Glutathione and K\(^+\) channel remodeling in postinfarction rat heart

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Rozanski, George J., and Zhi Xu. Glutathione and K\(^+\) channel remodeling in postinfarction rat heart. Am J Physiol Heart Circ Physiol 282: H2346–H2355, 2002. First published January 31, 2002; 10.1152/ajpheart.00894.2001.—Electrical remodeling of the diseased ventricle is characterized by downregulation of K\(^+\) channels that control action potential repolarization. Recent studies suggest that this shift in electrophysiological phenotype involves oxidative stress and changes in intracellular glutathione (GSH), a key regulator of redox-sensitive cell functions. This study examined the role of GSH in regulating K\(^+\) currents in ventricular myocytes from rat hearts 8 wk after myocardial infarction (MI). Colorimetric analysis of tissue extracts showed that endogenous GSH levels were significantly less in post-MI hearts compared with controls, which is indicative of oxidative stress. This change in GSH status correlated with significant decreases in activities of glutathione reductase and \(\gamma\)-glutamylcysteine synthetase. Voltage-clamp studies of isolated myocytes from rat hearts demonstrated that downregulation of the transient outward K\(^+\) current \((I_{\text{to}})\) could be reversed by pretreatment with exogenous GSH or N-acetylcysteine, a precursor of GSH. Upregulation of \(I_{\text{to}}\) was also elicited by dichloroacetate, which increases glycolytic flux through the GSH-related pentose pathway. This metabolic effect was blocked by inhibitors of glutathione reductase and the pentose pathway. These data indicate that oxidative stress-induced alteration in the GSH redox state plays an important role in \(I_{\text{to}}\) channel remodeling and that GSH homeostasis is influenced by pathways of glucose metabolism.

VENTRICULAR ARRHYTHMIAS ARE a major clinical problem contributing to the high incidence of sudden death in chronic disease states that cause cardiac electrical remodeling (23, 34). The mechanisms underlying this greater risk of arrhythmogenesis are not fully understood, but experimental studies suggest that downregulation of K\(^+\) currents is an important contributing factor (23, 34). A pathogenic decrease in K\(^+\) channel activity is proposed to lead to abnormal repolarization, which would increase dispersion of refractoriness and the likelihood of reentry or initiate triggered activity from afterdepolarizations (23, 25, 26). Moreover, long-term downregulation of repolarizing K\(^+\) channels may elevate intracellular Ca\(^{2+}\) concentration and accelerate the progression toward heart failure (15, 37).

At least three major K\(^+\) currents contribute to repolarization and action potential duration in ventricular myocardium, including the transient outward current \((I_{\text{to}})\), the delayed rectifier current \((I_{\text{K}})\), and the inward rectifier \((I_{\text{K}}\text{,i})\), each of which differs in density and voltage- and time-dependent properties (24). Of these currents, \(I_{\text{to}}\) is consistently decreased in chronic cardiac disorders characterized by electrical remodeling (12, 14, 15, 26, 27, 29, 35, 38–40) including the human heart (4, 13). Although some time- and voltage-dependent properties of \(I_{\text{to}}\) are altered, the major electrophysiological phenotype of the remodeled ventricle is a decrease in current density, which is probably due to a decrease in the number of functional \(I_{\text{to}}\) channels (14). Moreover, recent experimental studies of heart failure have determined that decreased \(I_{\text{to}}\) density and delayed repolarization are correlated with decreased mRNA expression and levels of channel protein that underlie \(I_{\text{to}}\), i.e., the voltage-gated K\(^+\) channels (Kv) Kv4.2, and Kv4.3 (9, 12, 13, 15, 40). Nevertheless, the cellular mechanisms by which Kv channels are downregulated in the remodeled heart are still unclear.

