Improving Glucose Metabolism and/or Sarcoplasmic Reticulum Ca^{2+} -ATPase Function is Warranted for Immature Pressure Overload Hypertrophied Myocardium

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The cellular mechanisms of abnormal calcium regulation and excitation–contraction coupling in relation to glucose metabolism in the hypertrophied heart are not well understood. The present study evaluated the myocardial mechanics of 6–7-week-old pressure overload hypertrophied rabbit hearts in response to dobutamine by (1) serial echocardiograms in vivo and (2) isolated Langendorff perfusion. Cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) and sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA2) expression were measured by fluorescence spectroscopy and Western immunoblotting, respectively. The effect of glycolytic inhibition by 2-deoxy-D-glucose $\pm$ pyruvate was also evaluated. Both systolic and diastolic $[\text{Ca}^{2+}]_i$ tended to be higher and diastolic calcium removal (Ca) significantly slower in the hypertrophied heart. The myocardial response to dobutamine was blunted and dobutamine insignificantly improved Ca . The SERCA2 protein level was higher in early hypertrophy, but was significantly reduced by 6 weeks of age, with progressive contractile failure. Inhibition of glycolysis or SERCA2 caused an increase in $[\text{Ca}^{2+}]_i$ as well as a slower Ca . Pyruvate completely preserved myocardial function and $[\text{Ca}^{2+}]_i$ handling during glycolytic inhibition. It was concluded that in this model of advanced pressure overload hypertrophy, contractile failure and inotrope insensitivity are associated with increased $[\text{Ca}^{2+}]_i$, slower Ca and reduced sensitivity of the contractile proteins to Ca^{2+} . These changes occur in association with downregulation of the SERCA2, probably caused by impaired glucose metabolism. (Jpn Circ J 2001; 65: 1064–1070)

Key Words: Calcium insensitive contractile dysfunction; Glucose metabolism; Myocardial hypertrophy

Myocardial hypertrophy is associated with numerous metabolic changes, including substrate preference¹ and calcium regulation, alterations in cellular Ca^{2+} transport and excitation–contraction coupling^{2,4} which may contribute to the inotropic-insensitive contractile dysfunction observed in cardiac hypertrophy. In particular, the preferred energy substrate of the hypertrophied myocardium is known to shift from fatty acids to glucose⁵ but the availability of glucose would be limited in the hypertrophied-failing heart. We previously demonstrated in a model of left ventricular (LV) hypertrophy that glucose transport improved myocardial contractile function, particularly post ischemia^{6–8}. Abnormal excitation–contraction coupling is known to occur in the hypertrophied failing heart and is related to impaired sarcoplasmic reticulum (SR) Ca^{2+} -ATPase (SERCA2)⁹, $\text{Na}^+/\text{Ca}^{2+}$ exchanger¹⁰ and/or contractile protein¹¹. However, the precise cellular mechanisms that are responsible for the changes in contractility and the alteration in cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) are largely unknown. Recent evidence suggests that glycolysis is important for the preservation of myocardial Ca^{2+} homeostasis during α -adrenergic stimulation¹² and inhibi-

tion of glycolysis contributes to diastolic dysfunction in LV hypertrophy.¹³ We also previously demonstrated in our model of LV hypertrophy that glucose transport into the myocytes was a critical limiting factor in the hypertrophied heart and that stimulation of glucose transport improved myocardial contractile function, especially post ischemia.⁷ We hypothesized that the impaired glucose metabolism that is known to occur in the hypertrophied myocardium contributes to the SR Ca^{2+} -ATPase dysfunction, which is subsequently followed by contractile dysfunction. To further determine the cellular mechanisms of abnormal calcium regulation and excitation–contraction coupling in the hypertrophied heart (aortic banding from 10 days of age), we evaluated myocardial mechanics by (1) serial echocardiograms in vivo; and (2) isolated Langendorff perfusion of 6–7-week-old hypertrophied hearts in response to dobutamine (20–150 ng/ml). The effects on myocardial contractility and intracellular calcium (Ca_i) handling of glycolytic inhibition with and without pyruvate, and inhibition of SR Ca^{2+} -ATPase were also evaluated in isolated Langendorff perfused age-matched control (AMC) hearts. $[\text{Ca}^{2+}]_i$ was measured using fluorescence spectroscopy (Rhod-2) and the rate of $[\text{Ca}^{2+}]_i$ removal ($\text{Ca}_i\text{T}_{1/2}$), and oxygen consumption (MVO_2) were also evaluated. SERCA2 expression was determined by Western immunoblotting.

