Cardiomyocytes from postinfarction failing rat hearts have improved ischemia tolerance

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ISCHEMIC HEART DISEASE is one of the most prevalent causes of morbidity and mortality in developed countries, with myocardial infarction and postinfarction congestive heart failure (CHF) as common serious consequences (7). The risk of ischemia is probably high in postinfarction CHF, and acute coronary events are frequent in heart failure patients who die suddenly, as shown by Uretsky et al. (56). Increased size of the heart, insufficient oxygen supply, and pathological structural alterations such as fibrosis and microvascular damage may expose the failing myocardium to continuous ischemia or repeated episodes of ischemia-reperfusion (39, 40).

During ischemia-reperfusion, an increase in cell Na+ causes excess Ca2+ entry via reverse (Ca2+/Na+ exchange (NCX) (51, 52). Both mRNA expression and protein levels of NCX are increased in the failing myocardium (12, 46), and Sjaastad et al. observed that CHF rat cardiomyocytes took up more Ca2+ in an experimental model of Na+-loaded cells (46). Intracellular Na+ is increased in both hypertrophied and failing myocardium (11, 35, 36), causing increased Ca2+ influx via reverse NCX (34). Ca2+ overload is a key mechanism behind ischemia-reperfusion damage of the myocardium. Excess Ca2+ activates intracellular degrading enzymes (5, 19, 27, 55, 57), damages mitochondria (25, 26, 37), and may induce arrhythmia (2, 52). Thus the alterations in Na+ and Ca2+ may render the failing myocardium more vulnerable to ischemia-reperfusion damage. There is evidence that procedures that limit Na+ accumulation (9, 16) and subsequent Ca2+ overload (44, 45, 58) during ischemia-reperfusion reduce cell injury.

Increased entry of Ca2+ via NCX might thus be expected to put the failing heart at exaggerated risk of damage during new ischemia-reperfusion episodes. In heart failure, it is mandatory to protect the heart against further damage, such as new infarcts and further reduction of contractile function. The tolerance to new ischemic episodes has not previously been investigated in postinfarction CHF. To test the hypothesis that myocardial tolerance to ischemia is reduced in CHF, we investigated the effects of hypoxia-reoxygenation on isolated cardiomyocytes from rats with postinfarction CHF. Surprisingly, we found that tolerance to hypoxia-reoxygenation was increased in CHF cardiomyocytes compared with control, suggesting that CHF myocardium had improved ischemia tolerance.

MATERIALS AND METHODS

Chemicals and materials. 45Ca2+ was obtained from American Radiolabeled Chemicals (St. Louis, MO). 22Na+ was obtained from Amersham Biosciences (Little Chalfont, UK). The following chemicals were obtained from Sigma Chemical (St. Louis, MO): bovine serum albumin (BSA; essentially fatty acid free), d,l-carnitine, taurine, creatine, insulin, dibutyl phthalate, ouabain, trypsin, ranolazine, and the increase in exchange (NCX) (51, 52). Both mRNA expression and protein levels of NCX are increased in the failing myocardium (12, 46), and Sjaastad et al. observed that CHF rat cardiomyocytes took up more Ca2+ in an experimental model of Na+-loaded cells (46). Intracellular Na+ is increased in both hypertrophied and failing myocardium (11, 35, 36), causing increased Ca2+ influx via reverse NCX (34). Ca2+ overload is a key mechanism behind ischemia-reperfusion damage of the myocardium. Excess Ca2+ activates intracellular degrading enzymes (5, 19, 27, 55, 57), damages mitochondria (25, 26, 37), and may induce arrhythmia (2, 52). Thus the alterations in Na+ and Ca2+ may render the failing myocardium more vulnerable to ischemia-reperfusion damage. There is evidence that procedures that limit Na+ accumulation (9, 16) and subsequent Ca2+ overload (44, 45, 58) during ischemia-reperfusion reduce cell injury.

