Sarcolemmal Alterations in Unloaded Rat Heart after Heterotopic Transplantation

Naoki Makino, MD¹ Paul Ganguly, MD² Vijayan Elimban, PhD³ Naranjan S. Dhalla, PhD³

¹Department of Molecular and Clinical Gerontology, Medical Institute of the Bioregulation, Kyushu University, Oita, Japan

²Department of Anatomy, College of Medicine, Alfaisal University, Riyadh, Kingdom of Saudi Arabia

³Department of Physiology and Pathophysiology, Institute of Cardiovascular Sciences, St. Boniface Hospital Albrechtsen Research Centre, University of Manitoba, Winnipeg, Canada

Address for correspondence Naranjan S. Dhalla, PhD, Institute of Cardiovascular Sciences, St. Boniface Hospital Albrechtsen Research Centre, 351 Tache Avenue, Winnipeg, Canada R2H 2A6 (e-mail: NSDhalla@sbr.ca).

Int J Angiol 2018;27:196–201.

Abstract

Keywords

- cardiac contractility
- sarcolemmal Ca^{2+} pump
- sarcolemmal Na^+ – Ca^{2+} exchange
- membrane phospholipids
- cardiac atrophy
- Ca^{2+} transport in myocardium
- transplanted heart

Following heterotopic transplantation, the rat heart undergoes atrophy and exhibits delayed cardiac relaxation without any changes in contraction and systolic Ca^{2+} transients. Furthermore, the sarcoplasmic reticular Ca^{2+} uptake and release activities were reduced and Ca^{2+} influx through L-type Ca^{2+} channels was increased in the atrophied heart. Since Ca^{2+} movements at sarcolemma are intimately involved in the regulation of intracellular Ca^{2+} concentration, the present study was undertaken to test if sarcolemma plays any role to maintain cardiac function in the atrophied heart. The characteristics of sarcolemmal Ca^{2+} pump and Na^+ – Ca^{2+} exchange activities were examined in 8 weeks heterotopically isografted rat hearts which did not support hemodynamic load and underwent atrophy. Sarcolemmal ATP (adenosine triphosphate)-dependent Ca^{2+} uptake and Ca^{2+} -stimulated ATPase (adenosine triphosphatase) activities were increased without any changes in Na^+ – K^+ ATPase activities in the transplanted hearts. Although no alterations in the Na^+ -dependent Ca^{2+} uptake were evident, Na^+ -induced Ca^{2+} release was increased in the transplanted heart sarcolemmal vesicles. The increase in Na^+ -induced Ca^{2+} release was observed at different times of incubation as well as at 5, 20, and 40 mM Na^+ . The sarcolemma from transplanted hearts also showed higher contents of phosphatidic acid, sphingomyelin, and cholesterol. These results indicate that increases in the sarcolemmal, Ca^{2+} transport activities in unloaded heart may provide an insight into adaptive mechanism to maintain normal contractile behavior of the atrophic heart.

Reverse myocardial remodeling in the failing heart, associated with cardiac atrophy, is considered to be an appropriate strategy for improving heart function upon implantation of left ventricular assist devices.¹ Various interventions such as hypophysectomy, hypothyroidism, adrenalectomy, castration, and protein-free diet have been shown to result in cardiac atrophy.² Likewise, heterotopically isografted rat hearts, which experience a long-term effect of reduced hemodynamic load, have been reported to exhibit a considerable decrease in muscle mass.^{1,3–5} Although no changes in the isometric contraction

phase of the transplanted cardiac muscle were found to occur, the relaxation phase of the papillary muscles was prolonged only at high frequencies of stimulation (> 24 stimuli/minute) and high concentration of calcium (> 2.0 mM Ca^{2+}).⁴ Furthermore, the abilities of sarcoplasmic reticulum to accumulate and release Ca^{2+} were found to be impaired in the transplanted hearts.^{4,5} Augmented Ca^{2+} -influx and increased density of L-type Ca^{2+} channels were also observed and were considered to maintain normal Ca^{2+} transient in the transplanted atrophied heart.⁵ These functional and biochemical observations suggest

that some important changes in calcium movements may occur in the cardiac cell of the atrophied heart. Since sarcolemmal Ca²⁺ pump and Na⁺-Ca²⁺ exchange mechanisms are considered to be intimately involved in the regulation of the intracellular concentration of calcium,⁶⁻⁸ it is possible that the sarcolemmal membrane may also undergo alterations during the development of atrophy in transplanted hearts. Accordingly, it was the purpose of this study to investigate the status of sarcolemma with respect to Ca²⁺ pump and Na⁺-Ca²⁺ exchange activities in atrophied, transplanted myocardium that undergoes hemodynamic unloading. Experiments were also performed to gain some information regarding the mechanisms of sarcolemmal alteration by monitoring the membrane composition with respect to phospholipid and cholesterol content in the transplanted hearts.