Methods

Hypertrophied Heart Model

The experiments were approved by the institutional

(Received May 25, 2001; revised manuscript received August 10, 2001; accepted August 29, 2001)

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Animal Care and Use Committee. New Zealand White rabbits (aged 7–10 days) were used to create the model of left ventricular (LV) hypertrophy. After anesthesia was induced with ketamine sulfate (30 mg/kg im) and xylazine (0.5 mg/kg im), a left thoracotomy was performed under sterile conditions. A 2-0 silk suture was placed around the descending aorta just distal to the ligamentum arteriosum, taking care to make the suture just snug without causing stenosis of the aorta. After chest closure, air was removed from the thoracic cavity by catheter aspiration. After recovery from anesthesia, the animals were returned to their mothers and allowed to feed and grow in the normal manner. Thus, as the animals grew, an aortic coarctation gradually developed. At 6–7 weeks of age, the animals were killed to measure cardiac function and Ca_i . Age-matched animals served as controls.

Echocardiography

At 3 weeks of age and then at 1-week intervals, 2-dimensional cross-sectional images and M-mode images of the left ventricle were obtained by echocardiography with simultaneous recording of the ECG and measurement of the LV short-axis dimensions by M-mode on hard copy at a paper speed of 100 mm/s. The LV epicardial and endocardial surfaces were traced by computer-aided off-line analysis on a bit-mapped digitizing tablet. End-diastolic (maximum dimension) and end-systolic (minimum dimension) LV wall thicknesses were determined using previously described methods.^{14–16} The Mass/Volume (M/V) ratio was calculated, and the serial evaluation of the M/V ratio was expressed as the percentage change from the baseline.

Isolated Perfused Heart Model

At 6–7 weeks of age, animals were killed with an overdose of ketamine (150 mg/kg iv); heparin was also given (500 U/kg iv). The hearts were rapidly excised and retrogradely perfused at 80 mmHg constant pressure in the Langendorff mode with modified Krebs-Henseleit (K-H) buffer solution, as described previously.⁶ Briefly, this buffer comprises: NaCl, 140 mmol/L; CaCl_2 , 1.25 mmol/L; KCl, 4.7 mmol/L; glucose, 11.0 mmol/L; MgSO_4 , 1.2 mmol/L; NaHCO_3 , 25 mmol/L; NaH_2PO_4 , 0.5 mmol/L; bovine regular insulin, 10 U/L, was also included. The hearts were then rapidly perfused retrogradely through the aorta in the Langendorff mode at 80 mmHg constant pressure with 37°C K-H buffer that had been equilibrated with 95% O_2 –5% CO_2 gas mixture and passed through a 0.2 μm filter. The final buffer pH was 7.35–7.45, PO_2 was 500–600 mmHg, and PCO_2 was 30–40 mmHg. The pulmonary outflow tract was incised and cannulated for venous effluent collection. A latex fluid-filled balloon connected to a micromanometry catheter (Millar Instruments, Houston, TX, USA) was used for isovolumic LV function measurements. The balloon was inserted into the left ventricle and sewn to the mitral annulus to prevent herniation; balloon volume was varied with a calibrated, fluid-filled syringe. The atrioventricular node was destroyed and the hearts were paced at 150 beats/min by atrioventricular pacing wires sutured to the right atrium and the free wall of the right ventricle.

Peak developed LV pressure (Peak DevP) was measured at an LV end-diastolic pressure of 5 mmHg. Coronary flow was measured by timed collection of the coronary effluent through the cannula placed in the pulmonary artery. Oxygen content was calculated from the measured oxygen

tension (ABL-3 Acid-Base Laboratory, Radiometer, Copenhagen, Denmark) of the aortic perfusate and the coronary venous effluent samples; myocardial oxygen consumption (MVO_2) was calculated as the measured arteriovenous oxygen difference multiplied by coronary flow and divided by the dry weight of the heart.

Experimental Protocol

After 30 min of stabilization, several experiments were performed. After dye loading and baseline data were obtained, 20–150 ng/min of dobutamine was infused for 2 min in both AMC and hypertrophied hearts. After a 5-min wash-out to clear the effects of the previous dobutamine infusion, another dobutamine infusion was performed. The effect of 2-deoxyglucose (2-DG), with and without pyruvate, and the selective SR Ca^{2+} -ATPase inhibitor, thapsigargin (10 $\mu\text{mol/L}$), were also tested in another 9 hearts. In the glycolysis inhibition experiment, modified K-H buffer containing 11 mmol/L glucose was replaced with low glucose K-H buffer in which 1 mmol/L glucose, and 3 mmol/L 2-DG with and without 10 mmol/L pyruvate were included as energy substrates.