Recent studies from our laboratory have shown that \(I_{\text{to}}\) density in isolated ventricular myocytes from rat hearts with chronic myocardial infarction (MI) is upregulated in vitro by compounds augmenting cellular levels of glutathione (30, 38), an endogenous regulator of redox-sensitive cell functions (1, 6, 7, 10, 22). We have also reported that \(I_{\text{to}}\) upregulation occurs in myocytes treated with metabolic agents that activate glucose utilization (29, 39). These findings suggest that oxidative stress and altered glucose metabolism may play an important role in K\(^+\) channel remodeling through alteration in cell redox state, which is controlled by the relative concentrations of reduced and oxidized glutathione (GSH and GSSG, respectively; see Refs. 6 and 10). Therefore, the present study was designed to examine the role of GSH in regulating \(I_{\text{to}}\) in myocytes from rat hearts with chronic MI and to determine whether glucose metabolism is functionally coupled to the myocyte GSH system. The results of our

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experiments suggest that oxidative stress-induced alteration in GSH redox state contributes to \( I_{\text{to}} \) channel remodeling and that GSH homeostasis in ventricular myocytes is supported by the pentose pathway of glucose metabolism.

**METHODS**

**Post-MI rat model: isolation of cardiac myocytes.** A chronic post-MI model of ventricular dysfunction was used in the present investigation, as described previously (29, 30). Briefly, male Sprague-Dawley rats (body wt 200–250 g) under brevital anesthesia (50 mg/kg ip) were intubated and artificially ventilated with a respirator. A left thoracotomy was performed, and the left coronary artery was ligated by positioning a suture between the pulmonary artery outflow tract and the left atrium. The thorax was closed, and the rats were allowed to recover for ~8 wk before in vitro experimentation. This coronary ligation protocol produced infarcts of 30–40% of the left ventricular free wall and was accompanied by physiological signs of heart failure after several weeks (29, 30).

In the present study, data were collected from a total of 25 post-MI rats and compared with 27 time-matched controls which were either sham operated (\( n = 11 \)) or unoperated (\( n = 16 \)). We found no significant differences in mean data from sham-operated and unoperated rats, and thus data control were pooled from these two groups.

Ventricular myocytes of epicardial and endocardial origin were dissociated from isolated, perfused hearts using a collagenase-digestion procedure described previously (27, 29, 30, 38, 39). Most electrophysiological experiments summarized in this report were done on myocytes isolated from the left ventricle, although in some studies the data were obtained from right ventricular myocytes. After collagenase digestion, dispersed myocytes were suspended in DMEM/Ham’s F-12 medium that contained penicillin (100 U) plus streptomycin (100 μg/ml), and were stored in an incubator at 37°C until used, which was usually within 6 h of isolation. For some experiments, myocytes were cultured for 18 h before study. Aliquots of myocytes were transferred to a cell chamber mounted on the stage of an inverted microscope and were superfused with standard external solution that contained (in mM) 138 NaCl, 4.0 KCl, 1.2 MgCl\(_2\), 1.8 CaCl\(_2\), 10 glucose, 5 HEPES, pH 7.4, and 0.5 CdCl\(_2\) to block Ca\(^{2+}\) channels. All voltage-clamp experiments were done at room temperature (22–25°C).

**Recording techniques.** Ionic currents were recorded using the whole cell configuration of the patch-clamp technique. Briefly, glass pipettes were pulled (model P-87, Sutter Instruments) to an internal tip diameter of ~2 μm and filled with a solution containing (in mM) 135 KCl, 3 MgCl\(_2\), 10 HEPES, 3 Na\(_2\)-ATP, 10 EGTA, 0.5 Na-GTP, and pH 7.2. Filled pipettes were coupled to a patch-clamp amplifier (Axopatch 1C; Axon Instruments) and the liquid junction potential was corrected. After series-resistance compensation in whole cell recording mode, capacitance (\( C_m \)) was calculated as the area under the capacitative transient divided by the amplitude of an applied test pulse. A computer program (pClamp; Axon Instruments) controlled command potentials and acquired current signals filtered at 2 kHz using a four-pole low-pass Bessel filter. Currents were sampled at 4 kHz by a 12-bit-resolution analog-to-digital converter and stored on the hard disk of a computer.