Methods

Experimental Animals

The protocol and experimental design for the use of animals in this study were approved by the University of Manitoba Animal Care Committee according to the guidelines of the Canadian Council on Animal Care and the Guide to the Care and Use of Laboratory Animals. Male Lewis inbred rat (Microbiological Associates) weighing 300 to 400 g was used in this study. The animals were anesthetized with pentobarbital (40 mg/kg, i.p.) and the heart from one animal was transplanted in the abdominal cavity of another rat for retrograde perfusion of coronary arteries from the aorta of the recipient according to the procedure described previously.²⁻⁴ The details for preparing this heterotopically isografted heart, as well as other characteristics of this experimental model have been outlined earlier.⁴ After a period of 4 to 6 weeks, the transplanted as well as the host (control) animals were transported from Ottawa to Winnipeg where these were maintained for approximately 4 weeks in the Animal Care Facility of the St. Boniface Hospital Research Centre before use for biochemical studies. The animals were sacrificed after pentobarbital (40 mg/kg, i.p.) anesthesia and hearts were removed. The ventricles were frozen in the liquid N₂ and stored at -70°C for biochemical analysis. Since the sham operated control hearts without transplant were found not to differ from the host hearts, the former group was not used for comparison.

Preparation of Sarcolemma

Sarcolemmal membranes were isolated from the ventricular tissue according to the method outlined by Pitts.⁹ Three to four hearts from control and transplanted groups were used for isolating the membrane preparations. Briefly, the ventricular tissue was homogenized in 4 volumes of 0.6 M sucrose, 10 mM imidazole-HCl (hydrochloric acid), pH 7.0, with a polytron PT-20 (5 × 20 second; dial setting 5) and centrifuged at 12,000 × g for 30 minutes. The supernatant was diluted with 5 volumes of 160 mM KCl (potassium chloride), 20 mM morpholinopropane-sulfonic acid (MOPS), pH 7.4 (KCl-MOPS solution) and centrifuged at 96,000 × g for 60 minutes. The pellet was resuspended in KCl-MOPS solution, layered over a 30% sucrose solution containing 0.3 M KCl, 50 mM sodium

pyrophosphate, 0.1 M Tris-HCl, pH 8.3, and centrifuged at 95,000 × g for 90 minutes. The band at the sample-sucrose interface was recovered, diluted with 3 volumes of KCl-MOPS solution and centrifuged at 100,000 × g for 30 minutes. The pellet was resuspended in 160 mM NaCl (sodium chloride), 20 mM MOPS, pH 7.4, at a concentration of approximately 2 mg protein/mL. The purity of this membrane preparation was checked by measuring the marker enzyme activities according to the procedure described elsewhere.¹⁰

Measurement of Ca²⁺ Pump Activities

Sarcolemmal vesicles (10–15 µg protein) were preincubated at 37°C in a medium (500 µL) containing 160 mM KCl, 20 mM Tris-MOPS, pH 7.4, 2 mM MgCl₂ and the required amounts of ⁴⁵Ca-EGTA (ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid) to produce 10 µM concentration of free Ca²⁺. Total Ca²⁺-uptake was initiated in the presence of 2 mM Tris-ATP (pH 7.4) whereas nonspecific Ca²⁺-binding was determined in the absence of ATP. After 1 min of the reaction, an aliquot of 100 µL was immediately filtered through Millipore filters (0.45 µm) and washed with 1 mL of ice cold 160 mM KCl, 20 mM Tris-MOPS and 1 mM LaCl₃ (lanthanum chloride), pH 7.4. The radioactivity remaining on the dried filter was determined by liquid scintillation and the nonspecific Ca²⁺ binding in the absence of ATP was subtracted from the total Ca²⁺ to calculate the ATP-dependent Ca²⁺ uptake. This technique is essentially similar to that described by Caroni and Carafoli.¹¹ For the Ca²⁺-stimulated ATPase assay,¹² sarcolemmal membranes (approximately 25 µg) were preincubated at 37°C in 1 mL medium containing 160 mM, 5 mM MgCl₂ (magnesium chloride), 5 mM NaN₃ (sodium azide), 20 mM Tris-MOPS, pH 7.4, for 10 minutes. Total ATPase and Mg²⁺ ATPases activities were determined for 5 minutes by measuring the hydrolysis of ATP (2 mM) in the presence and absence of 10 µM free Ca²⁺ respectively; when Mg²⁺ ATPase was determined, 0.2 mM EGTA was also added in the incubation medium. The Ca²⁺-stimulated ATPase activity was calculated by subtracting Mg²⁺ ATPase activity from the total ATPase activity.

