Preconditioning attenuates ischemia-reperfusion-induced remodeling of Na\(^+\)-K\(^+\)-ATPase in hearts

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Elmoselhi, Adel B., Anton Lukas, Petr Ostadal, and Naranjan S. Dhalla. Preconditioning attenuates ischemia-reperfusion-induced remodeling of Na\(^+\)-K\(^+\)-ATPase in hearts. Am J Physiol Heart Circ Physiol 285: 1055–1063, 2003.—The aim of this study was to determine whether changes in protein content and/or gene expression of Na\(^+\)-K\(^+\)-ATPase subunits underlie its decreased enzyme activity during ischemia and reperfusion. We measured protein and mRNA subunit levels in isolated rat hearts subjected to 30 min of ischemia and 30 min of reperfusion (I/R). The effect of ischemic preconditioning (IP), induced by three cycles of ischemia and reperfusion (10 min each), was also assessed on the molecular changes in Na\(^+\)-K\(^+\)-ATPase subunit composition due to I/R. I/R reduced the protein levels of the \(\alpha_2\), \(\alpha_3\), \(\beta_1\), and \(\beta_2\)-isoforms by 71%, 85%, 27%, and 65%, respectively, whereas the \(\alpha_1\)-isoform was decreased by <15%. A similar reduction in mRNA levels also occurred for the isoforms of Na\(^+\)-K\(^+\)-ATPase. IP attenuated the reduction in protein levels of Na\(^+\)-K\(^+\)-ATPase \(\alpha_2\), \(\alpha_3\), and \(\beta_2\)-isoforms induced by I/R, without affecting the \(\alpha_1\) and \(\beta_1\)-isoforms. Furthermore, IP prevented the reduction in mRNA levels of Na\(^+\)-K\(^+\)-ATPase \(\alpha_2\), \(\alpha_3\), and \(\beta_1\)-isoforms following I/R. Similar alterations in protein contents and mRNA levels for the Na\(^+\)/Ca\(^+\)+ exchanger were seen due to I/R as well as IP. These findings indicate that remodeling of Na\(^+\)-K\(^+\)-ATPase may occur because of I/R injury, and this may partly explain the reduction in enzyme activity in ischemic heart disease. Furthermore, IP may produce beneficial effects by attenuating the remodeling of Na\(^+\)-K\(^+\)-ATPase and changes in Na\(^+\)/Ca\(^+\)+ exchanger in hearts after I/R.

ischemic preconditioning; myocardium; Na\(^+\)-K\(^+\)-ATPase gene expression; Na\(^+\)/Ca\(^+\)+ exchanger

DEFINING THE NATURE AND MECHANISM of ischemia-reperfusion (I/R) injury of the heart has become important because of its clinical relevance in thrombolytic therapy, transhumeral coronary angioplasty, and coronary bypass surgery. Previous studies have shown a decrease in sodium pump (Na\(^+\)-K\(^+\)-ATPase) activity in the isolated rat heart subjected to I/R (2) and hypoxia-reoxygenation injury (9); however, the exact mechanisms of this reduction remain unclear. Na\(^+\)-K\(^+\)-ATPase is a heterodimer protein composed of \(\alpha\)- and \(\beta\)-subunits that plays a key role in regulating membrane potential and cation transport in the myocardium. The \(\alpha\)-subunit in the heart consists of three isoforms encoded by three distinct genes: \(\alpha_1\), \(\alpha_2\), and \(\alpha_3\). It is responsible for the catalytic activity of the enzyme because it contains the ATP and glycoside binding sites (37). These three isoforms vary in their affinity to cardiac glycosides; the \(\alpha_1\)-isoform exhibits a low affinity to ouabain, whereas \(\alpha_2\) and \(\alpha_3\)-isoforms have a much greater affinity. The \(\alpha_1\)-subunit is ubiquitous and present in abundance in cardiac muscle (~75%) (22). In contrast, \(\alpha_2\) and \(\alpha_3\)-subunits are localized in more strategic areas such as the conduction system and junctional complexes in the heart (43, 44). Besides their differences in expression during various developmental stages, the \(\alpha_2\) and \(\alpha_3\)-isoforms are more sensitive to oxidative stress than the \(\alpha_1\)-isoform; this may relate to their distinct structural features (14). Recently, the \(\alpha_2\)-subunit was found to play a specific role in intracellular Ca\(^2+\) signaling during cardiac contraction in genetically modified mouse hearts (15). Such studies not only indicate a difference in expression of \(\alpha\)-isoforms in different areas of the myocardium but have also revealed distinct functional roles of these isoforms. The \(\beta\)-subunit, on the other hand, is mainly responsible for the proper localization and insertion of the enzyme in the plasma membrane. It consists of three isoforms: \(\beta_1\), \(\beta_2\), and \(\beta_3\), but adequate information about differences in their functional properties is unavailable. Nevertheless, alterations in \(\beta\)-subunit content were shown to affect the activity of the enzyme (24). The present study was undertaken to test the hypothesis that a reduction in Na\(^+\)-K\(^+\)-ATPase activity in the heart due to I/R is associated with changes in protein content and mRNA levels for different subunits of the enzyme.