\( I_{\text{to}} \) was evoked in each cell by 500-ms depolarizing pulses to test potentials between −40 and +60 mV (0.2 Hz). The holding potential in all experiments was −80 mV, and a 100-ms prepulse to −60 mV was applied to inactivate the fast Na\(^+\) current. For each test pulse, \( I_{\text{to}} \) amplitude was measured as the difference between peak outward current and the steady-state current at the end of the depolarizing clamp pulse and was normalized as current densities by dividing measured current amplitude by \( C_m \). In addition to measuring current voltage (\( I-V \)) relations, voltage- and time-dependent properties of \( I_{\text{to}} \) were determined. First, steady-state activation parameters were derived from \( I-V \) relations by calculating conductance (\( G \)) at each test potential (\( V_m \)), normalized to maximum conductance at +60 mV (\( G_{\text{max}} \)), and plotting these values as a function of \( V_m \). Data were fitted by a Boltzmann distribution to calculate the activation parameters \( V_{1/2} \) and \( k \) according to the relationship

\[
G/G_{\text{max}} = 1/[1 + \exp(V/V_{1/2} - k)]
\]

where \( V_{1/2} \) is the voltage at half-maximal activation, and \( k \) is the slope factor at \( V_m = V_{1/2} \). Steady-state inactivation was examined by applying 500-ms prepulses from −100 to 0 mV before pulsing to +60 mV and inactivation curves were constructed by plotting normalized current (\( I/I_{\text{max}} \)) against prepulse voltage. These data were also fitted by a Boltzmann distribution. GSSG concentrations were determined by delivering two identical 500-ms depolarizing pulses from −80 to +60 mV and varying the interpulse interval from 50 to 3,000 ms. Reactivation curves were constructed by plotting the ratio of peak \( I_{\text{to}} \) elicited by the second pulse relative to the first (\( I_1/I_2 \)) against interpulse interval and were fitted to a single exponential to derive the time constant of recovery.

Alterations in \( I_{\text{to}} \) post-MI were also compared with two other major repolarizing K\(^+\) currents in rat ventricular myocytes. Specifically, data were compared with the inward rectifier current (\( I_{\text{k1}} \)) and the steady-state outward current (\( I_{\text{to}} \)), which is proposed to be carried by a delayed rectifier channel (24). \( I_{\text{k1}} \) was recorded with 100-ms test pulses applied to potentials of −120 to −40 mV (holding potential = −80 mV) and data were expressed as \( I-V \) relations. \( I_{\text{to}} \) was measured from current traces generated from \( I_{\text{k1}} \) clamp protocols as the steady-state current at the end of each depolarizing clamp pulse.

**Measurement of GSH and related enzyme activities.** The major intracellular redox buffer GSH was measured using the method of Floreani et al. (8). Briefly, tissue samples (50–100 mg) from the left ventricle were homogenized in 6% metaphosphoric acid, centrifuged at 4°C (3,000 g) for 10 min, and the supernatant was collected for assay. Total glutathione (GSH and GSSG) was measured in 100-μl samples of the supernatant by recording the formation of 2-nitro-5-thiobenzoic acid at 412 nm (25°C) in a spectrophotometer (Genesys II) in the presence of 0.25 mM 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB), 0.4 mM NADPH, and 2 U type III glutathione reductase (GR, Sigma). GSSG was determined by derivatizing 150-μl samples of supernatant with 3 μl of undiluted 2-vinylpyridine and assaying 100-μl aliquots of the derivatized sample as described for total GSH. Standard curves were constructed for GSSG concentration, and GSH concentration was calculated by subtracting the concentration of GSSG from the total glutathione (GSH and GSSG). Measured concentrations of GSH and GSSG were expressed per gram of wet tissue weight and as a ratio (GSH/GSSG).

Endogenous GR activity was measured by the method of Carlberg and Mannervik (5). Briefly, isolated tissue samples (50–100 mg) from the interventricular septum were homogenized in ice-cold Tris buffer (0.1 M, pH 8.0) with 2 mM EDTA and centrifuged at 4°C (6,000 g) for 30 min. A 200-μl aliquot of the supernatant was added to a 1-ml cuvette containing KH\(_2\)PO\(_4\) buffer (0.2 M, pH 7.0) with 2 mM EDTA,
20 mM GSSG, and 2 mM NADPH. The change in absorbance at 340 nm was monitored for 5 min at 30°C. A unit of GR activity was defined as the amount of enzyme catalyzing the reduction of 1 μM NADPH per minute. Specific activity was expressed in milliunits (mU) per milligram of protein, the latter of which was measured using a commercial kit (Pierce).