Measurement of Na⁺-Ca²⁺ Exchange Activities

The Na⁺-Ca²⁺ exchange activities in the sarcolemmal vesicles were measured out according to the procedures described by Reeves and Sutko¹³ with some modifications. For studying Na⁺-dependent Ca²⁺ uptake, the vesicles were suspended in 160 mM NaCl, 20 mM Tris-MOPS, pH 7.4, and incubated at 37°C for 60 minutes. These Na⁺-loaded vesicles (10–15 µg protein) were diluted 1:50 in 160 mM KCl containing 20 mM Tris-MOPS, pH 7.4, 10 µM CaCl₂ (calcium chloride) in a total volume of 500 µL and incubated at 37°C. Samples of 100 µL were filtered through Millipore filters (0.45 µm) and washed with 1 mL of ice cold 160 mM KCl, 20 mM Tris-MOPS, pH 7.4, and 1 mM LaCl₃. The radioactivity on the dried filters was counted for determining the total Ca²⁺ uptake activity. Nonspecific Ca²⁺ uptake in these experiments was measured by suspending the Na⁺-loaded vesicles in the above medium except 160 mM KCl was replaced by 160 mM NaCl; this value was subtracted from the total Ca²⁺ uptake to obtain Na⁺-dependent Ca²⁺-uptake activity. To measure Na⁺-induced

Ca^{2+} release, the sarcolemmal vesicles were loaded with Ca^{2+} as described for the Na^+ -dependent Ca^{2+} uptake for 30 minutes. Ca^{2+} efflux was initiated by diluting the incubation mixture into an equal volume of solution containing different concentrations of NaCl or KCl, 20 mM Tris-MOPS, pH 6.4, and 1 mM EGTA; the EGTA used in the incubation medium blocked Ca^{2+} uptake. The content of Ca^{2+} remaining in the vesicles at a desired time was determined by filtering 100 μL aliquot through Millipore filter as described above. Na^+ -induced Ca^{2+} release was calculated by subtracting Ca^{2+} contents of vesicles exposed to NaCl from that exposed to KCl.

Determination of Phospholipid and Cholesterol Contents

The phospholipid composition of the sarcolemmal preparations was determined by extracting phospholipids in a chloroform:methanol solution (2:1), separating phospholipid by thin layer chromatography and measuring inorganic phosphate (Pi) through spectrophotometer according to the procedure described elsewhere.¹⁴ The cholesterol content in the membrane was determined by cholesterol esterase method using cholesterol kit reagent of Sigma Chemical, St. Louis, MO.

Statistical Analysis

Statistical analysis of the data was performed by using the student's *t*-test and a *p*-value < 0.05 was taken to reflect a significant difference.

Results

Characterization of the Membrane Preparation

Although ventricular weight in the transplanted hearts was decreased markedly in comparison to the control hearts, no significant difference (*p* > 0.05) in sarcolemmal yields was

Table 1 Ventricular weights, sarcolemmal yields and marker enzyme activities of control and transplanted rat hearts

	Control	Transplanted
Ventricular weight (g)	0.95 ± 0.04	0.43 ± 0.06 ^a
Sarcolemmal yield (mg/g)	0.98 ± 0.10	1.12 ± 0.09
Na^+ - K^+ ATPase ($\mu\text{mol Pi/mg/h}$)	25.7 ± 3.12	27.0 ± 2.61
Purity factor	18.2 ± 1.13	17.6 ± 1.24
Ouabain sensitive Na^+ - K^+ ATPase ($\mu\text{mol Pi/mg/h}$)	2.5 ± 0.18	2.7 ± 0.31
Cytochrome C oxidase (nmol/mg/min)	43 ± 4.12	48 ± 5.14
NADPH cytochrome C reductase (nmol/mg/min)	4.8 ± 0.36	4.1 ± 0.29

Abbreviations: ATPase, adenosine triphosphatase; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

Note: Values are means ± SEM of five experiments. Purity factor was calculated as the ratio of Na^+ - K^+ ATPase activities in sarcolemma and heart homogenate. Ouabain sensitive Na^+ - K^+ ATPase activity was determined in the presence of 1 mM ouabain.

