Myocardial infarction in rat eliminates regional heterogeneity of AP profiles, $I_{\text{to}}$ K$^+$ currents, and [Ca$^{2+}$]$_i$ transients

ROGER KAPRIELIAN, RAJAN SAH, TIN NGUYEN, ALAN D. WICKENDEN, and PETER H. BACKX. Myocardial infarction in rat eliminates regional heterogeneity of AP profiles, $I_{\text{to}}$ K$^+$ currents, and [Ca$^{2+}$]$_i$ transients. Am J Physiol Heart Circ Physiol 283: H1157–H1168, 2002. First published May 2, 2002; 10.1152/ajpheart.00518.2001. —Transient outward K$^+$ current density ($I_{\text{to}}$) has been shown to vary between different regions of the normal myocardium and to be reduced in heart disease. In this study, we measured regional changes in action potential duration (APD), $I_{\text{to}}$, and intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) transients of ventricular myocytes derived from the right ventricular free wall (RVW) and interventricular septum (SEP) 8 wk after myocardial infarction (MI). At $+40\text{ mV}$, $I_{\text{to}}$ density in sham-operated hearts was significantly higher ($P < 0.01$) in the RVW (15.0 ± 0.8 pA/pF, $n = 47$) compared with the SEP (7.0 ± 1.1 pA/pF, $n = 18$). After MI, $I_{\text{to}}$ density was not reduced in SEP myocytes but was reduced ($P < 0.01$) in RVW myocytes (8.7 ± 1.0 pA/pF, $n = 26$) to levels indistinguishable from post-MI SEP myocytes. These changes in $I_{\text{to}}$ density correlated with Kv4.2 (but not Kv4.3) protein expression. By contrast, Kv1.4 expression was significantly higher in the RVW compared with the SEP and increased significantly after MI in RVW. APD measured at 50% or 90% repolarization was prolonged, whereas peak [Ca$^{2+}$]$_i$ transients amplitude was higher in the SEP compared with the RVW in sham myocytes. These regional differences in APD and [Ca$^{2+}$]$_i$ transients amplitude was higher in the SEP compared with the RVW in sham myocytes. These regional differences in APD and [Ca$^{2+}$]$_i$ transients were eliminated by MI. Our results demonstrate that the significant regional differences in $I_{\text{to}}$ density, APD, and [Ca$^{2+}$]$_i$ between RVW and SEP are linked to a variation in Kv4.2 expression, which largely disappears after MI.

Address for reprint requests and other correspondence: P. H. Backx, Heart and Stroke Richard Lewar Centre, Univ. of Toronto, Rm. 68, Fitzgerald Bldg., 150 College St., Toronto, Ontario, Canada M5G 2C4 (E-mail: p.backx@utoronto.ca).

There are marked differences in the action potential duration (APD) in different regions of the mammalian ventricle (4, 15, 18, 39, 58). This electrical heterogeneity in normal myocardium correlates with regional differences in the Ca$^{2+}$-independent transient outward K$^+$ current density ($I_{\text{to}}$) (5, 15, 18, 34, 35, 43) as well as in gene expression of K$^+$ channels (8, 16, 58). APD prolongation and reductions in $I_{\text{to}}$ density occur in rat heart after left anterior descending coronary artery ligation (2, 43, 58), aortic banding (5, 22, 55), as well as after treatment with either catecholamine (11) or monocrotaline (32, 33). Depending on the model, the extent of $I_{\text{to}}$ density changes in disease may not be uniform throughout the ventricle (2, 5, 11, 22, 55), thereby leading to possible losses of electrical heterogeneity and increased susceptibility to arrhythmias (3).

Aside from electrical heterogeneity, regional differences in other myocardial properties also exist. For example, systolic intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) is higher in the endocardium than in the epicardium (19, 54), consistent with the notion that the endocardium may play a more important role in contraction compared with the epicardium. The underlying basis for the regional differences in contraction is currently unknown but may be related to a heterogeneous transmural expression of Ca$^{2+}$ handling proteins (26, 31). Alternatively, APD might also play a key role because the action potential profile is an important determinant of the inotropic state of the heart in both normal (7, 42, 54) and hypertrophied (10, 30) rat ventricular myocytes.