Calcium Measurements

Measurement of beat-to-beat Ca_i transients was performed as previously described and validated in detail.¹⁷ For all spectroscopic measurements, the hearts were perfused in a fluid-sealed chamber specially designed to minimize movement artifacts, abate the curvature of the epicardium, and maintain physiological conditions. The chamber was built in-house; it consisted of a front frame equipped with a 1-mm-thick optical window (sapphire; Oriel, Stratford, CT, USA) that made contact with the LV free wall. The perfusion chamber was equipped with ports for a perfusion cannula, an intracavity balloon, and pacing wires. The excitation light beam was focused on the surface of the heart by a biconvex lens to provide a 5-mm excitation spot. The emission fluorescence light was collected with a biconvex lens positioned at 90° to the incident excitation light beam. Reflected excitation light was collected by a third biconvex lens focused onto a flexible liquid light guide (Oriel) coupled to a second photomultiplier tube. The light guide and focusing lens were both positioned at 45° on a vertical plane from the optical window and the plane of incident excitation light. A vertical plane was chosen to minimize the signal from the light reflex by the air–optical window and window–tissue interfaces (spectral reflection) and therefore increase the relative signal from the excitation light backscattered by the heart tissue itself. This backscatter reflectance signal was used to quantify the dye loading into the myocardium and to correct for the effects of motion. Similarly, the perfusion chamber with the optical window was positioned with a 30° angle of incidence (horizontal plane) to the excitation beam, which minimized the reflected excitation light from the air–window and window–tissue interfaces that was seen by the fluorescence emission detector. After a 30-min stabilization period, hearts were loaded with cell-permeable Rhod-2 acetoxymethyl ester (Rhod-2 AM; 0.5 mg per 0.25 ml 100% ethanol infused over 2 min into the aortic root without recirculation) by perfusing the heart at 37°C, followed by a 15-min washout period to remove non-hydrolyzed dye. A modified spectrofluorometer (SLM-Aminco, Springfield, IL, USA) provided excitation light at 524 nm and measured emission light at 589 nm. Real-time

Table 1 Left Ventricular (LV) Weight and Body Weight (BW) in the Aortic Banding Model

	LV weight (g)	Body weight (kg)	LV/BW ratio
Hypertrophied heart	7.73±1.86	1.85±0.27	4.25±0.89
Age-matched control	5.14±0.14*	2.45±0.10*	2.11±0.05*

* $p < 0.05$ vs hypertrophied heart.

Fig 1. Peak systolic stress and mass/volume (M/V) ratio. (A) Change in peak systolic stress in the aortic banding model. Aortic banding was performed at 1 week of age (age is expressed in weeks). * $p < 0.05$ vs age-matched control. Peak systolic stress led to an increase in the M/V ratio. (B) Change in the M/V ratio in the hypertrophied heart (Hyp) and age-matched control heart (AMC). A temporal increase in the peak systolic stress at 3 weeks of age was followed by significant increase of the M/V ratio at 4 weeks of age. * $p < 0.05$ vs age-matched control.

acquisitions of 4 ms each (ie, approximately 100 data points per cardiac cycle) recorded every 5 s were digitized and stored. Because Rhod-2 has no spectral shift when it binds calcium, it is necessary to account for changes in tissue Rhod-2 concentration over time (eg, leakage or photobleaching), as well as differences in dye loading. To do this, F_{589} was corrected for tissue absorbance as follows. Excitation light was scanned at wavelengths between 524 nm (peak Rhod-2 absorbance in cardiac tissue) and 589 nm (reference wavelength at which there is insignificant Rhod-2 absorbance) and the ratio of reflected light at 524:589 was used to estimate tissue dye concentration.¹⁵ The calcium fluorescence signal was also corrected for tissue autofluorescence by subtracting the fluorescence spectra of the heart prior to Rhod-2 loading from that of the Rhod-2 loaded heart. Instantaneous rationing of the fluorescent intensity (F) at 589 nm to reflected excitation light at 524 nm were done electronically to correct for heart motion. Calcium transient values were expressed as peak fluorescent intensity corrected for autofluorescence divided by the absorbance correction for dye concentration (F/A).