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and sodium dodecyl sulfate (SDS). Joklik minimum essential medium (MEM) and penicillin/streptomycin were obtained from Life Technologies (Paisley, UK). Collagenase and deoxyribonuclease were obtained from Worthington Biochemical ( Lakewood, NJ). Micro BCA protein assay reagent was obtained from Pierce (Rockford, IL). Di-isonomyl phthalate was obtained from Fluka Chemie (Buchs, Switzerland). Optifluor (high-flash point LCS cocktail) was obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). Dimethyl sulfoxide (DMSO) was obtained from E. Merck (Darmstadt, Germany). Laminin (natural mouse) was obtained from Invitrogen (Life Technologies, Carlsbad, CA). Acetoxymethyl (AM) ester of fluo-3 and the ATP determination kit (A-22066) were obtained from Invitrogen/Molecular Probes.

Buffers and solutions. Culture medium was medium 199 supplemented with 1 mg/ml BSA, 2 mmol/L d,l-carnitine, 5 mmol/L creatine, 5 mmol/L taurine, 100 μM insulin, 0.1 mM T₃, and 100 U/ml penicillin and streptomycin. Experiments were performed in a normal physiological phosphate bicarbonate buffer (NPB) with the following composition (in mmol/L): 120.0 NaCl, 3.3 KCl, 1.2 KH₂PO₄, 24.0 NaHCO₃, 1.0 CaCl₂, 0.8 MgSO₄, and 5.5 glucose supplemented with 1% BSA (wt/vol), equilibrated with 95% air-5% CO₂ at 37°C, pH 7.4. Cardioplegic solution contained (in mmol/L) 87.3 NaCl, 20 KCl, 1.2 KH₂PO₄, 24.0 NaHCO₃, 1.0 CaCl₂, 0.8 MgSO₄, and 5.5 glucose supplemented with 100 μM insulin.

Animals, surgical procedure, and isolation of cardiomyocytes. Male Wistar rats (Mollegaard Breeding and Research Centre, Skensved, Denmark) weighing ~320 g were cared for according to the Norwegian Animal Welfare Act, which conforms with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No 85-23, Revised 1996). The experiments and procedures concerning animals described in this article have been approved by the Norwegian Animal Research Authority. Animals were housed in a temperature-regulated room with a 12:12-h day-night cycle (2 animals per cage) with free access to water and feed. Animals were intubated and ventilated with 68% N₂O, 29% O₂, and 2–3% isoflurane (Isoflurane, Abbott Laboratories). An extensive myocardial infarction was induced by proximal ligation of the left coronary artery. Six weeks later, the rats were anesthetized and ventilated with 2.2% isoflurane, and left ventricular pressures were measured. Rats were considered to have CHF when BP, LVEDP, and systolic BP were measured. Rats were considered to have CHF if left ventricular end-diastolic pressure (LVEDP) was >15 mmHg (48). Sham-operated animals were subjected to the same surgical procedures but not to coronary artery ligation. Animal and organ characteristics are summarized in Table 1. Lungs and heart were excised and weighed, and the heart was mounted on a Langendorff apparatus for immediate perfusion for 2–3 min with cardioplegic solution containing elevated magnesium and potassium with a temperature of 5–10°C. The heart was kept in cardioplegic solution up to 30 min at most to initiate ordinary Langendorff perfusion of three hearts simultaneously with enzyme solution containing collagenase and trypsin to isolate cardiomycocytes as previously described (41), except that 5% CO₂ and 95% air were used for gassing the perfusion buffer. In addition, infarction area was removed from CHF hearts by dissection at the end of enzymatic perfusion of the hearts, before the final enzymatic dissolution of tissue pieces by shaking in water bath. Cells were cultured on Costar culture wells using laminin for attachment. After 2 h of incubation, culture medium was replaced by fresh culture medium to remove dead cells. Experiments were performed the day after cell isolation.

Experimental procedure. At the start of experiments, culture medium was aspirated and replaced by NPB buffer. Culture disks were incubated in an atmosphere of 5% CO₂ and 95% air (normoxia) or in 5% CO₂ and 95% nitrogen (hypoxia) with the use of a Galaxy R 300 CO₂ incubator (RS Biotech; www.rsbitech.com), achieving 0.1% O₂ tension within 20–30 min. At reoxygenation, the atmosphere was changed back to normoxic conditions. Hypoxia-reoxygenation experiments were performed by exposing the cells to hypoxia for 4 h without glucose present in the buffer, followed by 2 h of reoxygenation with 5.5 mmol/L glucose.

Enzyme release. Release of lactate dehydrogenase (LD) was determined using the method of Wroblewski and LaDue (60) in plated cells (without trypsinization) as enzyme activity in the buffer and after cell lysis with the Cobas Integra 800 (Roche Diagnostics).