Brief periods of ischemia are known to render the heart more resistant to the effects of longer periods of ischemia (25), and this phenomenon is termed ischemic preconditioning (IP). The protection offered by IP appears in two phases: one occurs soon after I/R (classic or early IP), whereas the second becomes evident after ~24 h (delayed or second window of protection) (23). IP-induced protection of the heart is manifest as a reduction in infarct size, prevention of ventricular arrhythmia, and reduction in reperfusion injury (28). It is believed that the protective effects of IP are due to a reduction in the release of cytochrome c (11) and other proinflammatory cytokines (6), and an improvement in mitochondrial function (21). In this study, we found that administration of a 30 min period of ischemia followed by 30 min of reperfusion (I/R) reduced the protein levels of the \(\alpha_2\), \(\alpha_3\), and \(\beta_2\)-isoforms by 71%, 85%, and 15%, respectively (19, 20). The present study was undertaken to test the hypothesis that a reduction in Na\(^+\)-K\(^+\)-ATPase activity in the heart due to I/R is associated with changes in protein content and mRNA levels for different subunits of the enzyme.

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rhythmas, and attenuation of abnormalities in cardiac performance following I/R (4, 13). Although the exact mechanism of IP is unknown, several pathways are implicated in IP such as activation of adenosine receptors, G protein-coupled receptors, protein kinase C, and ATP-sensitive K+ (KATP) channels, as well as synthesis and upregulation of various proteins such as heat shock protein 72, MnSOD, inducible nitric oxide synthase, and Bcl2 (21, 23, 31, 40, 42). In a recent study, Nawada et al. (26) demonstrated that IP protects the rabbit heart against the I/R-induced reduction in Na+-K+-ATPase activity. However, the mechanism of this protection is unclear, because these authors only measured ATPase activity. In the present study, we assessed the changes in Na+-K+-ATPase subunits induced by I/R and determined whether IP protects against these changes. The changes in different isoforms were measured at both the protein and mRNA levels. The effects of I/R and IP on the Na+/Ca2+ exchanger in isolated hearts were also assessed to determine whether changes in exchanger protein content and mRNA levels were similar to those seen for Na+-K+-ATPase.

METHODS

Isolated heart function. Experiments were conducted in accordance with the guide to the care and use of experimental animals issued by the Canadian Council in Animal Care. Male Sprague-Dawley rats (250–350 g) were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg). The heart was quickly removed and mounted on a Langendorff apparatus, where it was perfused with Krebs-Henseleit medium containing (in mM) 120 NaCl, 25 NaHCO3, 11 glucose, 4.7 KCl, 1.2 H3PO4, 1.2 MgSO4, and 1.25 CaCl2 at a constant rate of 10 ml/min (37°C). The perfusion solution was gassed with 95% O2-5% CO2 resulting in pH 7.4. The heart was kept in a humidified chamber maintained at 37°C. The atrioventricular node was crushed, and the heart was electrically stimulated at a rate of 300 beats/min by using a Phipps and Bird stimulator (Richmond, VA). A water-filled balloon was inserted into the left ventricle and connected to a pressure transducer to measure contractile parameters. Left ventricular (LV) developed pressure (LVDP) was measured as the systolic minus the diastolic pressure. The LV end-diastolic pressure (LVEDP) was adjusted to 10 mmHg at the beginning of the experiment, and the LV pressure was differentiated to estimate the rate of ventricular pressure development (+dP/dt) and the rate of ventricular pressure decay (−dP/dt). Data were acquired using Acqknowledge 3.03 software for Windows (Biopac System).