γ-Glutamylcysteine synthetase (γ-GCS) activity was determined using the method of Seelig and Meister (31). Briefly, tissue samples were prepared as described above and a 50-μl aliquot of supernatant was added to a reaction mixture containing 0.1 M Tris buffer, 150 mM KCl, 5 mM Na2-ATP, 2 mM phosphoenolpyruvate, 10 mM L-glutamate, 10 mM L-α-amino-nobutyrate, 20 mM MgCl2, 2 mM Na2-EDTA, 0.2 mM NADH, 17 μg pyruvate kinase, and 17 μg lactate dehydrogenase. The change in absorbance at 340 nm was monitored for 5 min at 37°C, and γ-GCS activity was expressed in milliunits, which are defined as the activity converting 1 nanomole of NADH to NAD per minute. Enzyme activity for each sample was normalized per milligram of protein.

**RESULTS**

The characteristics of post-MI rats used in the present investigation were qualitatively similar to our previous studies done 16 wk after coronary artery ligation (29). Briefly, the heart wt-to-body wt ratio was 65% greater in 8-wk post-MI rats compared with controls (post-MI, 5.6 ± 1.0 mg/g; n = 6; control, 3.4 ± 0.2 mg/g; n = 5; P < 0.05), which indicates the presence of compensatory cardiac hypertrophy. In agreement with this change in cardiac structure, the mean Cm of isolated left ventricular myocytes from post-MI hearts was 19% greater than control (post-MI, 205.3 ± 5.0 pF; n = 106; control, 171.8 ± 4.4 pF, n = 97; P < 0.05). Although we did not measure hemodynamic parameters in post-MI rats, previous studies using this model have documented marked elevations in left ventricular end-diastolic pressure and other indices of heart failure (11). Accordingly, the present study also found a significant increase in the lung wt-to-body wt ratio (post-MI, 6.4 ± 1.6 mg/g, n = 6; control, 3.5 ± 0.1 mg/g, n = 5; P < 0.05).

**GSH system in post-MI rat heart.** Figure 1 outlines the major components of the myocardial GSH system and identifies specific steps that were examined in the present study. In ventricular myocytes, as in most mammalian cells, the intracellular environment is normally maintained in a reduced state due to a high GSH/GSSG ratio, which is controlled by the activities of two major enzymes: 1) GR, which catalyzes the reduction of GSSG to GSH using NADPH as a source of reducing equivalents; and 2) γ-GCS, the rate-limiting step in GSH synthesis (5, 22, 31). It is also proposed that glucose-6-phosphate dehydrogenase (G6PDH), the rate-limiting enzyme of the pentose pathway, is the major source of NADPH utilized by GR (1, 41). Under conditions of oxidative stress, GSH-mediated inactivation of reactive oxygen species increases GSSG production, which decreases the GSH/GSSG ratio and leads to a more oxidized intracellular environment. Accordingly, significant changes in intracellular levels of GSH and GSSG have been documented in post-MI models of heart failure, which indicates that the noninfarcted, surviving myocardium is under marked oxidative stress (11). To assess the status of the GSH system in our model, we first measured GSH and GSSG concentrations in tissue samples obtained from the left ventricle. Figure 2, A and B, illustrates that the mean GSH concentration in post-MI hearts was 50% less than control (P < 0.05), whereas the GSSG level was twofold higher in post-MI hearts (P < 0.05). Therefore, as summarized in Fig. 2C, the GSH/GSSG ratio, which is indicative of the cell-redox state, was decreased by 76% in the post-MI rat heart compared with control (P < 0.05). Second, to explore the mechanisms responsible for the change in GSH status post-MI, the activities of GR and γ-GCS were measured in tissue samples of interventricular septum. Figure 3 shows that the basal activities of both GSH-related enzymes were significantly decreased in post-MI hearts. Specifically, GR activity in post-MI hearts was 31% less than control (P < 0.05), whereas the activity of γ-GCS (Fig. 3B) in the post-MI group was decreased 26% from control (P < 0.05).