^aSignificantly different from control (*p* < 0.05).

evident in two groups (►Table 1). The activities of Na^+ - K^+ ATPase in both control and experimental group preparations were approximately 18-fold enriched with respect to the heart homogenates and were not different from each other. Ouabain (1 mM) was found to inhibit Na^+ - K^+ ATPase activities in sarcolemmal vesicles by approximately 10%; however, the enzyme activities in both control and experimental preparations were inhibited completely when the sarcolemmal vesicles were treated with 0.2 mg/mL deoxycholate. On the basis of ouabain sensitive and total Na^+ - K^+ ATPase activities in the sarcolemmal preparations, it appears that approximately 90% of the membrane vesicles exhibited inside-out orientation and there was no difference in the degree of sidedness of the control and transplanted preparations (►Table 1). The data on cytochrome C oxidase and reduced nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome C reductase indicated minimal but equal extent of contamination by mitochondria and fragments of the sarcoplasmic reticulum in both sarcolemmal preparations, respectively (►Table 1). Furthermore, K^+ -EDTA (ethylenediaminetetraacetic acid) activity, which is a marker for myofibrils,⁴ was not detectable in control and experimental members.

Sarcolemmal Ca^{2+} Pump Activities

The data shown in ►Table 2 indicate that Ca^{2+} -stimulated ATPase and ATP-dependent Ca^{2+} -uptake activities in the transplanted membranes were approximately 50% higher than the control values. No difference in the Mg^{2+} ATPase and nonspecific Ca^{2+} -binding activities under the same experimental conditions was evident in the control and transplanted sarcolemmal vesicles. Control values for ATP-dependent Ca^{2+} uptake and Ca^{2+} -stimulated ATPase activities are similar to those reported elsewhere.¹² The Ca^{2+} -stimulated ATPase activities in control and experimental preparations were inhibited by 4 μM vanadate by 75 to 80% of the initial values. It should be pointed out that 4 μM vanadate is known to have no effect on the sarcoplasmic reticular Ca^{2+} -stimulated ATPase.¹⁵

Table 2 Ca^{2+} pump activities in sarcolemmal preparations from control and transplanted rat hearts

	Control	Transplanted
Mg^{2+} ATPase ($\mu\text{mol Pi/mg/h}$)	125.8 ± 6.57	131.2 ± 7.42
Ca^{2+} -stimulated ATPase ($\mu\text{mol Pi/mg/h}$)	6.67 ± 0.42	10.14 ± 0.85 ^a
ATP-dependent Ca^{2+} uptake (nmol/mg/min)	15.2 ± 1.13	21.47 ± 1.81 ^a
Nonspecific Ca^{2+} binding (nmol/mg/min)	2.7 ± 0.36	2.1 ± 0.41

Abbreviations: ATP, adenosine triphosphate; ATPase, adenosine triphosphatase.

Note: Values are means ± SEM of four experiments.

^aSignificantly different from control (*p* < 0.05).

Sarcolemmal Na⁺-Ca²⁺ Exchange Activities

In one series of experiments, Na⁺-dependent Ca²⁺ uptake and nonspecific Ca²⁺-binding were measured at different times of initiating the reaction. The data in ►Fig. 1 show no difference in the Na⁺-dependent Ca²⁺-uptake activities between control and transplanted hearts. Nonspecific Ca²⁺ binding under these experimental conditions varied between 4 to 6 nmol Ca²⁺/mg protein and showed no significant difference between two groups. In one experiment, Na⁺-dependent Ca²⁺-uptake activities in sarcolemmal vesicles were studied at 5 to 60 μM concentrations of Ca²⁺ but no changes were seen between control and experimental groups. On the other hand, Na⁺-induced Ca²⁺ release was more in the transplanted heart sarcolemmal vesicles at different times of initiating the release by Na⁺ in comparison to the control (►Fig. 1). Na⁺-induced Ca²⁺ release from the

Table 3 Na⁺-induced Ca²⁺ release by different concentrations of Na⁺ from the Ca²⁺-loaded sarcolemma vesicles prepared from control and transplanted rat hearts

Na ⁺ (mM)	Na ⁺ -induced Ca ²⁺ release (nmol/mg/min)	
	Control	Transplanted
5	9.3 ± 0.71	15.2 ± 1.14 ^a
20	19.1 ± 0.92	25.7 ± 1.82 ^a
40	26.7 ± 0.81	32.6 ± 0.75 ^a
80	33.9 ± 1.12	35.4 ± 1.16

Note: Values are means ± SEM of four experiments. Sarcolemma vesicles were loaded with Ca²⁺ by incubating with 10 μM Ca²⁺ for 3 min according to the method described for the Na⁺-Ca²⁺ exchange mechanism. Ca²⁺-release was induced by exposing these vesicles to different concentration of Na⁺ for 1 min.
^aSignificantly different from control values (*p* < 0.05).