Control hearts were perfused for 120 min. For the I/R group, hearts were perfused for 60 min and then subjected to 30 min of global ischemia followed by reperfusion for 30 min. IP hearts were subjected to three 10-min cycles of ischemia and reperfusion. For the IP + I/R group, hearts were subjected to three 10-min cycles of I/R, followed by 30 min of global ischemia and 30 min of reperfusion. At the end of each experiment, the heart was immediately frozen by being clamped, immersed in liquid nitrogen, and then stored at −70°C before use.

Isolation of cardiac sarcolemmal preparations. Ventricular tissue of three hearts were pooled, and purified sarcolemmal membrane fraction was isolated according to the method of Pitts (30) as modified by Kaneko et al. (16). The final pellet was suspended in 0.25 M sucrose-10 mM histidine (pH 7.2), quickly frozen, and stored at −70°C. Marker enzyme activities (10, 16, 29) revealed a 16- to 18-fold purification of the membrane with respect to Na+-K+-ATPase activity in the heart homogenate and minimal cross contamination with other subcellular organelles such as mitochondria, sarcoplasmic reticulum, and myofibrils.

Measurement of ATPase enzyme activities and Na+-dependent Ca2+ uptake. Na+-K+-ATPase activity was measured using a method described previously with some modifications (10). Briefly, the sarcolemma membrane (40 μg) was incubated for 5 min at 37°C with the following (in mM): 1.0 EGTA (Tris) (pH 7.4), 5 NaCl, 6 MgCl2, 100 NaCl, and 10 KCl. An ATP-regenerating system was added to the incubation medium to maintain the ATP concentration; this consisted of 2.5 mM phosphoenolpyruvate and 10 IU/ml pyruvate kinase. The reaction was started by adding 0.025 ml of 80 ml Tris-ATP (pH 7.4) and terminated after 5 min with 0.5 ml cold 12% trichloroacetic acid. The liberated phosphate was estimated by the method of Tausk and Shorr (38). Different concentrations of Mg-ATP were used by being preincubated with the amounts of Mg2+ and ATP required to achieve the final concentration of Mg-ATP in the incubation medium were determined according to the “SPECS” FORTRAN program developed by Fabiato (11). Na+-K+-ATPase activity was calculated as the difference between activities with and without Na+ plus K+. Mg2+-ATPase activity was determined as the difference between the activities with and without Mg2+ in the absence of Na+ and K+ in the incubation medium. The Na+-dependent Ca2+ uptake was measured as described previously (9). Briefly, sarcolemmal vesicles from various groups (7.5 μg protein/tube) were preloaded with NaCl-MOPS buffer at 37°C for 30 min; it was then rapidly diluted (50 times) with Ca2+-free uptake medium containing 140 mM KC1, 20 mM MOPS, 0.4 μM valinomycin, 0.3 μM CaCl2, and 20 μM CaCl2 concentration in pH 7.4. After 2 s, the reaction was stopped by the addition of 0.03 ml ice-cold stopping buffer containing (in mM) 140 KCl, 1 LaCl3, and 20 MOPS at pH 7.4. Samples of the total reaction mixture were filtered through 0.45-μm Millipore filters and washed twice with 2.5 ml ice-cold washing solution containing (in mM) 140 KCl, 0.1 LaCl3, and 20 MOPS in pH 7.4. The radioactivity of the filters was measured using a Beckman LS 1701 counter. Na+-dependent Ca2+ uptake activity was corrected by subtraction of the nonspecific Ca2+ uptake values.