Cell content of Ca²⁺ and Na⁺ and uptake of ⁸⁶Rb⁺. Cell content of Ca²⁺ was determined as ⁴⁵Ca²⁺ uptake in cells detached by trypsinization and centrifuged through an oil layer as previously described (41) with some minor modifications. Briefly, cell Ca²⁺ was determined as rapidly exchangeable Ca²⁺ by uptake of ⁴⁵Ca²⁺. A 20-ml Falcon tube (Oxnard, CA) containing 500 μl of oil mixture (dibutyl phthalate and di-isonomyl phthalate, 45–55% wt/wt) below 8.5 ml of NPB was kept in ice water (0–5°C). A sample of cell suspension (500 μl) was added to the buffer phase, and the tube was centrifuged (2,000 g, 2 min) within 5 min, allowing cardiomyocytes to pass through the oil to the bottom of the tube. The tip of the tube (containing the cell pellet) was cut off, and the pellet was dissolved in 1 ml of 1% SDS. Radioactivity was determined by liquid scintillation counting, and protein content was determined using the Micro BCA protein assay reagent (with BSA as standard) from each cell pellet. The same method was used for Na⁺ uptake (using ²²Na⁺) and for K⁺ uptake (using ⁸⁶Rb⁺ as a K⁺ analog). All isotopes were added in trace amounts, and specific activity of the isotope was measured for each experiment. ⁴⁵Ca²⁺ and ⁸⁶Rb⁺ were determined by scintillation counting and ²²Na⁻ by using a gamma counter.

ATP measurement. Cells were centrifuged through an oil layer into a perchloric acid (PCA) solution. After the overlying buffer and oil phases were aspirated, the remaining PCA phase was rapidly frozen in liquid nitrogen and stored at ~80°C until analysis by luminescence using firefly luciferase.

Flow cytometric measurement of intracellular Ca²⁺ and cell death. Levels of intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) were measured in CHF and sham cells exposed to either hypoxia and reoxygenation or normoxia by determining the fluorescence of fluo-3 in a FACSsort (Becton Dickinson, Rutherford, NJ) flow cytometer equipped with a 488-nm argon ion laser and supplied with the Cell Quest software as previously described (43). Propidium iodide (PI; 5 μg/ml) was added 2 min before determination of cell viability to CHF and sham cells exposed to either hypoxia-reoxygenation or normoxia. Population of cells emitting red fluorescence was gated and defined as dead cells in a FACSDiVa SE flow cytometer equipped with a 488-nm argon ion laser and supplied with the BD FACSDiVa software. Fluo-3 fluorescence in CHF and sham cells exposed to either hypoxia and reoxygenation or normoxia was also measured in separate experiments where dead cells were excluded by PI staining. Each determination was based on mean fluorescence intensity of 2,000–3,000 cells in arbitrary units.

Table 1. Animal and organ characteristics

<table>
<thead>
<tr>
<th>Animal and Organ Characteristics</th>
<th>Sham</th>
<th>CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Weight, g</td>
<td>383±7</td>
<td>368±6</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.3±0.1</td>
<td>2.5±0.2*</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>1.5±0.1</td>
<td>3.9±0.2*</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>106±11.1</td>
<td>102±1.38</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>2.2±0.6</td>
<td>27.8±1.6*</td>
</tr>
<tr>
<td>dP/dtmax, mmHg/h</td>
<td>8,122±626</td>
<td>5,171±249*</td>
</tr>
<tr>
<td>dP/dtmin, mmHg/h</td>
<td>−8,293±1,109</td>
<td>−3,914±235*</td>
</tr>
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Values are means ± SE; n = no. of sham-operated and congestive heart failure (CHF) rats. BP, blood pressure; LVEDP, left ventricular end-diastolic pressure; dP/dtmax and dP/dtmin, maximum and minimum first derivative of LV pressure, respectively. *P < 0.05 compared with sham rats.
Statistics. All experiments were performed in parallel design including both CHF and sham cells. Statistical analysis was performed using Statgraphics Plus software (version 5.0; Manugistics, Rockville, MD). Statistical analysis of multigroup comparisons was performed with one-way analysis of variance (ANOVA) followed by Fisher’s least significance difference method to discriminate among the means or the Kruskal-Wallis test (a nonparametric method that tests the assumption that the medians of samples are equal). The Kruskal-Wallis test was performed when the variances of results obtained from different groups were too different to use ANOVA. Statistical analysis of two-sample comparisons was performed using Student’s t-test (2-sided). \( P < 0.05 \) was considered to be significant.