In this study, we examined the regional changes in APD, $I_{\text{to}}$ density, and [Ca$^{2+}$]$_i$ transient magnitude and MI and correlated these differences with the expression of K$^+$ channel genes encoding for $I_{\text{to}}$ (i.e., Kv1.4, Kv4.2, and Kv4.3). Our results show that gradients in APD, $I_{\text{to}}$ density, Kv4.2 expression, and peak [Ca$^{2+}$]$_i$ exist between the right ventricular free wall (RVW) and interventricular septum (SEP), and these differences are largely eliminated after myocardial infarction (MI).

METHODS

Induction of MI and isolation of ventricular myocytes. Male Lewis Brown Norway rats (Harlan; Indianapolis, IN) weighing 220–250 g underwent left anterior coronary artery ligation, as described previously (40). Sham-operated rats were handled in the same manner except the coronary artery was not ligated. After the surgical procedure, the rats were housed in a climate-controlled environment at an ambient temperature of 21°C with 12:12-h light/dark cycle. Water and

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standard Purina rat chow were given ad libitum. Eight weeks after surgery, the animals were euthanized and the myocytes were superfused with the same modified Tyrode solution composed of (in mmol/l) 140 NaCl, 1 MgCl₂, 10 HEPES, 4 CsCl, 1 CaCl₂, and 10 d-glucose, pH adjusted to 7.4 with NaOH. The pipette solution for I_{ca,L} recordings was prepared with 50 mg/ml bovine serum albumin. Infarct size was assessed by dissection of the left ventricular free wall and measurement of the fraction of the left ventricular free wall, which was replaced with fibrous tissue (30).

**Single cell physiological studies.** Current densities and action potentials were recorded using the whole cell patch-clamp technique with an amplifier (Axopatch 200A, Axon Instruments). Microelectrodes were prepared with the use of a 1.5-mm thin-walled borosilicate glass (World Precision Instruments, Sarasota, FL). After the pipettes were polished, the typical resistances were 3–4 Ω when filled with the pipette solutions. Series resistance compensation was typically ~75–85%. After membrane rupture, cell capacitance was estimated by integrating the area of the capacitance transients after a 5-mV step from a holding potential of ~70 mV. The measured currents were divided by the cell capacitance to normalize currents for cell size.

To measure L-type Ca²⁺ currents (I_{ca,L}), voltage-clamp recordings were made in myocytes superfused with a modified Tyrode solution composed of (in mmol/l) 140 NaCl, 1 MgCl₂, 10 HEPES, 4 CsCl, 1 CaCl₂, and 10 d-glucose, pH adjusted to 7.4 with NaOH. The pipette solution for I_{ca,L} recordings contained (in mmol/l) 150 CsCl, 10 HEPES, 1 MgCl₂, 5 EGTA, and 5 MgATP, pH adjusted to 7.2 with CsOH. I_{ca,L} was estimated as the Ca²⁺-sensitive current (0.3 mmol/l CdCl₂). For K⁺ currents and action potential recordings, myocytes were superfused with the same modified Tyrode solution, except that CsCl was replaced with KCl. CdCl₂ (0.3 mmol/l) was routinely added to block I_{ca,L} when recording K⁺ currents. Pipette solutions for K⁺ current and action potential recordings contained (in mmol/l) 130 K-Aspartate, 20 KCl, 10 HEPES, 1 MgCl₂, 5 NaCl, 5 EGTA, and 5 MgATP, pH adjusted to 7.2 with Trizma base. Action potentials were corrected, but voltage-clamp recordings were not corrected for the measured liquid junction potential (~8.6 mV) between the pipette and the bath solution.

[Ca²⁺] was recorded with the same pipette and extracellular solutions used in the K⁺ current measurements except that intracellular EGTA was replaced with 75 μM fura 2 pentapotassium salt and CdCl₂ was absent. Fluorescence measurements were performed using light from a 75-W xenon lamp (Oriel; Stratford, CT) passed through band-pass filters (Omega Optical) centered at 340 or 380 nm via an epifluorescence port and a ×40 Fluor objective microscope (Nikon; Tokyo, Japan). The emitted fluorescence was collected by the objective and passed through a 510-nm filter to a photomultiplier detection unit (Hamamatsu; Bridgewater, NJ). The photomultiplier output was filtered at 100 Hz and stored in the computer for later analysis. In our experimental setup, background fluorescence was measured at both wavelengths after a gigaohm seal formation and before rupturing the cell membrane. The ratio of the background subtracted fluorescent signal (340/380) was used to estimate [Ca²⁺], using the equation (24)

\[ [\text{Ca}^{2+}]_i = K_d \cdot (R - R_{\min})/(R_{\max} - R) \]

where \( K_d \) is the apparent dissociation constant and \( R \) is the ratio of the background-subtracted fluorescence at 340-nm excitation to that at 380-nm excitation. The effective \( K_d \) was estimated in current-clamp and voltage-clamp conditions, as previously described (30). In voltage-clamp studies, myocytes were depolarized to +10 mV (100 ms in duration) from a holding potential of ~80 mV. All experiments were performed at room temperature (19–21°C) within 18 h of cell isolation. [Ca²⁺], measurements were always made under steady-state conditions by stimulating the myocyte at 0.25 Hz and recording fluorescence at both wavelengths between the 17th and 20th beat.