RNA isolation and Northern blot analysis. Total cellular RNA was extracted from the ventricular tissue of six hearts from each group by using guanidinium thiocyanate methods (7). Samples normalized to 20 μg of the total RNA were denatured with formaldehyde and run on a 1% agarose-formaldehyde gel. The fractionated mRNA transcripts were transferred to a charged nylon membrane with respect to Na+-K+-ATPase activity in the heart homogenate and minimal cross contamination with other subcellular organelles such as mitochondria, sarcoplasmic reticulum, and myofibrils.
Na+/Ca2+ exchanger (courtesy of Dr. K. D. Philipson; Los Angeles, CA). The cDNA fragments were radiolabeled with α-[32P]dCTP (NEN Life Sciences Products; Boston, MA) with a Random Primers DNA Labeling Kit (GIBCO). For 18S rRNA measurement, we used a 25-mer synthetic oligonucleotide complementary to the rat 18S RNA sequence (1,046–1,070 nt) (5). Synthetic oligonucleotides were 5′-end labeled using T4 polynucleotide kinase and [α-32P]ATP (GIBCO). The hybridized blots were exposed to X-ray film (Kodak-X-Omat), and radiolabeled mRNA bands were scanned using an image densitometer (model GS-670, Bio-Rad; Mississauga, ON, Canada) and quantified with Image Analysis software. The densitometric value for the band was divided by the corresponding 18S rRNA value to obtain the value normalized for loading.

Western blot analysis. The relative protein contents of Na+-K+-ATPase isoforms and Na+/Ca2+ exchanger were determined as previously described (17, 18). Sarcolemmal membranes (20 μg of total protein/lane) were separated on a 10–12% SDS-PAGE gel and electroblotted to polyvinylidene difluoride membranes (Boehringer Mannheim). The Na+-K+-ATPase isoforms and Na+/Ca2+ exchanger were detected using the following primary antibodies: monoclonal anti-α1 mouse IgG (0.05 μg/ml); polyclonal anti-α2 rabbit IgG (1:1,000); polyclonal anti-α3 rabbit IgG (1 μg/ml); monoclonal anti-β1 mouse IgG (0.8 μg/ml); polyclonal anti-β2 rabbit IgG (1:1,000) (Upstate Biotechnology; Lake Placid, NY); and monoclonal anti-Na+-Ca2+ mouse IgG (1:2,000) (Research Diagnostic; Flanders, NJ). Secondary antibodies consisted of biotinylated anti-mouse IgG (1:3,000) for α1, β1, and Na+/ Ca2+ exchanger (Amersham Life Science) and biotinylated anti-rabbit IgG (1:3,000) for α2, α3, and β2 (Upstate Biotechnology). Membranes were incubated for 1 h with streptavi-
din-conjugated horseradish peroxidase (1:5,000) and then processed for chemiluminescence (ECL Kit) on hyperfilm-ECL (Amersham Life Science). An imaging densitometer (model GS-670, Bio-Rad; Hercules, CA) was used to scan the bands, which were quantified using the Image Analysis Software (version 1.3). A purified microsomal preparation from a rat brain (Upstate Biotechnology) was used as a positive control to identify different Na+-K+-ATPase isoforms. No standards are commercially available for any of the Na+-K+-ATPase isoforms; thus we could not measure absolute levels of any subunit. To calculate the distribution of Na+-K+-ATPase isoforms, we determined the percentage of each subunit with respect to the total protein content for the α1-, α2-, α3-, β1-, and β2-isoforms in each group.

Statistical analysis. Values are reported as means ± SE unless otherwise indicated. Statistical analysis among groups was performed using one-way ANOVA and confirmed by two-way Student’s t-test. P < 0.05 was considered significant.

RESULTS

Contractile performance, ATPase enzyme activities, and Na+-dependent Ca2+ uptake. Cardiac contractile function was evaluated by measuring LVDP, LVEDP, +dP/dt, and −dP/dt in isolated hearts from various groups (Table 1). There was no significant change in LVDP between the control and IP hearts. However, LVDP was decreased by 77% in the I/R hearts compared with controls (P < 0.05). This decrease was prevented by IP before the I/R (IP + IR) (P < 0.05). The I/R hearts showed a significant increase (50-fold) in LVEDP, which was prevented by IP (IP + IR) (P < 0.05).

Table 1 also shows the results of Na+-K+-ATPase, Mg2+-ATPase activities, and Na+-dependent Ca2+ uptake in the various groups. Na+-K+-ATPase activity fell by 27% in the I/R hearts compared with controls; this reduction was prevented in the IP + IR hearts. The reduction of Mg2+-ATPase in the I/R hearts was even more severe (54%) compared with controls but was also completely prevented in the IP + IR hearts. Na+-dependent Ca2+ uptake did not change in IP hearts but was decreased by 33% in the I/R hearts versus control (P < 0.05). The I/R-induced reduction was completely prevented in the IP + IR hearts.