**Effects of exogenous GSH and N-acetylcysteine on I_K.** K+ channel remodeling 8 wk post-MI was characterized by measuring basal properties of three major K+ currents that control repolarization in rat myocardium: I_{to}, I_{ss}, and I_{K1}. Figure 4A compares superimposed current traces that have been normalized to whole cell capacitance in a control left ventricular myocyte with another myocyte isolated from a post-MI heart. The top traces illustrate that I_{to} density was markedly less in the myocyte from the post-MI heart compared with control, whereas I_{K1} density (bottom traces) was simi-
lar for both myocytes despite a marked difference in $C_m$ in these examples (control, 155 pF; post-MI, 206 pF). Figure 4B shows that the mean $I$-$V$ relation for $I_{to}$ in myocytes from post-MI hearts was shifted downward from control, with maximum $I_{to}$ density (+60 mV) being 50% less than control ($P < 0.05$). This change in electrophysiological phenotype was not accompanied by major alterations in voltage- or time-dependent properties of $I_{to}$ (Table 1) with the exception of the midpoint of the steady-state activation curve ($V_{1/2}$), which was shifted ~8 mV in the negative direction in the post-MI group ($P < 0.05$). Moreover, analyses of mean $I$-$V$ relations for $I_{ss}$ and $I_{K1}$ (Fig. 4, C and D) indicated that the densities of these $K^+$ currents were not significantly altered in the post-MI heart, which suggests that $I_{to}$ downregulation in the left ventricle post-MI is not a manifestation of a general decrease in $K^+$ channel activity.

Basal electrophysiological data were also collected from right ventricular myocytes isolated from three post-MI and two control hearts. Qualitatively similar changes post-MI were observed in the right ventricle as in the left. Briefly, maximum $I_{to}$ density in right ventricular myocytes from post-MI hearts was 67% less than control (post-MI, 15.8 ± 2.6 pA/pF; n = 15; control, 35.4 ± 4.5 pA/pF; n = 9; $P < 0.05$), whereas mean $C_m$ was 93% greater in the post-MI group (139.7 ± 10.4 pF vs. 72.2 ± 3.2 pF, respectively; $P < 0.05$). Moreover, no significant changes were observed for mean $I$-$V$ relations of $I_{ss}$ or $I_{K1}$ (data not shown).

Because the myocardial GSH concentration in post-MI hearts was significantly less than control (see Fig. 2A), we assessed the potential role of GSH in regulating $I_{to}$ channel density by incubating myocytes with GSH to replenish intracellular stores (1, 7, 19, 22). In initial experiments, incubation periods <2 h with 10 mM GSH did not affect $I_{to}$ in myocytes from post-MI hearts, but longer exposures eventually increased $I_{to}$ density to control levels. This response is summarized in Fig. 5A, which compares superimposed, normalized current traces recorded from an untreated myocyte in the post-MI group (left) with another myocyte pretreated with 10 mM GSH for 4 h. The markedly larger current density in the GSH-treated myocyte supports the notion that alteration in GSH status par-

![Fig. 2. GSH status in the left ventricle.](http://ajpheart.physiology.org/)

![Fig. 3. GSH-related enzyme activities.](http://ajpheart.physiology.org/)
The time course for upregulating $I_{to}$ by GSH is summarized in Fig. 5B, which plots mean maximum $I_{to}$ density (+60 mV) versus GSH (10 mM) incubation time. These data show that GSH treatment significantly increased maximum $I_{to}$ density compared with the untreated group (bar labeled 0) with a time delay of 2–3 h. Figure 5C compares mean $I-V$ relations recorded from control myocytes with untreated and GSH-treated (3–4 h) myocytes from post-MI hearts. In this latter group of experiments, GSH treatment increased maximum $I_{to}$ density in myocytes from post-MI hearts by 75% compared with untreated myocytes from the same group ($P < 0.05$).

The electrophysiological role of GSH in the post-MI heart was further probed by testing the effects of a GSH precursor, N-acetylcysteine (NAC). This compound supplies cysteine, the rate-limiting amino acid in GSH synthesis, after being metabolized by intracellular $N$-deacetylase (1). These experiments were similar to those described above for GSH, with myocytes being incubated with 10 mM NAC for up to 5 h. Figure 6A illustrates that NAC treatment also upregulated $I_{to}$ density, but this effect did not reach significance until 3–4 h of incubation. As shown in Fig. 6B, NAC-treated myocytes (3–4 h) from post-MI hearts exhibited a mean $I-V$ relation that was similar to control and markedly different from untreated myocytes from the post-MI group, such that maximum $I_{to}$ density in NAC-treated myocytes was increased by 95% compared with untreated myocytes from the same group ($P < 0.05$).