**Ribonuclease protection assay.** Immediately after removing the hearts, the right ventricle and septum were dissected (5 rats per group), rinsed briefly in 0.9% NaCl (wt/vol) and snap-frozen in liquid nitrogen. Ventricular tissue was powdered and RNA extracted by the one-step acid guanidium phenol method. The concentration of RNA was measured spectrophotometrically and confirmed by agarose gel electrophoresis. RNase protection assays were performed as previously described (30, 59).

**Western blot analysis.** Rat hearts were quickly removed and retrogradely perfused with Tyrode solution for 20 s. Immediately after this procedure, the RVW and SEP were dissected (4 rats per group) and stored at ~80°C before isolation of membrane proteins, as previously described (58). Total heart protein (50–100 μg) and 10–20 μg of total brain protein were resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. After transfer, the membrane was rinsed with Tris-buffered saline (TBS; 150 mM NaCl and 20 mM Tris, pH 7.5). Blots were blocked with 10% (Carnation) instant milk powder in TBS for 1 h and probed with anti-Kv4.2, Kv4.3, and Kv1.4 antibodies diluted in 3% milk-TBS overnight at 4°C. After the membrane was washed with TBS to remove excess primary antibody, blots were incubated with secondary antibody (donkey anti-rabbit-IgG conjugated to horseradish peroxidase, Amersham) in blocking buffer for 1 h at room temperature. The membrane was washed again with TBS containing 0.05% Tween 20 and 1% Triton X-100 and developed by enhanced chemiluminescence (ECL reagent, Amersham). We checked the gel loading by staining total proteins with Ponceau S, whereas molecular weights were determined using prestained markers (Kaleidoscope, Bio-Rad). Western blots were repeated 2–3 times per sample. Protein abundance was quantified by integrated densitometry of the bands (GS670 Imaging Densitometer, Bio-Rad). The integrated density of the protein samples was normalized by the corresponding value in the RVW of sham hearts for comparison.

**Statistical analysis and curve fitting.** All data are expressed as means ± SE. Steady-state activation (g) and inactivation (h) data were fit with the following Boltzmann functions

\[ g = g_{\text{max}} \left( 1 + \exp \left( -\left( V - V_{\text{h}} \right)/k \right) \right) \]

\[ h_x = 1/(1 + \exp \left( (V - V_{\text{x}})/k \right)) \]

where \( V \) is the step or conditioning potential, \( V_{\text{h}} \) is the midpoint of the function, and \( k \) is the slope factor. Biexponential functions were used to fit recovery from inactivation data using the equation below

\[ I/I_{\text{to}} = 100 - [A_{\text{fast}} \exp(-x/\tau_{\text{fast}})] + \left[ (100 - A_{\text{fast}}) \times \exp(-x/\tau_{\text{slow}}) \right] \]

where \( I/I_{\text{to}} \) is the fraction of current recovered, \( A_{\text{fast}} \) and \( \tau_{\text{fast}} \) are the amplitude and time constant for the fast component.
of recovery, 100 - $A_{fast}$ (i.e., $A_{slow}$) and $\tau_{slow}$ represent the amplitude and time constant for the slow component, and $x$ is the time spent at the recovery potential. Correlation between APD and $[Ca^{2+}]$, was performed by linear regression. Statistical comparisons were made with one-way analysis of variance (ANOVA) using the SPSS program (version 7.0 for Windows, SPSS). When ANOVA showed statistical significance with the use of the $F$ test, intergroup comparisons were made with the Student-Newman-Keuls procedure. A value of $P < 0.05$ was considered significant.