RESULTS

Animal characteristics. All CHF rats included in the study had severe heart failure as evidenced by increased LVEDP (Table 1), pulmonary congestion, and cardiac hypertrophy. The lung weight-to-body weight ratio was 2.7-fold higher and the heart weight-to-body weight ratio was 2-fold higher in CHF than in sham rats. Tachypnea, increased left atrial dimensions, and pleural effusion were also observed in the CHF group (data not shown).

Elongated isolated cardiomyocytes are viable cells, whereas spherical cells might be alive or dead. The percentage of elongated cells right after isolation was 57 \pm 2.2\% in CHF and 71.7 \pm 3.4\% in sham rats (means \pm SE of 11 CHF hearts and 7 sham hearts from 6 separate isolation days). Spherical cells do not attach to the laminin-coated culture wells. Viability of the cultured cells on the experimental day was almost similar in CHF and sham cells after 4 h of normoxic incubation, showing 87.6 and 90.1\% viable cells, respectively (see Fig. 2A). CHF cells in this experimental model have been shown to be larger based on a 23\% increase in capacitance from 176 pF in sham to 217 pF in CHF cells and a 15\% increase in length from 118 \mu m in sham to 136 \mu m in CHF cells (47). Electron scanning micrographs of sham (Fig. 1A) and CHF cells (Fig. 1B) were taken at the end of the experimental protocol, i.e., after 4 h of hypoxia and 2 h of reoxygenation.

CHF cardiomyocytes showed less cell death and better preservation of ATP during hypoxia-reoxygenation. The proportion of dead cells increased less in CHF (from 12 to 26\%) than in sham cardiomyocytes (from 10 to 35\%), measured as the increase in PI-positive cells following 4 h of hypoxia and 15 min of reoxygenation (Fig. 2A). Accordingly, in CHF cardiomyocytes, LD release after hypoxia-reoxygenation was merely 1.7 times higher than in normoxic CHF cells (\( P < 0.05 \)). In contrast, LD release from sham cardiomyocytes was 6.3-fold higher after hypoxia-reoxygenation compared with normoxic sham cells. Thus LD release from reoxygenated CHF cardiomyocytes was 30\% less (\( P < 0.05 \)) compared with reoxygenated sham cells (Fig. 2B). This indicates less hypoxia-reoxygenation-induced injury in CHF than in sham cardiomyocytes. ATP content was reduced by 32\% in CHF cardiomyocytes and by 50\% in sham cardiomyocytes after 4 h of hypoxia (both \( P < 0.05 \)). In hypoxic CHF cardiomyocytes, no recovery of ATP content was observed at the end of the 2-h reoxygenation period compared with the end of hypoxia, whereas reoxygenation partially restored ATP content in hypoxic sham cardiomyocytes (Fig. 3). Normoxic CHF cells contained 50\% less ATP than normoxic sham cells (\( P < 0.05 \)).

Cell Ca\(^{2+}\) and \([Ca^{2+}]_i\) increased less in CHF than in sham cardiomyocytes during hypoxia-reoxygenation. Exchangeable cell Ca\(^{2+}\) has been shown to increase during reoxygenation of hypoxic cardiomyocytes (41). In line with this, the present results showed that cell Ca\(^{2+}\) increased three- to sixfold during the 2-h reoxygenation in sham cells (\( P < 0.05 \)). In contrast, no significant increase in cell Ca\(^{2+}\) was detected in CHF cells during the reoxygenation period (Fig. 4A). It is known that hypoxia leads to an increase in cardiomyocyte \([Ca^{2+}]_i\) that partly recovers during subsequent reoxygenation (43). Accordingly, in our experiments, fluo-3 signal reflecting \([Ca^{2+}]_i\) increased significantly in sham cardiomyocytes during hypoxia, but in striking contrast, we did not detect a significant increase in fluo-3 signal of the CHF cardiomyocytes during hypoxia when the cell populations were analyzed without separating live and dead cells (Fig. 4B). During the 2-h reoxygenation period, the fluo-3 signal recovered by 47\% in sham cells, still markedly higher than in normoxic sham cells at the end of the experiment (Fig. 4B). Since the increase in fluo-3 fluorescence could be partly due to loss of Ca\(^{2+}\) homeostasis in dead cells, we used flow cytometry to analyze fluo-3 fluorescence in PI-negative (live) cells in a separate set of experiments, as shown in Fig. 4C. In PI-negative cardiomyocytes, fluo-3 fluorescence increased 1.8-fold in CHF and 4.6-fold in sham cells (Fig. 4D), demonstrating a significantly less increase of \([Ca^{2+}]_i\) in live CHF cells after hypoxia-reoxygenation. In both CHF and sham PI-negative cardiomyocytes, there was a slight decrease of fluo-3 fluorescence from 2 to 25 min of reoxygenation (Fig. 4D).