Fig. 1 Time course of Na⁺-dependent Ca²⁺ uptake (A) and Na⁺-induced Ca²⁺ release (B) in sarcolemmal vesicles from control (■) and transplanted (●) rat hearts. The concentration of Ca²⁺ was 10 μM for uptake studies whereas that of Na⁺ was 20 mM for release studies. Nonspecific Ca²⁺ binding for the control and experimental preparations varied between 4 to 6 nmol Ca²⁺/mg under the conditions employed for the measurement of Na⁺-Ca²⁺ exchange activities; this value was subtracted from total Ca²⁺ uptake to calculate Na⁺-dependent Ca²⁺ uptake activities. Each value is a mean ± Standard Error of the Mean of five experiments. *significantly different from the control value (*p* < 0.05).

experimental preparations was also higher than the control values when monitored by using different concentrations of Na⁺ (5–40 mM) except 80 mM Na⁺ (►Table 3). It should be pointed out that the control values for Na⁺-dependent Ca²⁺ uptake and Na⁺-induced Ca²⁺ release are comparable to those reported earlier.¹⁶ In two experiments, sarcolemmal vesicles were loaded via ATP-dependent Ca²⁺ mechanism as described earlier for 5 minutes and Na⁺-induced Ca²⁺ release was initiated by 40 mM Na⁺. ATP-dependent Ca²⁺-uptake values were 28.5 and 36.8 nmol Ca²⁺/5 minutes and the Na⁺-induced Ca²⁺ release values were 22.6 and 31.4 nmol Ca²⁺/min in the control and transplanted heart sarcolemmal preparations (*n* = 2), respectively.

Sarcolemmal Phospholipid and Cholesterol

To gain some information regarding sarcolemmal changes in transplanted hearts, the phospholipid composition and cholesterol content of the membrane preparations employed, in this study, were examined. The data in ►Table 4 indicate no alteration in the composition of any major phospholipids except that sphingomyelin and phosphatidic acid contents were increased in the transplanted membranes. Furthermore, cholesterol content and cholesterol/phospholipid ratio in the experimental preparations were markedly higher than those in the control membranes (►Table 4).

Discussion

In this study, we have shown that ATP-dependent Ca²⁺-uptake and Na⁺-induced Ca²⁺-release activities in sarcolemmal vesicles were increased in transplanted atrophied rat hearts. These changes do not appear to be due to any alterations in the permeability characteristics of the membrane vesicles because the nonspecific Ca²⁺ binding of transplanted membranes was not different from the control. In fact, the augmented sarcolemmal Na⁺-induced Ca²⁺-release activity seems to be due to changes at some specific site because Na⁺-dependent Ca²⁺ uptake was not altered in the experimental preparations. It should be mentioned that electrophysiological studies have

Table 4 Phospholipids composition, cholesterol content and cholesterol to phospholipid ratio of sarcolemma from control and transplanted rat hearts

	Control	Transplanted
A. Phospholipids (nmol Pi/mg)		
Phosphatidylcholine	204 ± 7.1	190 ± 10.4
Lysophosphatidylcholine	3.7 ± 0.6	5 ± 0.8
Phosphatidylethanolamine	143 ± 9.3	144 ± 12.1
Spingomyelin	53 ± 2.2	76 ± 4.1 ^a
Phosphatidylserine	40 ± 2.6	48 ± 3.4
Phosphatidylinositol	16 ± 1.7	21 ± 1.9
Diphosphatidylglycerol	23 ± 2.6	30 ± 3.4
Phosphatidic acid	3.8 ± 0.5	6.5 ± 0.7 ^a
Unidentified phospholipids	2.5 ± 0.8	4.6 ± 1.7
Total phospholipids	489 ± 13.1	525 ± 16.3
B. Cholesterol (nmol/mg)		
	126 ± 4.9	339 ± 21.7 ^a
C. Cholesterol: Phospholipid (molar ratio)		
	0.258 ± 0.025	0.645 ± 0.036 ^a