Effect of I/R and IP on protein expression. The protein levels of Na+-K+-ATPase isoforms in the various groups (n = 6 for each group) were measured using Western blot analysis and are shown in Fig. 1. The bands of the Na+-K+-ATPase α1-, α2-, and α3-isoforms are located at ~110 kDa, with α1 being the most prominent isoforms. The β1- and β2-bands are located at ~55 and ~45 kDa, respectively. A β3-band was also detected at ~40 kDa, but it was too faint to accurately assess any change among the various groups. There was also a nonspecific band that always appeared between 66 and 90 kDa, which may be due to a cross-reaction with secondary antibodies.

The protein level of the Na+-K+-ATPase α1-isoform decreased to a lesser extent (<15%) in the I/R hearts as compared with controls. There was also a decrease in Na+-dependent Ca2+ uptake in I/R hearts as compared with controls. The autophosphorylation activity was also decreased in the I/R hearts as compared with controls.
relative to other isoforms, and this decrease was not prevented by IP. The Na\(^+\)-K\(^+\)-ATPase \(\alpha_2\), \(\alpha_3\), \(\beta_1\), and \(\beta_2\)-protein levels, on the other hand, were decreased in the I/R hearts by 71%, 85%, 27%, and 65%, respectively, compared with control hearts. However, only the changes in protein levels of \(\alpha_2\), \(\alpha_3\), and \(\beta_2\)-protein levels, on the other hand, were decreased in the I/R hearts by 71%, 85%, 27%, and 65%, respectively, compared with control hearts. However, only the changes in protein levels of \(\alpha_2\), \(\alpha_3\), and \(\beta_2\)-protein levels, on the other hand, were decreased in the I/R hearts by 71%, 85%, 27%, and 65%, respectively, compared with control hearts.

Thus a change of the relative percentage of various isoforms occurred following I/R. However, IP preceding I/R prevented these changes, whereas IP alone did not have much effect. The bands of the Na\(^+\)-Ca\(^{2+}\) exchanger are located at 120, 160, and 70 kDa (29, 30). In Fig. 2A, the density of the 120-kDa band was compared among various groups. This band alone showed a decrease of 72% in the I/R group, and this reduction was almost completely prevented in the IP + I/R group (P < 0.05). A similar pattern was seen when we compared other bands of the Na\(^+\)-Ca\(^{2+}\) exchanger.

**Effect of I/R and IP on gene expression.** We measured the steady-state level of mRNA of Na\(^+\)-K\(^+\)-ATPase isoforms in various groups (n = 6 for each group) (Fig. 3). Northern blot analysis showed that I/R reduced the level of Na\(^+\)-K\(^+\)-ATPase \(\alpha_2\), \(\alpha_3\), and \(\beta_1\) mRNA by 55, 67, and 38% compared with controls, respectively (P < 0.05). The band for the \(\alpha_1\)-isoform was reduced to a lesser extent (~15%) compared with other isoforms, and this reduction was unaffected by IP. The \(\alpha_1\) mRNA level increased in the IP group, but the reason for this increase is not clear. IP preceding I/R (IP + IR) atten-

