded specific Ca^{2+} uptakes in the range of 0.65 ± 0.005 and 0.85 ± 0.06 nmol/mg per 2 s in hypertrophied and sham-operated cardiac preparations, respectively (not shown).

The involvement of mitochondrial membranes known to have a Na^+/Ca^{2+} exchange activity [16] was very unlikely. Indeed, the very low level of azide-sensitive ATPase activity (less than 2% of the total ATP hydrolysis) suggested that, in the two cardiac preparations, the amount of submitochondrial vesicles was too low to interfere with our assay of the sarcolemmal activity.

Three more possibilities had to be excluded before concluding that there was a decreased Na^+-dependent Ca^{2+} uptake in hypertrophied rat heart preparations.

First, the lower activity revealed might be due to a lower yield in sarcolemmal vesicles from hypertrophied hearts. The preparations used in the present work were highly enriched in sarcolemma vesicles with specific activity of Na^+,K^+-ATPase in the range of 103 ± 14 and 100 ± 22 μmol P_i per h per mg of proteins in hypertrophied and normal heart preparations, respectively (table 1). In agreement with the results of Lelievre et al. [5], no significant difference in specific activity could be detected between normal and hypertrophied rat heart vesicles. The protein yields in microsomes were similar in both cardiac preparations (table 1). Thus both similar Na^+,K^+-ATPase activity and protein yields suggested that the same amount of sarcolemmal vesicles was isolated from the two heart groups.

Second, both a difference in the orientation of the vesicles isolated from either normal or hypertrophied hearts and a nonsymmetric Na^+/Ca^{2+} exchange in rat hearts might explain the result observed. This possibility has to be excluded since the orientation of the vesicles was similar in hypertrophied and normal heart preparations. In both groups, 40% of the vesicle population was impermeable and inside-out and 60% was leaky to either ouabain or ATP as revealed by SDS pretreatments. It is likely that this latter subpopulation of vesicles was also leaky to Ca^{2+} and consequently was unable to show any Ca^{2+} transport activity. Whatever the physiological state of the myocardium, no significant population of impermeable right-side-out vesicles could be detected in all the preparations tested. As a conse-

Table 1

Isolation of sarcolemmal vesicles

	Protein (mg/4 g of tissue)	Na^+,K^+-ATPase (μmol $P_i \cdot h^{-1} \cdot mg^{-1}$)	Na^+/Ca^{2+} exchange (nmol $\cdot mg^{-1} \cdot 2\ s^{-1}$)
Sham-operated			
S1 fraction	127 ± 20	16 ± 5 (100%)	undetectable
Microsomes	5.7 ± 0.7	100 ± 22 (28%)	2.77 ± 0.14
Hypertrophied			
S1 fraction	119 ± 2	17 ± 5 (100%)	undetectable
Microsomes	5.1 ± 0.8	103 ± 14 (26%)	1.67 ± 0.48[a]

Values are means ± SE. Results were obtained from duplicate experiments for Na^+,K^+-ATPase and ten experiments for Na^+/Ca^{2+} exchange (at 50 μM free Ca^{2+}) per membrane preparation ($n = 5$). Number in parentheses are recovery of enzyme activity. Value different from control: [a] $p < 0.005$ as determined by the Student's t-test

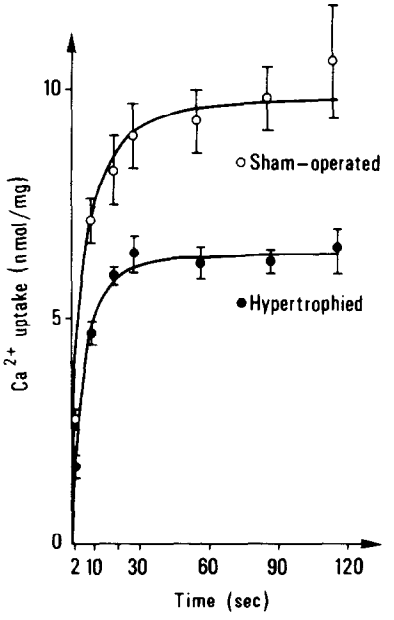

Fig.1. Na^+-dependent Ca^{2+} uptake in sarcolemmal vesicles from hypertrophied (●) and sham-operated (○) rat hearts. The uptake was carried out at 50 μM free Ca^{2+}. The results depicted in this figure are means ± SE from duplicate experiments on five different membrane preparations from either hypertrophied or normal rat hearts. The two curves are significantly different ($p<0.01$ by Student's t-test).

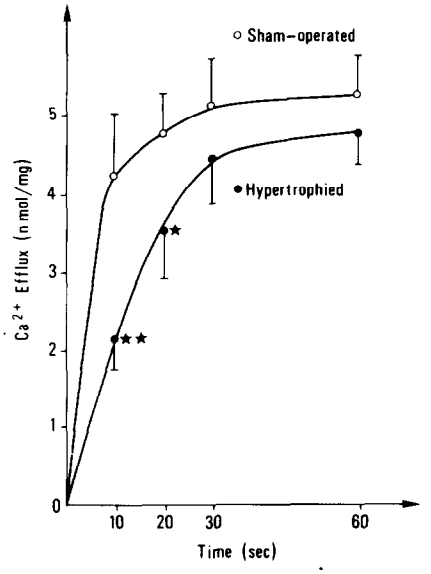

Fig.2. Time course of Na^+-dependent Ca^{2+} efflux. Passively accumulated $^{45}Ca^{2+}$ were exchanged with Na^+ added at time zero. For details see legend to fig.1. Value different from sham-operated rat heart preparations: $^{**}p<0.01$ and $^{*}p<0.05$ by Student's t-test.

quence, in this homogeneous population of vesicles, the Na^+-dependent Ca^{2+} uptake was unidirectional since only sealed inside-out vesicles could exhibit a transport activity.