Note: Values are means ± SEM of five experiments.
^aSignificantly different from control ($p < 0.05$).

also shown unaltered density of the Na^{+} - Ca^{2+} exchange current in atrophied heart.⁵ However, several studies have reported changes in the Na^{+} -dependent Ca^{2+} uptake and Na^{+} -induced Ca^{2+} release activities independent of each other under a variety of different experimental conditions.^{10,16-21} On the other hand, the observed increase in ATP-dependent Ca^{2+} uptake activity may be due to higher Ca^{2+} -stimulated ATPase activity in the transplanted heart, since this enzyme is considered to serve as Ca^{2+} pump in the sarcolemmal membrane.⁸ Such changes in the sarcolemmal Ca^{2+} transport system may be considered as an adaptive mechanism for maintaining the intracellular concentration of Ca^{2+} within normal range because Ca^{2+} influx through L-type Ca^{2+} channels is increased⁵ and Ca^{2+} uptake by sarcoplasmic reticulum is reduced^{4,5} in the transplanted atrophied heart. Increased sarcolemmal Ca^{2+} pump activity has also been observed in the hypothyroid atrophied rat heart.²²

It is unlikely that the high Na^{+} - Ca^{2+} exchange and Ca^{2+} pump activities in transplanted sarcolemma are due to any differential cross contamination because the purity factor with respect to Na^{+} - K^{+} ATPase activity in the experimental preparation was similar to that for the control membranes. Furthermore, marker enzyme activities indicated minimal but equal extent of cross contamination by mitochondria, sarcoplasmic reticulum, and myofibrils in both preparations. No difference in the sidedness of membrane vesicles from control and transplanted hearts was apparent because the sensitivity of Na^{+} - K^{+} ATPase to ouabain was same in both preparations. On the other hand, changes in the sarcolemmal phospholipid composition may explain the observed alterations in Na^{+} - Ca^{2+} exchange and Ca^{2+} pump activities because sphingomyelin and phosphatidic contents were increased in membranes from the atrophied hearts. Although the significance of changes in sphingomyelin content with respect to alterations in the

functions of sarcolemma is not clear at present, phosphatidic acid content has been shown to increase in sarcolemma from hypertrophied hearts exhibiting high Na^{+} -induced Ca^{2+} release and ATP-dependent Ca^{2+} -uptake activities.¹⁰ Since cholesterol/phospholipid ratio is believed to be a gauge of membrane fluidity,²³ it is possible that the observed changes in the sarcolemmal membrane activities in transplanted hearts may also be due to the observed increase in the cholesterol/phospholipid ratio. Although this study did not examine the status of gene expressions for the Ca^{2+} pump and Na^{+} - Ca^{2+} exchange proteins, it appears that the observed changes in their activities may be due to remodeling of the sarcolemmal membrane with respect to its phospholipid content.

In view of the role of sarcolemmal ATP-dependent Ca^{2+} -uptake and Ca^{2+} -stimulated ATPase activities in lowering the cytoplasmic concentration of Ca^{2+} in the myocardium,⁶ increased Ca^{2+} pump activities in the transplanted preparations can be seen to increase Ca^{2+} efflux from the cardiac cell. Likewise, if it is assumed that Na^{+} - Ca^{2+} exchange in the myocardial cell is operating in the mode for Ca^{2+} efflux,^{8,24} then the observed increase in this mechanism can be seen to favor the removal of Ca^{2+} from the transplanted myocardium. Such an alteration in the sarcolemmal function may be adaptive in nature because Ca^{2+} pump mechanism in the sarcoplasmic reticulum, which is considered to play a major role in lowering the cytoplasmic Ca^{2+} concentration,^{6,8} has been shown to be impaired in the transplanted atrophied hearts.^{4,5} Augmentation of the mechanisms in sarcolemma for lowering the cytoplasmic concentration of Ca^{2+} and depression of those at the sarcoplasmic reticulum have also been reported for the aging myocardium²⁵ and stable cardiac hypertrophy.^{10,26} Such differential changes in Ca^{2+} transport activities at the sarcolemma and sarcoplasmic reticulum may explain the ability of cardiac muscle from the transplanted hearts to exhibit normal contractile activity at stimulation frequencies less than 24 stimuli/minute or at calcium concentrations less than 2 mM calcium.⁴