RESULTS

The effects of MI in rat hearts were assessed 8 wk after left anterior descending coronary artery ligation. Left ventricular free wall infarct sizes of hearts used in our studies were $47.5 \pm 2.6\%$ (range $34.4-64.0\%$). Hearts with infarct sizes <30\% were not included in our analysis because small infarcts are not associated with significant hemodynamic changes (30, 47). As summarized in Table 1, MI was associated with global and cellular hypertrophy. Specifically, tissue weight-to-body weight ratios and myocyte capacitance were increased in both the RVW and SEP after MI (Table 1).

Regional changes in membrane potential after MI.

Figure 1 shows representative action potentials measured in RVW and SEP myocytes derived from sham-operated (Fig. 1A) and post-MI (Fig. 1B) hearts. For sham-operated hearts, 50% and 90\% APD (APD$_{50}$ and APD$_{90}$, respectively) in RVW myocytes (APD$_{50}$ = 4.8 ± 0.6 ms, $n = 31$, and APD$_{90}$ = 28.9 ± 2.7 ms, $n = 31$) were shorter ($P < 0.01$) in duration than in SEP myocytes (APD$_{50}$ = 9.7 ± 1.1 ms, $n = 26$, and APD$_{90}$ = 49.4 ± 4.5 ms, $n = 26$). In SEP myocytes, APD$_{50}$ was unchanged (12.2 ± 1.3 ms, $n = 25$, $P = 0.1$), whereas APD$_{90}$ was slightly increased (71.2 ± 7.0 ms, $n = 25$, $P < 0.05$) after MI. In RVW myocytes both APD$_{50}$ (13.2 ± 1.6 ms, $n = 26$, $P < 0.05$) and APD$_{90}$ (70.9 ± 7.0, $n = 26$, $P < 0.05$) were increased by MI. More important, MI entirely eliminated differences in APD between RVW and SEP myocytes.

Frequency histograms of APD$_{50}$ for myocytes are summarized in Fig. 1C and reveal notable differences in the distribution of APD$_{50}$ magnitudes between RVW (solid bars) and SEP (open bars) myocytes derived from sham-operated hearts. Indeed, for the sham group ~65\% of the RVW myocytes had APD$_{50}$ magnitudes <5 ms compared with <25\% for SEP myocytes. A comparison of the distribution of APD$_{50}$ magnitudes reveals that substantial changes occur in RVW, but not SEP, myocytes after MI. Consequently, the APD$_{50}$ frequency histograms of RVW and SEP myocytes became very similar after MI with only 12\% of RVW myocytes compared with 23\% of SEP myocytes exhibiting APD$_{50}$ values <5 ms. These results suggest that action potential profiles in RVW myocytes take on characteristics of SEP myocytes in response to MI. Similar patterns were observed for the APD$_{90}$ magnitudes (data not shown). These findings are consistent with the APD changes observed previously after MI (55) and aortic stenosis (22) and demonstrate that MI leads to a loss of electrical heterogeneity of repolarization.

Despite regional differences of APD, no differences in resting membrane potential were observed between RVW myocytes and SEP myocytes. However, post-MI RVW myocytes were somewhat more depolarized (corrected for junction potentials) compared with sham RVW myocytes (sham, −84.1 ± 1.3 mV, $n = 31$; post-MI, −76.9 ± 1.1 mV, $n = 26$, $P < 0.01$) (see Table 1). $I_{\text{to}}$ and the sustained current. To investigate the basis for the loss of electrical heterogeneity between the RVW and SEP after MI, we initially focused on voltage-dependent $K^+$ currents because our previous studies revealed that $K^+$ channel expression is altered in this heart disease (30). Figure 2A shows that $I_{\text{to}}$ density, measured in response to depolarizing steps to +40 mV, was larger in RVW myocytes compared with SEP myocytes [15.0 ± 0.8 pA/pF ($n = 47$) vs. 7.0 ± 0.9 pA/pF ($n = 18$)]. After MI, $I_{\text{to}}$ density was reduced far more in RVW myocytes to 8.7 ± 1.0 pA/pF ($n = 26$, $P < 0.01$) than in SEP myocytes (5.1 ± 0.6 pA/pF, $n = 20$) compared to sham.

As summarized in Table 2, the variation in $I_{\text{to}}$ density at +40 mV between the groups was not related to measurable differences in activation and inactivation gating parameters (i.e., $V_{1/2}$ and $k$), suggesting that differences in the maximal conductance of $I_{\text{to}}$ ($G_{\text{max}}$) exist. Indeed, $G_{\text{max}}$ was reduced ($P < 0.05$) from 138.3 ± 7.6 pS/pF ($n = 47$) to 78.1 ± 8.1 pS/pF ($n = 26$) in RVW myocytes, whereas $G_{\text{max}}$ was not changed ($P = 0.12$) in the SEP (sham, 67.8 ± 3.6 pS/pF, $n = 18$, and post-MI, 48.6 ± 5.9 pS/pF, $n = 20$) (Table 2).