### Table 2. Remodeling effect of I/R and IP on protein contents of Na\(^+\)-K\(^+\)-ATPase subunits

<table>
<thead>
<tr>
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<th>C</th>
<th>IP</th>
<th>I/R</th>
<th>IP + I/R</th>
</tr>
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<tbody>
<tr>
<td>(\alpha_1)</td>
<td>38.1 ± 3.4</td>
<td>38.6 ± 3.5</td>
<td>52.3 ± 4.8</td>
<td>42.5 ± 3.5</td>
</tr>
<tr>
<td>(\alpha_2)</td>
<td>18.8 ± 4.1</td>
<td>18.6 ± 4.3</td>
<td>8.7 ± 1.4</td>
<td>17.8 ± 3.4</td>
</tr>
<tr>
<td>(\alpha_3)</td>
<td>6.0 ± 1.4</td>
<td>5.9 ± 1.4</td>
<td>1.2 ± 0.3</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>(\beta_1)</td>
<td>28.2 ± 4.6</td>
<td>28.7 ± 4.9</td>
<td>32.8 ± 2.5</td>
<td>28.0 ± 5.4</td>
</tr>
<tr>
<td>(\beta_2)</td>
<td>8.9 ± 1.2</td>
<td>8.9 ± 0.9</td>
<td>5.0 ± 1.7</td>
<td>7.5 ± 0.8</td>
</tr>
</tbody>
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Values are the percentage of protein contents of Na\(^+\)-K\(^+\)-ATPase isoforms relative to total protein measured for both \(\alpha\)- and \(\beta\)-subunits in the different experimental groups (n = 6). *P < 0.05 vs. C; †P < 0.05 vs. I/R.
uated the reduction of mRNA levels of the α2-, α3-, and β1 isoforms to 20, 30, and 32%, respectively. This pattern was also in agreement with the protein levels of the various isoforms except that the protein level of β1 was not significantly affected by IP. The mRNA level of the Na\(^{+}/\)Ca\(^{2+}\) exchanger decreased by 43% in the I/R group, and this reduction was completely prevented by IP (\(P < 0.05\)) (Fig. 2B).

The assays for mRNA and protein levels of Na\(^{+}/\)K\(^{-}\)-ATPase subunits used in this study are not quantita-
tive. Thus differential changes in the levels of subunits could potentially be due to an underestimation of the changes in some of the subunits as a consequence of oversaturation of the density of these bands. However, assays for each of the isoforms in the control, IP, I/R, and IP + I/R groups were performed under identical conditions and argues against this being the case. Furthermore, the band for any one isoform should not be compared with other bands because the density of the band depends on the quality of the antibody (or molecular probe) and the exposure time. Thus caution needs to be exercised when interpreting these data. Nonetheless, the relation between protein content and optical density was linear for the Western blots (Fig. 4). It should be noted that the purified rat brain microsomal Na\(^+\)/K\(^+\)-ATPase preparation has at least 10 times higher activity than the heart sarcolemmal preparation. Thus the amount of sarcolemmal protein used in this study should be well within the linear range of the Western blot assay. The Northern blots in Fig. 5 also indicate that the amount of mRNA used was within the linear range. Although it is not readily apparent in Figs. 1 and 2, the data in Table 2 clearly indicate that both the α2- and α3-isoform levels in the control and IP hearts are expressed at markedly lower levels than the α1-isoform. This is consistent with previous observations by our group and others (22, 43, 44).

**DISCUSSION**

The present study demonstrates that I/R reduces myocardial contractile function and Na\(^+\)/K\(^+\)-ATPase activity in the isolated rat heart. I/R induced a large decrease in Na\(^+\)/K\(^+\)-ATPase α2- and α3-protein levels and a lesser reduction in α1-protein levels. Also, β2-protein levels were reduced to a greater extent than β1-protein levels. A similar pattern was seen at the mRNA level. A change in the percentage of various isoforms relative to the total subunits also occurred following I/R for both protein and mRNA levels. Thus differential changes in Na\(^+\)/K\(^+\)-ATPase subunits seem to occur following I/R, and this change in the molecular structure of the enzyme may represent remodeling of the Na\(^+\)/K\(^+\)-ATPase. Our differential remodeling

![Fig. 4. Relation between protein content and optical density (OD) for the α1-, α2-, α3-, β1-, β2-, and β3-Na\(^+\)/K\(^+\)-ATPase isoforms obtained using purified rat brain microsomal Na\(^+\)/K\(^+\)-ATPase. Regression squared (r\(^2\)) values (n = 4) as well as representative Western blots are shown for each isoform.](http://ajpheart.physiology.org/)