Fig. 1. Electron scanning micrograph of sham (A) and congestive heart failure (CHF) cardiomyocytes (B) fixed with 1\% paraformaldehyde at the end of 4 h of hypoxia and 2 h of reoxygenation.
It is known that ischemia leads to increased Na$^+$ content in cardiomyocytes during hypoxia. ATP content was measured in CHF and sham cardiomyocytes. ATP content was reduced by 35% in normoxic sham cells after 4 h of hypoxia compared with normoxic CHF cells and was therefore ~66% lower than in sham cells after hypoxia (P < 0.05). In CHF cells, the Na$^+$ content did not change significantly during the first 10 min of reoxygenation. Na$^+$ content was not significantly different in CHF and sham cardiomyocytes after 250 min in normoxia.

**Ranolazine, the inhibitor of late Na$^+$ current, preferably inhibited Ca$^{2+}$ accumulation and cell damage in sham cardiomyocytes during hypoxia-reoxygenation.** Ranolazine (100 μM) present from the start of hypoxia significantly attenuated the increase in exchangeable Ca$^{2+}$ in sham cells measured 2 h after reoxygenation without affecting exchangeable Ca$^{2+}$ in CHF cells (Fig. 6A). This observation suggests that Na$^+$ accumulation was important for the difference in ischemia tolerance between CHF and sham cells. In parallel with the inhibitory effect of ranolazine on reoxygenation-mediated Ca$^{2+}$ accumulation in sham cells, LD release was also significantly reduced, as shown in Fig. 6B.
Ca\textsuperscript{2+} uptake caused by intracellular Na\textsuperscript{+} loading (by Na\textsuperscript{+}-K\textsuperscript{+}-ATPase inhibition) was not different in CHF and sham cardiomyocytes. The ability of CHF and sham cardiomyocytes to accumulate Ca\textsuperscript{2+} under conditions with elevated cell Na\textsuperscript{+} was tested by blocking the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase with 1 mmol/l ouabain under normoxic conditions (Fig. 7). Under these conditions, both CHF and sham cardiomyocytes showed a 50- to 70-fold increase in Ca\textsuperscript{2+} content compared with cardiomyo-
cytes that were not exposed to ouabain, and Ca\(^{2+}\) content did not differ between the cell types.

**CHF and sham cardiomyocytes took up the K\(^{+}\) analog 86Rb\(^{+}\) to the same degree during hypoxia.** Differences in Na\(^{+}\) accumulation during hypoxia between CHF and sham could be a result of altered Na\(^{+}\)-K\(^{+}\)-ATPase activity during hypoxia-reoxygenation. Thus uptake of the K\(^{+}\) analog 86Rb\(^{+}\) through the Na\(^{+}\)-K\(^{+}\)-ATPase was measured in CHF and sham cardiomyocytes exposed to either 4 h of hypoxia or 4 h of normoxia. Bumetanide (0.5 mmol/l) was added to inhibit the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter. Hypoxia resulted in a 60% reduction of 86Rb\(^{+}\) uptake in both CHF and sham cardiomyocytes, as shown in Fig. 8, and 86Rb\(^{+}\) uptake in hypoxia and normoxia did not differ between CHF and sham cells.