Moreover, the effect of NAC on myocytes from post-MI hearts was concentration-dependent when tested for an incubation period of 4–5 h (Fig. 6C).
contrast to their significant effects on myocytes from post-MI hearts, neither GSH nor NAC altered the mean $I-V$ relations for $I_{to}$ in control myocytes treated for 4–5 h. Specifically, the maximum $I_{to}$ density values in untreated ($n = 15$), GSH-treated (10 mM, $n = 8$), and NAC-treated (10 mM, $n = 7$) myocytes from control hearts were 28.3 ± 2.0, 25.8 ± 4.4, and 26.9 ± 4.7 pA/pF, respectively ($P > 0.05$).

The increase in $I_{to}$ density elicited by GSH and NAC in the post-MI group of myocytes was not accompanied by significant changes in mean $I-V$ relations for $I_{ss}$ or $I_{K1}$, nor did these redox agents alter the inactivation kinetics of $I_{to}$ at +60 mV (data not shown). There was, however, a normalization of steady-state activation. Specifically, $V_{1/2}$ values for untreated ($n = 16$), GSH-treated (10 mM, $n = 7$), and NAC-treated (10 mM, $n = 5$) myocytes from post-MI hearts were 0 ± 2.4, 11.6 ± 1.8 ($P < 0.05$), and 11.2 ± 3.2 ($P < 0.05$) mV, respectively (control, 7.8 ± 1.1 mV; see Table 1). Mean values for slope factor $k$ were not different among the three groups (untreated, 12.1 ± 0.7 mV; GSH treated, 13.5 ± 0.3 mV; NAC treated, 13.8 ± 0.3 mV; $P > 0.05$).

Relationship of glucose metabolism and GSH. The GSH/GSSG ratio in most mammalian cells is largely controlled by the activity of GR, which catalyzes the reduction of GSSG to GSH using NADPH as the source of reducing equivalents (5, 16, 17, 41; see Fig. 1). Moreover, the principal source of NADPH is postulated to be the pentose pathway of glucose metabolism (5, 17, 41). In this regard, we previously reported that metabolic activators of glucose utilization normalize $I_{to}$ density after a time delay of several hours in myocytes isolated from 8- and 16-wk post-MI rat hearts (28–30).

### Table 1. $I_{to}$ properties in control and 8-wk post-MI rat hearts

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<tr>
<th></th>
<th>Control</th>
<th>Post-MI</th>
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<tr>
<td><strong>Inactivation kinetics</strong></td>
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<tr>
<td>$n$</td>
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<tr>
<td>$\tau_1$, ms</td>
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<td>32.6±3.6</td>
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<td>$\tau_2$, ms</td>
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<td>16</td>
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<tr>
<td>$V_{1/2}$, mV</td>
<td>7.8±1.1</td>
<td>0±2.4*</td>
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<tr>
<td>$k$, mV</td>
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<td><strong>Steady-state inactivation</strong></td>
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<td>$k$, mV</td>
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<td><strong>Recovery from inactivation</strong></td>
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<tr>
<td>$n$</td>
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<tr>
<td>$\tau_{rec}$, ms</td>
<td>79.3±8.3</td>
<td>88.6±5.7</td>
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Values are means ± SE. Inactivation kinetics were measured at +60 mV. $n$, no. of myocytes; $\tau_1$ and $\tau_2$, components of inactivation; $V_{1/2}$, voltage at 50% activation or inactivation; $k$, slope factor. *$P < 0.05$ compared with control.

Fig. 5. Upregulation of $I_{to}$ density by GSH. Superimposed, normalized current traces recorded at test potentials of −40 to +60 mV are shown for myocytes from post-MI hearts untreated and treated for 4 h with 10 mM GSH (A). Maximum $I_{to}$ density measured at +60 mV is compared for myocytes from post-MI hearts treated with 10 mM GSH for different durations (B). *$P < 0.05$ compared with untreated myocytes (0). Mean $I-V$ relations of control myocytes and post-MI myocytes untreated or treated with 10 mM GSH for 3–4 h (C). *$P < 0.05$ compared with control.