*Fig. 4. Relation between protein content and optical density (OD) for the α1-, α2-, α3-, β1-, β2-, and β3-Na\(^+\)/K\(^+\)-ATPase isoforms obtained using purified rat brain microsomal Na\(^+\)/K\(^+\)-ATPase. Regression squared (r\(^2\)) values (n = 4) as well as representative Western blots are shown for each isoform.*
agrees with the literature with respect to expression of various Na⁺-K⁺-ATPase isoforms in the myocardium during development and in different pathological conditions. The nonsignificant change in α₁ expression and upregulation of protein and mRNA following I/R correlates well with its role as the ubiquitous maintenance isofrom in the rat heart. Cardiac hypertrophy is also associated with no change in α₁-protein and mRNA expression (6). Alternatively, α₂- and α₃-isoforms, the ouabain-sensitive isoforms, were significantly reduced after I/R. A similar reduction of α₂-isoform levels was also reported in cardiac hypertrophy (6) and hypokalemia (1). The β₁-protein level was decreased following I/R in the present study but to a lesser extent than the β₂-protein levels. Similarly, β₁ mRNA levels are unchanged in cardiac hypertrophy (6). Thus β₁ may share a comparable role to α₁ in terms of “housekeeping” of the heart. In agreement with the differential remodeling in our results, a study on rat cardiac hypertrophy showed that the α-isofroms are independently regulated at the pretranslational level, and Na⁺-K⁺-ATPase expression is controlled by distinct regulatory mechanisms (3, 6). However, extensive studies need to be carried out to fully understand the differential changes in Na⁺-K⁺-ATPase isoforms in the I/R hearts.

The reason for the alteration of various isoforms in our study is still speculative, but their sensitivity to I/R-induced environmental changes such as oxidative stress, Ca²⁺ overload, and proteolysis, as well as their distinct structure, may play a key role. Oxidative stress is involved in I/R damage (19, 33). Previous studies reported that α₂- and α₃-isoforms have a greater sensitivity to H₂O₂ and hydroxyl radical than α₁-isoforms (14, 41), and these differences in sensitivities of various Na⁺-K⁺-ATPase isoforms are attributed to distinct structural features (14). These results are consistent with our findings of a greater reduction of α₂- and α₃-isoform levels compared with α₁ following I/R. Na⁺-K⁺-ATPase α₂- and α₃-isoforms mRNA in the rat heart are expressed at higher levels in the conduction system and junctional complexes, whereas α₁ is distributed ubiquitously in both atrial and ventricular tissues (43). Thus the α₂- and α₃-isoforms may play a distinct role in impulse transmission (44). In this perspective, we can hypothesize areas of the heart containing abundant α₂- and α₃-isoforms may be more susceptible to damage by I/R than other areas of the myocardium. Furthermore, it is known that oxidative stress during I/R is implicated in altering cellular protein structure and function in the heart (14, 39). Specifically, the importance of protein and nonprotein sulfhydryl groups in affecting Na⁺-K⁺-ATPase function was recently demonstrated by depleting the protein sulfhydryl oxidation and glutathione, which led to depressed Na⁺-K⁺-ATPase activity (12). The distribution of protein sulfhydryl groups and reduced glutathione in the various α- and β-isoforms is not clear. However, several reports suggest higher numbers of free sulfhydryl groups for α₂- and β2-isoforms than other isoforms.
Thus a differential remodeling in the protein content of the different isoforms following I/R may reflect their relative distribution of protein sulf-hydryl groups and/or reduced glutathione.

Human heart failure is also associated with a reduction of Na\(^+\)-K\(^-\)-ATPase activity and \(\alpha_1\), \(\alpha_3\), and \(\beta_1\)-protein levels, whereas \(\alpha_2\) and Na\(^+\)/Ca\(^{2+}\)-exchanger protein levels did not change (32). This led to the suggestion that the failing heart may have an enhanced sensitivity to cardiac glycosides. Our data showed a different pattern of alterations of Na\(^+\)-K\(^-\)-ATPase and Na\(^+\)/Ca\(^{2+}\) exchanger following I/R. It is unclear why our findings in the rat differ from those in human hearts, but this may reflect differences in the etiology of the insults or species differences. In any case, there appears to be import structural and functional differences among Na\(^+\)-K\(^-\)-ATPase isoforms and the Na\(^+\)/Ca\(^{2+}\) exchanger during various myocardial insults. Further investigation is needed to evaluate the change in sensitivity of Na\(^+\)-K\(^-\)-ATPase isoforms to cardiac glycosides, especially after ischemia or I/R injury.