**DISCUSSION**

The present study showed that compared with sham cells, CHF cardiomyocytes responded to hypoxia-reoxygenation with less LD release, less increase in cell death and less relative reduction in ATP, less increase in cell Ca\(^{2+}\) and in [Ca\(^{2+}\)]\(_i\), and less Na\(^{+}\) accumulation during hypoxia. Thus, in several respects, CHF cardiomyocytes appeared to have improved tolerance to hypoxia-reoxygenation compared with sham cardiomyocytes. Ranolazine, an inhibitor of late Na\(^{+}\) current, significantly attenuated both the increase in exchangeable Ca\(^{2+}\) and the increase in LD release and exchangeable Ca\(^{2+}\) in CHF cells, suggesting that differences between CHF and sham cells in late Na\(^{+}\) current during hypoxia explain the differences in ischemia tolerance.

The low ATP level that was observed in CHF cells during normoxia compared with sham myocytes is in accordance with results published by others (31) and may be responsible for in vivo contractile dysfunction and the elevated LD release from CHF cells compared with sham cells during normoxia observed in the present study. This is in line with the observed increase in LD release from fibroblasts during ATP depletion (22). The surprising result showing relatively less increase in LD release during hypoxia-reoxygenation from CHF cardiomyocytes than sham cells, supported by the observation of less increase in dead CHF cells than sham cells during hypoxia-reoxygenation, demonstrates convincingly that there was less cell injury induced by hypoxia-reoxygenation in CHF cells. Our observation that the CHF cells had relatively less reduction...
improved tolerance to hypoxia-reoxygenation. It has been reported that Ca\(^{2+}\) extrusion mainly depends on glycolytically derived ATP (21), possibly linking the differences in LD release, ATP level, and Ca\(^{2+}\) accumulation between CHF and sham cells all to differences in glycolytic metabolism.

The much smaller increase in intracellular Na\(^+\) level in CHF cells than in sham cells during hypoxia likely explains the much lower Ca\(^{2+}\) uptake by CHF cells during the reoxygenation period. The intracellular Na\(^+\) concentration is an important determinant of the Ca\(^{2+}\) homeostasis in cardiomyocytes through the NCX. Increased intracellular Na\(^+\) is suggested as a major cause of NCX to run in Ca\(^{2+}\) influx mode and causes Ca\(^{2+}\) accumulation in the cardiomyocytes during reperfusion. The possible mechanisms for the increase in intracellular Na\(^+\) during ischemia (53) are decreased Na\(^{+}\)-K\(^{+}\)-ATPase activity (1, 17, 18, 29, 32), tissue acidosis, and increased tissue CO\(_2\) (8, 49) with increased Na\(^{+}\)/H\(^+\) exchange activity (1, 20, 23, 24, 33, 54) and Na\(^{+}\) entry via Na\(^{+}\) channels and possibly other cation channels. The markedly larger inhibitory effect of ranolazine, the inhibitor of late Na\(^{+}\) current (50), on the increase in exchangeable Ca\(^{2+}\) and the increase in LD release in sham cells than in CHF cells after reoxygenation indicates that differences between CHF and sham cells in late Na\(^{+}\) current during hypoxia might explain the differences in Na\(^{+}\) uptake during hypoxia and the differences in ischemia tolerance observed in this study. The present results in quiescent cells showed no difference between CHF and sham cells in cell Na\(^{+}\) during normoxic control conditions. An increase in intracellular Na\(^{+}\) that has previously been observed in both hypertrophic and failing contracting myocardium, causing secondary increase in intracellular Ca\(^{2+}\) via NCX (34), is probably dependent on electromechanical activity of the cells.

Less Na\(^{+}\) accumulation in CHF than in sham cells could theoretically be a result of more effective Na\(^{+}\) transport out of the cells by Na\(^{+}\)-K\(^{+}\)-ATPase. The possibility that higher than sham myocytes in ATP level during hypoxia also supports the idea that CHF cardiomyocytes had increased tolerance to hypoxia-reoxygenation. Hypoxic cell damage with enzyme release has previously been linked to metabolic inhibition and reduction in ATP (22, 59). Increase in enzyme release has been more closely correlated to inhibition of glycolysis than to inhibition of oxidative phosphorylation (4, 14). A sharp switch of fatty acid metabolism toward carbohydrate metabolism has been shown in late-stage canine failing myocardium (38). Better preservation of glycolytic metabolism during hypoxia and reoxygenation may explain smaller ATP reduction and less enzyme release in CHF cells.