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we examined the electrophysiological effects of an exogenous activator of glucose utilization, dichloroacetate (DCA), with and without inhibitors of GSH-related enzymes. Figure 7, A–C, shows that 1.5 mM DCA for 3–4 h significantly increased $I_{to}$ density in myocytes from post-MI hearts compared with untreated myocytes. Moreover, as shown in Fig. 7A, normalization of $I_{to}$ density by DCA was blocked in a separate group of myocytes by the addition of an inhibitor of GR, 1,3-bischloroethyl-nitrosourea (BCNU, 10 μM; 5–7). In a second, related series of experiments, we examined the effects of dehydroepiandrosterone (DHEA), an inhibi-
tior of G6PDH, which is the rate-limiting enzyme of the pentose pathway that generates NADPH (19; see Fig. 1). As for BCNU, the effects of DCA were blocked by the addition of 10 μM DHEA to the culture medium (Fig. 7B). Finally, to determine whether the effects of DCA included an increase in de novo GSH synthesis, DCA was restested in the presence of an inhibitor of γ-GCS, buthionine sulfoximine (BSO; 6, 7, 18, 22, 31). Because previous studies have shown that prolonged exposure to BSO is required to effectively inhibit γ-GCS (18), myocytes from post-MI hearts were pretreated with 30 μM BSO for 18 h before the addition of DCA for an additional 3–4 h. This pretreatment protocol did not significantly affect maximum basal $I_{to}$ density compared with untreated myocytes cultured for 18 h without BSO (BSO treated, 17.0 ± 2.0 pA/pF, n = 9; untreated, 14.6 ± 2.3 pA/pF, n = 11; $P > 0.05$). More importantly however, as shown in Fig. 7C, BSO did not block the normalizing effect of DCA on $I_{to}$ density in myocytes from post-MI hearts.

**DISCUSSION**

**Redox state and $I_{to}$ remodeling.** Of the major $K^+$ currents that control repolarization and action potential duration in ventricular myocardium, $I_{to}$ density is consistently decreased in chronic cardiac disorders associated with electrical remodeling (12, 14, 15, 26, 27, 29, 34, 35, 38–40). The mechanisms eliciting this change in electrophysiological phenotype are still unclear, but recent studies indicate that alterations in mRNA levels and expression of channel proteins that underlie $I_{to}$ are involved (9, 12, 13, 18, 40). However, pathogenic changes in $K^+$ channel expression and current density are dynamic and heterogeneous. For example, downregulation of fast ($I_{to-f}$) and slow ($I_{to}$) components of $I_{to}$ are evident in rat left ventricular myocytes as early as 3 days post-MI, before significant hypertrophy of the noninfarcted myocardium is detectable (12). These electrophysiological changes correlate with decreased mRNA and protein levels of Kv4.2/Kv4.3 and Kv2.1, respectively (12). At this early stage of electrical remodeling, however, $I_{to}$ and $I_K$ densities in right ventricular myocytes are increased compared with control (40), although there are no significant changes in mRNA or Kv channel protein levels (12). Our laboratory has examined changes in $K^+$ currents in ventricular myocytes 8 and 16 wk post-MI (28–30, present study), at somewhat later stages of remodeling where significant hypertrophy and hemodynamic indices of failure are manifest (11, 29). In these cases of chronic left ventricular dysfunction, downregulation of $I_{to}$ is more uniform throughout different regions of the heart. Nevertheless, our data show that $I_{to}$ remodeling is reversible in vitro within a time frame of several hours (29, 30, present study) despite the prolonged duration of ventricular dysfunction after coronary occlusion.

The present study provides new information suggesting that endogenous GSH plays an important role in regulating $I_{to}$ channels, presumably through the control of the myocyte redox state. As for alterations in $I_{to}$ density and Kv channel expression, changes in GSH status in the intact heart post-MI are complex and regionally specific. For instance, in noninfarcted areas of the rat left ventricle after coronary ligation, there is a progressive antioxidant deficit and a decrease in the GSH/GSSG ratio (11). In the right ventricle, however, there is an initial increase in the GSH/GSSG ratio 1 wk after MI, but thereafter the antioxidant reserve and redox state decline as in the left ventricle (11). The biphasic change in the GSH/GSSG ratio observed in the right ventricle post-MI suggests that portions of the myocardium initially upregulate GSH-related enzymes to compensate for the enhanced production of reactive oxygen species, but that a deficit eventually occurs with chronic oxidative stress. Although parallel regional changes in the GSH/GSSG ratio and $I_{to}$ density are apparent in the post-MI heart, additional studies are required to more clearly define the functional link between $K^+$ channel activity and the cardiac GSH system.