It may be noted that intracellular Ca^{2+} concentration which is pivotal to electromechanical coupling in heart were found to be unaffected in cardiac atrophy.⁵ Although the mechanisms underlying the maintenance of the Ca^{2+} transient in cardiac atrophy are unresolved, a decrease in sarcoplasmic reticulum Ca^{2+} uptake⁴ and subsequently an increase intracellular Ca^{2+} may be compensated by a concurrent upregulation of Ca^{2+} efflux system via cardiac sarcolemma. The remodelling of these sarcoplasmic and sarcolemmal-associated properties of cardiac atrophy therefore appears to be opposite to that in cardiac hypertrophy.²⁷ While cardiac hypertrophy is typically related to increase intracellular Ca^{2+} transients, atrophic hearts have identical Ca^{2+} transients as control hearts.⁵ Thus, it is safe to predict that load-dependent remodeling of systolic Ca^{2+} transients as seen in cardiac hypertrophy may be increased whereas these are unchanged in decreased cardiac workload to conserve the functional properties of contraction as seen in atrophic heart.

It has been shown that failing heart has decreased sarcoplasmic reticulum Ca^{2+} uptake as well decreased sarcolemmal Ca^{2+} efflux mechanism to develop a situation of intracellular

Ca²⁺ overload.²⁷ Since left ventricular assist device implantation is known to result in volume unloading of the ventricle with reverse remodeling and improved cardiac function of the failing myocardium, it has been suggested that the functional recovery in heart failure is possible through concerted alterations of sarcoplasmic reticulum and sarcolemmal Ca²⁺ transport systems. It should be noted that heterotrophic rat heart transplantation has been considered to be a relevant model to mimic unloading-induced changes in the failing heart, especially for investigating molecular mechanisms of myocardial remodeling.¹ Our data regarding the increased activities of sarcolemmal Ca²⁺ pump and Na⁺-Ca⁺ exchange activities can thus be seen to serve as an adaptive mechanism for the maintenance of intracellular Ca²⁺ concentration in heterotopic transplanted heart and support the view that unloading of the failing left ventricle plays a critical role for the improvement of heart function. It is also likely that the reversal of cardiac remodeling and improved heart function in failing hearts due to myocardial infarction upon treatments with diverse interventions, such as losartan, prazosin, and metoprolol may be occurring through unloading of the left ventricle and subsequent reduction in the ventricular wall tension.^{28–30}

Conflict of Interest

None.

Acknowledgments

This article is dedicated to Late Dr. Borivoj Korecky, Professor of Physiology, University of Ottawa, Ottawa, Canada. We are also grateful to the members of his laboratory for preparing and providing these animals for this biochemical study. This work was supported by funds provided by the St. Boniface Hospital Research Foundation, Winnipeg, Canada.