Third, the passive Ca^{2+} permeabilities of Ca^{2+}-loaded vesicles could be larger in hypertrophied than in normal cardiac preparations. The passive Ca^{2+} loading capacity was similar in Ca^{2+}-loaded vesicles from hypertrophied and normal heart (12.3 ± 0.7 and 14.3 ± 1.7 nmol/mg, respectively). Furthermore, there was no significant difference in the passive Ca^{2+} efflux from Ca^{2+}-loaded vesicles. The passive permeabilities to Ca^{2+} were similar in the two heart groups.

We have also investigated whether the decrease in the initial velocities of Na^+-dependent Ca^{2+} uptakes was associated with a decline in in Ca^{2+}-accumulation capacity (fig.1). It has been shown that in sarcolemmal vesicles from ischemic hearts, there was a decrease in the initial velocity of Na^+/Ca^{2+} exchange without any change in Na^+-dependent Ca^{2+}-accumulation capacity [17]. A steady-state

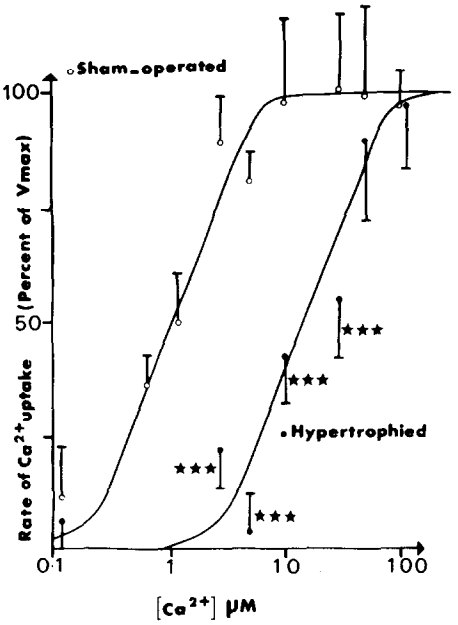

Fig.3. Initial rate of Na^+-dependent Ca^{2+} uptake as function of $[Ca^{2+}]$ from 0.1 to 100 μM. Results are expressed as percent of the respective maximal velocities measured at 100 μM free Ca^{2+} in both heart groups. These velocities are arbitrarily set to 100%. Values represent the means ± SE from 10 independent measurements from three preparations in each heart group. $^{***}p<0.005$.

level of accumulation was achieved within 70 s in both heart groups. In hypertrophied hearts, the plateau value represented 68 ± 15% of that reached with vesicles from normal hearts. Thus, there is a parallel decrease in both the initial velocity of Ca^{2+} uptake and the Ca^{2+} accumulation carried out by Na^+/Ca^{2+} exchange.

A decrease in Ca^{2+} efflux could be expected if the Na^+/Ca^{2+} exchange, a reversible transport system, is affected in cardiac hypertrophy. Addition of a large amount of Na^+ would differently release Ca^{2+} that has been passively accumulated. As illustrated in fig.2, the Na^+-dependent Ca^{2+} efflux was 50% slower in vesicles from hypertrophied hearts than in vesicles from sham-operated rat hearts (2.14 ± 0.40 vs 4.25 ± 0.70 nmol/mg per 10 s, respectively).

Since there was no difference in sarcolemma yields, vesicle orientation and passive Ca^{2+} permeabilities between normal and hypertrophied cardiac preparations, the decreased Na^+-dependent Ca^{2+} influx and efflux truly reflected changes in the Na^+/Ca^{2+} exchange activity. Note that the three criteria chosen to characterize the exchanger, initial velocity of Na^+-dependent Ca^{2+} uptake, Na^+-dependent Ca^{2+} accumulation and efflux, decreased by a similar percentage (about 40%) in rat cardiac hypertrophy.

The molecular basis of these changes occurring in hypertrophy could be quantitative (reduced number of functional exchanger and/or lower turnover) and/or qualitative, i.e. altered sensitivies to Ca^{2+}, Na^+ or membrane potential. The data presented in fig.3 show that the Na^+/Ca^{2+} exchange in hypertrophied heart preparations was 13-fold less sensitive to Ca^{2+} than that in normal hearts. The Ca^{2+} concentrations needed for half-maximal stimulation were 15.8 ± 0.6 and 1.15 ± 0.58 μM in hypertrophied and normal cardiac sarcolemma, respectively.

These qualitative (and maybe also quantitative) alterations of the Na^+/Ca^{2+} exchange will give new insights into the involvement of the ionic transports in heart physiology.

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