A major functional impact of a decreased GSH/GSSG ratio is oxidation of regulatory proteins at cysteine residues, through formation of mixed disulfides with GSSG or intramolecular disulfides (6, 10). This type of modification is reversible by reestablishing normal intracellular GSH levels for enzymatic and nonenzymatic reduction of oxidized proteins (6, 7, 10, 32). In the present study, the upregulation of $I_{to}$ density in myocytes from post-MI hearts by GSH or NAC (see Figs. 5 and 6) supports the hypothesis that redox state controls $I_{to}$ density in the remodeled ventricle. However, our experiments do not identify the key regulatory steps in overall channel activity involved. In light of the correlation between $I_{to}$ density, channel message, and protein levels (9, 12, 13, 18, 40), it is possible that the redox state of ventricular myocytes controls the transcription or posttranslational processing of Kv channel proteins or associated subunits. Indeed, the time delay for upregulating $I_{to}$ density by GSH and NAC (>2 h) is consistent with these mechanisms.

**Modulating cell GSH.** Our data also suggest that therapies aimed at augmenting myocyte GSH restore cell function in the post-MI heart at least in terms of $I_{to}$ channels. When intracellular GSH concentrations drop in pathophysiological states, one approach for restoration is to supplement cells with exogenous GSH as we did in our experiments (see Fig. 5). However, mammalian cells do not take up intact GSH in significant amounts (1, 7, 19, 22). Instead, it is proposed that extracellular GSH increases cellular uptake of cysteine, the rate-limiting amino acid in GSH synthesis, by its initial extracellular enzymatic degradation and subsequent amino acid uptake and resynthesis of GSH in the cytoplasm (1, 7, 19, 36). An increase in cysteine availability is also the proposed mechanism for the effects of exogenous NAC, which after entering a cell is hydrolyzed by N-deacetylase to release cysteine (1, 7, 36). The lack of effect of GSH and NAC on $I_{to}$ in control myocytes is consistent with the finding that intracellular GSH concentration is high under physiological
conditions and is controlled by feedback inhibition of de novo synthesis (1, 7, 19, 22). Nevertheless, further studies are necessary to measure the kinetics of intracellular GSH repletion by extracellular GSH and NAC in the post-MI heart and to correlate these data with the time course for \( I_{\alpha} \) upregulation by these redox agents.

A second approach to modulate cell GSH is to augment endogenous pathways of the GSH system (1, 7, 17, 19), and data from our study imply that metabolic activators of glucose utilization may play such a role. In particular, we found that upregulation of \( I_{\alpha} \) by DCA is mediated by the GR (see Fig. 7A) and G6PDH (see Fig. 7B) components of the GSH system but not by \( \gamma \)-GCS (see Fig. 7C). Although DCA increases the cardiac activity of pyruvate dehydrogenase, a key regulator of glucose metabolism (3, 33), it has been postulated that this effect causes an enhanced glycolytic flux to be diverted to the pentose pathway (21) where G6PDH generates NADPH required by GR to convert GSSG to GSH (1, 6, 7, 22). Alternatively, DCA may directly increase the activity of G6PDH, as has been shown in the liver (1). Finally, the lack of effect of BSO on DCA responsiveness of \( I_{\alpha} \) (see Fig. 7C) suggests that GSH synthesis is not influenced by this metabolic activator, even though \( \gamma \)-GCS most likely mediated the effects of exogenous GSH and NAC to upregulate \( I_{\alpha} \).

In summary, our data identify GSH as a key regulator of \( I_{\alpha} \) channels and suggest that electrical remodeling of the heart post-MI involves oxidative stress that profoundly affects the cell redox state. Myocardial GSH homeostasis is functionally linked to glucose metabolism, which importantly participates in protecting vulnerable cell proteins from oxidation. The relevant pathways and molecular signals involved in redox control of \( I_{\alpha} \) or other cardiac ion channels are not well defined and necessitate further study. Nevertheless, our studies suggest that therapies targeted to GSH and glucose metabolism may effectively reverse pathogenic K\(^+\) channel remodeling in the diseased ventricle.

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