References

- 1 Fu X, Segiser A, Carrel TP, Tevaearai Stahel HT, Most H. Rat heterotopic heart transplantation model to investigate unloading-induced myocardial remodeling. *Front Cardiovasc Med* 2016;3:34
- 2 Korecky B, Rakusan K. Physiological and morphological aspects of cardiac atrophy. In: Alpert N, ed. *Perspective in Cardiovascular Research*. New York, NY: Raven Press; 1983:293–309
- 3 Tolnai S, Korecky B. Lysosomal hydrolases in the heterotopically isografted heart undergoing atrophy. *J Mol Cell Cardiol* 1980;12(09):869–890
- 4 Korecky B, Ganguly PK, Elimban V, Dhalla NS. Muscle mechanics and Ca²⁺ transport in atrophic heart transplants in rat. *Am J Physiol* 1986;251(5, Pt. 2):H941–H948
- 5 Schwoerer AP, Neef S, Broichhausen I, et al. Enhanced Ca²⁺ influx through cardiac L-type Ca²⁺ channels maintains the systolic Ca²⁺ transient in early cardiac atrophy induced by mechanical unloading. *Pflugers Arch* 2013;465(12):1763–1773
- 6 Dhalla NS, Pierce GN, Panagia V, Singal PK, Beamish RE. Calcium movements in relation to heart function. *Basic Res Cardiol* 1982;77(02):117–139
- 7 Fabiato A, Fabiato F. Calcium and cardiac excitation-contraction coupling. *Annu Rev Physiol* 1979;41:473–484
- 8 Carafoli E. The homeostasis of calcium in heart cells. *J Mol Cell Cardiol* 1985;17(03):203–212
- 9 Pitts BJ. Stoichiometry of sodium-calcium exchange in cardiac sarcolemmal vesicles. Coupling to the sodium pump. *J Biol Chem* 1979;254(14):6232–6235
- 10 Heyliger CE, Takeo S, Dhalla NS. Alterations in sarcolemmal Na⁺-Ca²⁺ exchange and ATP-dependent Ca²⁺-binding in hypertrophied heart. *Can J Cardiol* 1985;1(05):328–339
- 11 Caroni P, Carafoli E. An ATP-dependent Ca²⁺-pumping system in dog heart sarcolemma. *Nature* 1980;283(5749):765–767
- 12 Panagia V, Okumura K, Makino N, Dhalla NS. Stimulation of Ca²⁺-pump in rat heart sarcolemma by phosphatidylethanolamine N-methylation. *Biochim Biophys Acta* 1986;856(02):383–387
- 13 Reeves JP, Sutko JL. Sodium-calcium ion exchange in cardiac membrane vesicles. *Proc Natl Acad Sci U S A* 1979;76(02):590–594
- 14 Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957;226(01):497–509
- 15 Alto LE, Dhalla NS. Role of changes in microsomal calcium uptake in the effects of reperfusion of Ca²⁺-deprived rat hearts. *Circ Res* 1981;48(01):17–24
- 16 Makino N, Jasmin G, Beamish RE, Dhalla NS. Sarcolemmal Na⁺-Ca²⁺ exchange during the development of genetically determined cardiomyopathy. *Biochem Biophys Res Commun* 1985;133(02):491–497
- 17 Takeo S, Elimban V, Dhalla NS. Modification of cardiac sarcolemmal Na⁺-Ca²⁺ exchange by diltiazem and verapamil. *Can J Cardiol* 1985;1(02):131–138
- 18 Trosper TL, Philipson KD. Effects of divalent and trivalent cations on Na⁺-Ca²⁺ exchange in cardiac sarcolemmal vesicles. *Biochim Biophys Acta* 1983;731(01):63–68
- 19 Makino N, Dhruvarajan R, Elimban V, Beamish RE, Dhalla NS. Alterations of sarcolemmal Na⁺-Ca²⁺ exchange in catecholamine-induced cardiomyopathy. *Can J Cardiol* 1985;1(03):225–232
- 20 Makino N, Dhalla KS, Elimban V, Dhalla NS. Sarcolemmal Ca²⁺ transport in streptozotocin-induced diabetic cardiomyopathy in rats. *Am J Physiol* 1987;253(2, Pt. 1):E202–E207
- 21 Dixon IMF, Eyolfson DA, Dhalla NS. Sarcolemmal Na⁺-Ca²⁺ exchange activity in hearts subjected to hypoxia reoxygenation. *Am J Physiol* 1987;253(5, Pt. 2):H1026–H1034
- 22 Daly MJ, Dzurba A, Tuana BS, Dhalla NS. Sarcolemmal Ca²⁺-binding and enzyme activities in myocardium from hypothyroid rat. *Can J Cardiol* 1986;2(06):356–361
- 23 Cooper RA. Abnormalities of cell-membrane fluidity in the pathogenesis of disease. *N Engl J Med* 1977;297(07):371–377
- 24 Reuter H. Exchange of calcium ions in the mammalian myocardium. Mechanisms and physiological significance. *Circ Res* 1974;34(05):599–605
- 25 Narayanan N. Differential alterations in ATP-supported calcium transport activities of sarcoplasmic reticulum and sarcolemma of aging myocardium. *Biochim Biophys Acta* 1981;678(03):442–459
- 26 Heyliger CE, Ganguly PK, Dhalla NS. Sarcoplasmic reticular and mitochondrial calcium transport in cardiac hypertrophy. *Can J Cardiol* 1985;1(06):401–408
- 27 Dhalla NS, Panagia V, Makino N, Beamish RE. Sarcolemmal Na⁺-Ca²⁺ exchange and Ca²⁺-pump activities in cardiomyopathies due to intracellular Ca²⁺-overload. *Mol Cell Biochem* 1988;82(1,2):75–79
- 28 Babick A, Chapman D, Zieroth S, Elimban V, Dhalla NS. Reversal of subcellular remodeling by losartan in heart failure due to myocardial infarction. *J Cell Mol Med* 2012;16(12):2958–2967
- 29 Babick A, Elimban V, Dhalla NS. Reversal of cardiac remodeling and subcellular defects by prazosin in heart failure due to myocardial infarction. *J Clin Exp Cardiol* 2012;S5:1–7
- 30 Babick A, Elimban V, Zieroth S, Dhalla NS. Reversal of cardiac dysfunction and subcellular alterations by metoprolol in heart failure due to myocardial infarction. *J Cell Physiol* 2013;228(10):2063–2070