IP prevents many defects induced by I/R (4, 39). In the present study, IP alone did not alter myocardial contractile function, Na\(^-\)-K\(^-\)-ATPase activity, or protein mRNA levels compared with controls. IP preceding I/R, however, protected against myocardial dysfunction and the I/R-induced decrease in Na\(^+\)-K\(^-\)-ATPase activity. Our results agree with a recent finding that IP protects Na\(^-\)-K\(^-\)-ATPase activity in rabbit hearts subjected to 20 min of sustained ischemia in vivo (26). At the protein level, we found that IP preceding I/R prevented the reduction of Na\(^+\)-K\(^-\)-ATPase \(\alpha_2\), \(\alpha_3\), and \(\beta_2\)-isoforms but not the decrease in \(\beta_1\)-isoform induced by I/R. A similar pattern was also seen at the mRNA levels, except that the decrease in the \(\beta_1\)-isoform levels was completely prevented by IP. Prevention of the \(\beta_1\)-isoform reduction at the gene, but not the protein level, during the short duration of this study suggests that this protection may occur only at the transcriptional level and that the \(\beta_1\)-isoform may play more of a role in the second window of IP than in the acute classic phase of IP. From the available data, it is difficult to predict whether Na\(^+\)-K\(^-\)-ATPase isoforms in the I/R and IP + I/R hearts are regulated independently or if there is cross talk between the subunits.

We also measured the Na\(^+\)/Ca\(^{2+}\) exchanger protein and mRNA levels to assess the specificity of the effects of I/R and IP on Na\(^+\)-K\(^-\)-ATPase isoforms in our study. I/R significantly reduced the protein and mRNA levels of the Na\(^+\)/Ca\(^{2+}\) exchanger. The reduction of Na\(^+\)/Ca\(^{2+}\) exchanger protein and mRNA levels correlate with the decrease in Na\(^+\)/Ca\(^{2+}\) exchanger activity seen here and previously (3, 9). Moreover, our results show that IP prevents the I/R-induced decrease in Na\(^+\)/Ca\(^{2+}\) exchanger at both the protein and mRNA levels. In agreement with our data, Nawada et al. (26) reported that IP preserved the Na\(^+\)/Ca\(^{2+}\) exchanger activity and that Na\(^+\)/Ca\(^{2+}\) exchanger activity was actually increased in ischemic versus nonischemic areas of preconditioned, but not control, rabbit hearts. Thus protection of Na\(^+\)/Ca\(^{2+}\) exchanger activity may be due to prevention of the alterations in protein content and mRNA levels induced by I/R.

Prevention of the I/R-induced remodeling of Na\(^+\)-K\(^-\)-ATPase isoforms by IP is probably a functional modification and consequently a mediator of IP, rather than the final end effector. IP prevents the loss of Na\(^+\)-K\(^-\)-ATPase activity during the sustained ischemia, and this may underlie its protective effects in the myocardium. IP hearts would be better able to handle the intracellular [Na\(^+\)] and intracellular [Ca\(^{2+}\)] overload that occurs during I/R because Na\(^+\)-K\(^-\)-ATPase activity is essentially preserved in these hearts. In this regard, it is emphasized that the present study reports two major and novel findings. First, I/R rapidly induces differential changes of Na\(^+\)-K\(^-\)-ATPase isoforms at both the protein and mRNA level. This remodeling of Na\(^+\)-K\(^-\)-ATPase may be responsible, at least in part, for the decline in enzyme activity following I/R and suggests distinct roles and differential sensitivity of individual isoforms to I/R. Second, IP prevents the changes in Na\(^+\)-K\(^-\)-ATPase isoforms induced by I/R at both the protein and mRNA levels. These changes are consistent with the IP-induced protection of myocardial contractile dysfunction and Na\(^+\)-K\(^-\)-ATPase activity. Inhibition of Na\(^+\)-K\(^-\)-ATPase activity is known to reduce the infarct limiting size of IP (26), but our results do not provide any information regarding the cause and effect of IP-mediated protection on Na\(^+\)-K\(^-\)-ATPase subunits or enzyme activity. Indeed, our data indicate that IP has no effect on Na\(^+\)-K\(^-\)-ATPase activity or isoform content. Thus it is unlikely that the IP-mediated protection against the I/R induced remodeling of Na\(^+\)-K\(^-\)-ATPase is due to a direct effect of IP.

DISCLOSURES

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