Following stabilization of dye loading and baseline signal acquisition, beating hearts were perfused in a step-wise fashion for 2 min each with K-H buffer containing 20, 50, 80 or 150 ng of dobutamine. In this way, the relationship of the inotropic effects on Ca_i and Ca_i to contractile force could be demonstrated. LV pressure and Rhod-2 fluorescence data were recorded simultaneously. All data analyses were performed using commercially available software (Sigma Plot, Jandel Scientific). The rate of the Ca_i transient decline during diastole, Ca was assessed by curve fitting to a monoexponential function as described previously¹⁸ using the following equation:

$$Ca_i(t) = (Ca_{zero} - Ca_{infin}) * \exp(-t / \tau_{Ca}) + Ca_{infin}$$

where Ca_{zero} is Ca_{infin} at minimum $d[Ca]/dt$ and Ca_{infin} is Ca_i extrapolated to infinite time, τ_{Ca} was calculated using this

equation between 20 and 70% decline of transient amplitude.

Statistical Analysis

All values are reported as mean $\pm$ SEM. Data are taken from 6–8 experiments. Multiple group comparisons were made using analysis of variance followed by the Bonferroni procedure. Differences were considered significant at $p < 0.05$ with adjustment made for multiple comparisons.

Results

Evaluation of the Hypertrophied Heart

The ratio of LV:body weight, which was used as an index of LV hypertrophy, was significantly higher in the hypertrophied hearts ($4.3 \pm 0.9 \times 10^{-3}$) than in the AMC hearts ($2.1 \pm 0.05 \times 10^{-3}$), suggesting well-developed LV hypertrophy (Table 1).

Echocardiography

Serial echocardiography revealed that systolic peak wall stress had fallen to within the normal range at 4 weeks after the aortic banding and was accompanied by significantly increased M/V ratio (1.1 ± 0.06 at 4 weeks vs 0.82 ± 0.06 at 3 weeks; ie, compensated hypertrophy). Thereafter the M/V ratio progressively fell, resulting in an elevation of peak systolic wall stress, reflecting a failure of adequate hypertrophy (Fig 1). Fractional shortening was also significantly reduced in the 5-week hypertrophied heart ($22.0 \pm 4.0\%$ vs $32.4 \pm 1.6\%$ in AMC) with further deterioration in the following time course ($15.1 \pm 4.0\%$ in 7-week hypertrophied heart).

Cardiac Function and Oxygen Consumption ($\dot{M}\dot{V}O_2$)

Peak DevP tended to be lower in the hypertrophied heart (78 ± 24 mmHg vs 99 ± 2 mmHg in AMC), but was not statis-

Fig2. Change in the developed pressure reaction to dobutamine in both hypertrophied and age-matched control hearts. The response to dobutamine was weaker in the hypertrophied heart than in the age-matched control heart, particularly with low-dose dobutamine. * $p < 0.05$ vs age-matched control.

Fig3. Typical calcium transient from age-matched control heart (Left) and hypertrophied heart (Right). Y-axis scale is intracellular calcium (Cai) calculated from the F/A rhod-2 fluorescence. Increased systolic and diastolic Cai , as well as a prolonged diastolic decline in Cai , are evident in the hypertrophied heart.

tically significant (Fig2) because of the variability of DevP, suggesting a transition from compensated hypertrophied heart to decompensated heart (ie, failing heart). However, the myocardial contractile response to the step-wise change in dobutamine concentration was significantly different; for example, although there was an insignificant change in the hypertrophied heart (98 ± 33 mmHg), a 30% increase was seen in the AMC heart (130 ± 5 mmHg) with 150 ng of dobutamine. The maximal positive dp/dt and minimum dp/dt were also insignificantly lower in the hypertrophied heart because of variable data. The rate-pressure product (RPP) over the MVO_2 , which was used as an index of mechanical efficiency, was not statistically different between the groups, suggesting that mechanical efficiency remained intact at the baseline in this model of the pressure overload hypertrophied myocardium (Table 2) with dobutamine challenge.