These regional changes in $I_{\text{to}}$ density after MI mirrored the changes in APD. Inspection of the $I_{\text{to}}$ density

<table>
<thead>
<tr>
<th>Table 1. Changes associated with MI in RVW and SEP myocytes</th>
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<tr>
<td><strong>Sham</strong></td>
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<td>RVW</td>
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<td><strong>HW/BW, %</strong></td>
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<tr>
<td><strong>Capacitance, pF</strong></td>
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<td><strong>APD$_{50}$, ms</strong></td>
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<td><strong>V$_{\text{m,rest}}$, mV</strong></td>
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<td><strong>V$_{m,peak}$, mV</strong></td>
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Values are means ± SE. MI, myocardial infarction; RVW, right ventricular wall; SEP, interventricular septum; BW, body weight; HW/BW, heart weight-to-body weight ratio; APD$_{50}$ and APD$_{90}$, action potential duration at 50\% and 90\% repolarization; V$_{\text{m,peak}}$, peak of the action potential; V$_{m,rest}$, resting membrane potential. *$P < 0.05$, RVW sham myocytes vs. RVW post-MI myocytes; †$P < 0.05$, SEP sham myocytes vs. SEP post-MI myocytes; §$P < 0.05$, RVW sham myocytes vs. SEP sham myocytes.
frequency histograms in Fig. 2G reveals that only ~17% of the RVW myocytes (solid bars) compared with 83% of SEP myocytes (open bars) exhibited current densities <9 pA/pF in sham-operated hearts. After MI, 67% of the RVW myocytes and 100% for SEP myocytes (Fig. 2H) displayed $I_{\text{to}}$ densities <9 pA/pF, suggesting again a convergence of cell populations after MI.

Figure 3 shows typical recovery from inactivation traces from sham-operated (Fig. 3A) and post-MI (Fig. 3B) hearts using a double-pulse protocol. Table 2 summarizes the mean data for recovery kinetics after fitting the data using a biexponential function. No differences were uncovered in the magnitudes of $\tau_{\text{fast}}$ or $\tau_{\text{slow}}$ between the different groups. In sham-operated hearts, the fast recovering component (i.e., $\tau_{\text{fast}}$) accounted for virtually all of the recovering current in RVW myocytes versus in SEP myocytes where it accounts for only 91.4 ± 0.9% ($n = 14$) of the recovering current. In post-MI hearts, $\tau_{\text{fast}}$ accounted for only 92.7 ± 0.8% ($n = 14$) of the current in RVW myocytes, which was not statistically different ($P = 0.2$) from SEP myocytes (92.4 ± 0.9%, $n = 17$).

To investigate the molecular basis for the changes in $I_{\text{to}}$ density, we examined the expression of Kv4.2, Kv4.3, and Kv1.4 subunits, which represent candidate voltage-dependent K$^+$ channels encoding for $I_{\text{to}}$-like currents previously shown to be expressed in rat heart (16). Figure 4 shows typical Western blots for Kv4.2, Kv4.3, and Kv1.4. In sham-operated hearts, Kv4.2 expression levels were significantly lower in SEP (0.77 ± 0.05, $n = 4$, $P < 0.01$) compared with RVW. MI induced a more than twofold reduction (0.46 ± 0.11, $n = 4$, $P < 0.01$) in Kv4.2 protein within the RVW with more modest reductions seen in SEP (0.56 ± 0.05, $n = 4$, $P < 0.01$), thereby eliminating the differences in Kv4.2 protein between the RVW and SEP.