The markedly lower Ca\(^{2+}\) content and lower fluo-3 fluorescence (reflecting lower [Ca\(^{2+}\)]\(_i\)) in CHF compared with sham cardiomyocytes in this study indicates a better tolerance of CHF cardiomyocytes to hypoxia-reoxygenation. The large reoxygenation-induced Ca\(^{2+}\) uptake observed in sham cells in the present study was similar to that previously seen in normal rat cardiomyocytes exposed to hypoxia-reoxygenation (41–43) and in agreement with data from other groups showing markedly increased myocardial Ca\(^{2+}\) uptake in hearts exposed to ischemia-reperfusion (44, 45, 58) and in cells exposed to hypoxia-reoxygenation (13, 30). The flow cytometry results on fluo-3 fluorescence obtained using PI to separate dead and live cells clearly demonstrated that live CHF cells had significantly less increase of [Ca\(^{2+}\)]\(_i\) than live sham cells after hypoxia-reoxygenation and that the difference between CHF and sham cells was not due to contamination with dead cells. Morris et al. (28) and Brecht et al. (3) found a significant increase in cytosolic Ca\(^{2+}\) before enzyme release and loss of viability in cardiomyocytes and hepatocytes, respectively. In relation to the likely role of Na\(^{+}\) and Ca\(^{2+}\) in ischemia-reperfusion damage, lower reoxygenation-induced Ca\(^{2+}\) accumulation in CHF is a candidate mechanism to explain the increase in cytosolic Ca\(^{2+}\) and cell death during reoxygenation (41–43) and in agreement with data from other groups showing markedly increased myocardial Ca\(^{2+}\) uptake in hearts exposed to ischemia-reperfusion (44, 45, 58) and in cells exposed to hypoxia-reoxygenation (13, 30). 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Na\(^+\)-K\(^+\)-ATPase activity in CHF than in sham cells could contribute to less Na\(^+\) accumulation in hypoxia was investigated, but the results of \(^{86}\)Rb\(^+\) uptake experiments did not support this hypothesis. In fact, reduced Na\(^+\)-K\(^+\)-ATPase activity could be expected based on lower ATP content in CHF. The role of different ATP levels in CHF and sham cells in relation to Na\(^+\)-K\(^+\)-ATPase activity and increase in Na\(^+\) during hypoxia is difficult to elucidate. The Na\(^+\)-K\(^+\)-ATPase has both catalytic and regulatory sites for ATP (6). In addition, its function is regulated by phospholipase, which is reported to be increased in the rat heart after myocardial infarction (6), and subject to increased phosphorylation in ischemia (10). Altogether, the present results indicate that reduced Na\(^+\) entry via Na\(^+\) channels is the most likely explanation for less Na\(^+\) accumulation in CHF cells during hypoxia. This is an interesting, and to our knowledge, new observation.

Our findings that CHF cells have improved ischemia tolerance are corroborated by the results of Hoskins et al. (15) showing that dogs with pacing-induced failing heart had much smaller infarcts than control animals after ischemia-reperfusion. Thus, despite different pathological stimuli (pacing vs. postinfarction heart failure) and different biological levels (whole heart vs. isolated cardiomyocytes), these two studies show the same phenomenon of better ischemic tolerance of failing myocardium. The mechanism for increased ischemia tolerance in heart failure is unknown and should be elucidated in further experiments. We speculate that factors like intermittent or continuous low-grade ischemia might increase ischemia tolerance by preconditioning the failing myocardium or that phenotypic alterations in heart failure in energy metabolism, oxygen consumption, and/or ion transport might increase ischemia tolerance. Further experiments are needed to answer these questions.

In conclusion, CHF cardiomyocytes have improved tolerance to hypoxia-reoxygenation compared with sham cardiomyocytes as shown by reduced LD release, less cell death, and less relative reduction in ATP. Reduced increase in free intracellular Ca\(^{2+}\) and less increase in Ca\(^{2+}\) content after hypoxia-reoxygenation support and may underlie the better viability and tolerance of CHF during hypoxia-reoxygenation. Furthermore, CHF cardiomyocytes show less increase in Na\(^+\) during hypoxia, which may be the reason for reduced Ca\(^{2+}\) uptake via NCX during reoxygenation. These findings suggest that CHF myocardium has improved tolerance to ischemia compared with nonfailing myocardium.

GRANTS

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