Intracellular Calcium

Systolic and diastolic Cai were both significantly higher in the hypertrophied heart than in the AMC heart (61 ± 24 vs 38 ± 3.0 ; 46 ± 20 vs 26 ± 1.5 , respectively). The % change in Cai in response to the different doses of dobutamine was similar in both the hypertrophied and AMC hearts. The Ca was significantly slower in the hypertrophied heart (183 ± 38 ms) than in the AMC heart (98 ± 5 ms) (Fig4) and

Table 2 Myocardial Function and Oxygen Consumption at Baseline

	+dp/dt	-dp/dt	MVO_2/RPP
Hypertrophied heart	958 ± 419	-762 ± 204	378 ± 110
Age-matched control	1311 ± 44	-891 ± 48	339 ± 39

MVO_2 ; oxygen consumption, $\mu\text{l} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ dry weight; RPP, rate-pressure product.

Fig4. The change in calcium tau with a high dose of dobutamine (150 ng) was significantly greater in the hypertrophied heart than in the age-matched control heart, suggesting slower cytosolic calcium removal. Dobutamine insignificantly improved calcium tau in the hypertrophied heart. * $p < 0.05$ vs age-matched control heart.

Fig5. Relationship of peak developed LV pressure (DevP) and intracellular calcium (Cai). Points depict the DevP- Cai relationship at perfusate dobutamine concentrations of 0, 20, 50, 80 and 150 ng. (Closed circle) Hypertrophy; (open circle) age-matched control hearts. DevP is plotted versus the magnitude of the calcium transient (Fluorescence (F)/Absorbance (A)) sys-F/Adias . The hypertrophied heart displayed a blunted response to dobutamine with lower DevP at an almost equivalent magnitude of the intracellular calcium transient.

did not improve with dobutamine administration in the hypertrophied heart (Fig5).

Western Immunoblotting

The level of SERCA2 protein was increased in the early hypertrophied heart at 5 weeks (Fig6), but then it significantly decreased (41 ± 6.7 U.OD vs 33 ± 3.0 U.OD AMC) by 6 weeks of age and was further decreased at 7 weeks of age (7.5 ± 0.5 U.OD) (Fig6).

Glucose Inhibition by 2-DG and SR ATPase Inhibition by Thapsigargin

Chemical inhibition of glycolysis using 2-DG impaired

Fig 6. (A) SR Ca^{2+} -ATPase (SERCA2) Western immunoblotting. Age is expressed in weeks. (B) Summary of the SERCA2 protein level measured by densitometer. It was significantly lower in the 6- and 7-week hypertrophied hearts than in age-matched control hearts. * $p<0.05$ vs age-matched control heart.

Fig 7. Effect of glycolytic inhibition by 3 mmol/L 2-deoxy-D-glucose and 1 mmol/L glucose with (B) or without (A) 10 mmol/L pyruvate infused into the Langendorff perfused normal heart. This was compared with the normal heart perfused with modified Krebs-Henseleit solution before glycolytic inhibition (pre). Pyruvate is obviously effective in protecting myocardial function from glycolytic inhibition.

Ca_i , including a slower Ca_i (152 ± 14 ms vs 98 ± 8 ms in AMC), and increased Ca_i (46 ± 24 vs 36 ± 21 in AMC) within 20 min, followed by functional deterioration of the myocardium (Fig 7). Pyruvate, which is an intermediate product of glycolysis, caused almost no further deterioration in the effects on cardiac function and Ca_i from the 2-DG administration (Fig 7).

The specific SR Ca^{2+} -ATPase inhibitor, thapsigargin, increased the Ca_i level (43 ± 15 vs 34 ± 12 in AMC) as well as slowing calcium removal from the cytosol (167 ± 24 ms vs 100 ± 8 ms in AMC), which mimicked the effects seen in the pressure overload hypertrophied myocardium (Fig 8).

Discussion

An important piece of information from this study is that the immature pressure overload hypertrophied heart has functional deterioration of the myocardium, along with progressive impairment of Ca_i handling associated with a significant decrease of SERCA2 protein. Acute inhibition of SERCA2 resulted in slower $[\text{Ca}^{2+}]_i$ removal, as well as an increase in the Ca_i level, effects that which mimicked the pressure overload hypertrophied heart. Glucose inhibition also impaired Ca_i handling and pyruvate administration almost completely overcame this impairment.

Glucose is a very important substrate for energy production in pathological conditions such as myocardial hyper-

Fig 8. Typical intracellular calcium trace from a normal heart with or without thapsigargin (10 $\mu\text{mol/L}$) treatment. Increased systolic and diastolic Ca_i , as well as a prolonged diastolic decline in Ca_i , are evident and were similar to the effects seen in the hypertrophied heart created by aortic banding.

trophy, ischemia-reperfusion and inflammatory reactions. In particular, the substrate preference for energy production in the hypertrophied heart is known to shift more towards glucose than fatty acids! We previously reported that impaired glucose transporter activity was an early indicator of progression of heart failure in the pressure overload hypertrophied heart⁸ ATP from glycolysis is important for

maintaining various protein functions on the sarcolemma, such as the ATP-sensitive K^+ channel¹⁹ and SERCA2,¹² and impairment of these proteins may cause ionic imbalances.

Calcium is an important molecule for myocardial contraction and is regulated by many membrane proteins, such as the ryanodine receptor, SR Ca^{2+} -ATPase, voltage gated calcium channel (dihydropyridine (DHP) sensitive receptors) and the Na^+/Ca^{2+} exchanger.²⁰ Intracellular calcium is mostly stored in the SR and mitochondria, and the major calcium release channel is the ryanodine receptor, and Ca_i is restored by SR Ca^{2+} -ATPase.²¹ Sumida and et al²² reported that in myocytes from a compensated hypertrophy heart model there was an adaptive increase in the amplitude of Ca_i transients with reduced myofilament responsiveness to increased Ca_i , which is interesting because this may be an early phenomenon in the transition from compensation to decompensation caused by the SR dysfunction associated with impaired glucose metabolism. It is believed that the function of the Na^+/Ca^{2+} exchanger is supplemental to the function of SR Ca^{2+} -ATPase, but the function of Na^+/Ca^{2+} is enhanced in the failing heart after the loss of SERCA2 as a compensatory mechanism in the end-stage failing heart.^{23,24} There have been several reports of a change in Ca_i levels in the hypertrophied failing heart in association with contractile dysfunction, such as impaired calcium handling, and less sensitivity of contractile proteins to calcium. Downregulation⁹ or decreased activity¹⁰ of SERCA2 is found in the decompensated failing heart and might be associated with contractile dysfunction. Glycolytic ATP is very important for maintaining SERCA2 function^{12,17,18} as well as other ionic channels such as K^+ -ATPase.¹⁹ Increased ADP concentration through the inhibition of glycolysis contributes to diastolic dysfunction in normal rat hearts.¹³ Functional coupling of glycolytic ATP and SR Ca^{2+} -ATPase in the normal heart has been suggested by Xu et al²⁵ We previously reported that there was significant change in glucose uptake and phosphorylation activity in the early decompensated hypertrophied heart, but no significant change in the glucose transporter protein GLUT4.^{7,8} The most plausible explanation for these results is that the decreased glucose utilization affects SERCA2 activity as well as the SERCA2 protein. Interestingly, this is true in other cell type: inhibition of glycolysis by 2-deoxy-D-glucose increases Ca_i in pulmonary arterial smooth muscle cells.²⁶ Vanadyl sulfate, which is +IV valence vanadate, has an insulin-like action that promotes glucose uptake and was used experimentally to restore glucose metabolism. It has also been used clinically for non-insulin dependent diabetes patients. We previously reported that this compound effectively promoted glucose uptake and phosphorylation and improved ischemic tolerance in the pressure overload hypertrophied heart.

Pyruvate, a metabolic product of glycolysis, is known to have variable actions, including increasing the energy potential, increasing cardiac mechanical performance, chelating inorganic phosphates and oxyradical scavenging via direct neutralization of peroxide and/or enhancement of the intracellular glutathione/NADPH antioxidant system. It was ascertained that myocardial function as well as Ca_i handling was restored by simultaneous pyruvate administration with glycolytic inhibition. Pyruvate is a final product of glycolysis and is oxidized and decarboxylated to form an acetyl group, which, then combined with coenzyme A (acetyl-CoA), enters the TCA cycle. This suggests that the total energy potential is important for maintaining

the calcium handling in myocytes rather than locally produced ATP.

We conclude that in a model of advanced pressure overload hypertrophy that is similar to many congenital heart defects, contractile failure and inotrope insensitivity were associated with increased $[Ca^{2+}]_i$ slow decline or removal of calcium during diastole by SR, and reduced sensitivity of the contractile proteins to Ca^{2+} (decreased calcium-activated force generation). These changes occur in association with downregulation of SR Ca^{2+} -ATPase. Strategies known to restore SR Ca^{2+} -ATPase function and/or expression (eg, triiodothyronine) or improve glycolysis (eg, pyruvate or vanadate) may be useful in improving functional recovery in the hypertrophied heart, especially after ischemia.

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