The relative amount of Kv1.4 protein expression in SEP myocytes (i.e., 0.60 ± 0.04, $n = 4$) was significantly ($P < 0.05$) different than in RVW myocytes. After MI, Kv1.4 protein increased significantly in both RVW (2.09 ± 0.4, $n = 4$, $P < 0.05$) and SEP (1.29 ± 0.39, $n = 4$, $P < 0.05$). The pattern of Kv1.4 channel expression changes correlated with the presence of a slow component in the recovery from inactivation, suggesting that Kv1.4 encodes for the slowly recovering component of $I_{\text{to}}$ in rat myocytes (58).
Unlike $I_{to}$, no regional differences in $I_{sus}$ density, recorded at the end of a 500-ms depolarizing pulse, existed between RVW and SEP myocytes. After MI, $I_{sus}$ density at +40 mV was reduced by similar extents in SEP (sham, 6.6 ± 0.4 pA/pF, n = 18, and post-MI, 4.7 ± 0.4 pA/pF, n = 47, and post-MI, 5.7 ± 0.5 pA/pF, n = 23, $P > 0.05$) and RVW (sham, 7.5 ± 0.6 pA/pF, n = 47, and post-MI, 5.7 ± 0.5 pA/pF, n = 23, $P > 0.05$) myocytes. These results are consistent with a previous study showing that $I_{sus}$ is significantly reduced in the left ventricular endocardium after short-term infarction (61). The molecular correlates of $I_{sus}$ remains uncertain, but three candidate K$^+$ channel genes (Kv1.2, Kv1.5, and Kv2.1) are expressed in the rat heart (16, 51, 53). In the right ventricle, RNase protection assays (data not shown) revealed that the percentage reductions in mRNA levels after MI did not reach significance for Kv1.2, (23.7 ± 15.3%, n = 5, $P = 0.19$), Kv1.5 (22.4 ± 11.4%, n = 5, $P = 0.11$) or Kv2.1 (27.0 ± 9.8%, n = 5, $P = 0.06$) genes. In SEP, mRNA levels for Kv1.2 and Kv2.1 were decreased by 39.7 ± 4.5% ($P < 0.05$), respectively, whereas Kv1.5 mRNA levels were unchanged ($n = 5, 18.7 ± 11.8, P = 0.17$) after MI. Collectively, these RNase protection assay results suggest that there is a general reduction in the expression of candidate genes encoding for $I_{sus}$ in both ventricles. The relevance of these observations will require further investigation.

The inward rectifier and $I_{Ca,L}$. The differences in APD between the groups might also be associated with variations in other currents. Unlike the differences observed in $I_{to}$ density, inward rectifier current ($I_{K1}$)
Table 2. Regional differences in characteristics and biophysical properties of transient outward current in RVW and SEP myocytes after MI

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>SEP</th>
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<th>SEP</th>
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<tr>
<td>At +40 mV</td>
<td>RVW</td>
<td>SEP</td>
<td>RVW</td>
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<td></td>
<td>47</td>
<td>18</td>
<td>23</td>
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<tr>
<td>$I_{\text{peak}}$</td>
<td>22.7±1.3§</td>
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<td>14.2±1.0†</td>
<td>9.8±0.7*</td>
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<td>$I_{\text{on}}$</td>
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<td>6.6±0.4</td>
<td>5.7±0.5</td>
<td>4.7±0.4‡</td>
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<tr>
<td>$I_{\text{off}}$</td>
<td>15.0±0.8§</td>
<td>7.0±0.9</td>
<td>8.7±1.0†</td>
<td>5.1±0.6*</td>
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Steady-state activation

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<tr>
<td>n</td>
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<td>$G_{\text{max}}$</td>
<td>138.3±7.6§</td>
<td>67.8±8.6</td>
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<td>$V_{1/2}$, mV</td>
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<td>$k$, mV</td>
<td>15.3±0.6</td>
<td>13.1±0.6</td>
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Steady-state inactivation

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<tr>
<td>n</td>
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<tr>
<td>$V_{1/2}$, mV</td>
<td>−31.4±0.7</td>
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<td>$k$, mV</td>
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Recovery from inactivation

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<tr>
<td>n</td>
<td>12</td>
<td>14</td>
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<tr>
<td>$A_{\text{fast}}$, %</td>
<td>99.9±0.01</td>
<td>91.4±0.9</td>
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<td>$\tau_{\text{fast}}$, ms</td>
<td>34.8±0.7</td>
<td>34.9±1.9</td>
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<tr>
<td>$A_{\text{slow}}$, %</td>
<td>8.6±0.3</td>
<td>7.3±0.5†</td>
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<tr>
<td>$\tau_{\text{slow}}$, ms</td>
<td>1,684.7±151.2</td>
<td>1,913.3±208.4†</td>
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</table>

Values are means ± SE; n, no. of rats. $I_{\text{peak}}$, peak outward current; $I_{\text{on}}$, sustained current measured at the end of the 500-ms pulse; $I_{\text{off}}$, transient outward current; $G_{\text{max}}$, maximal conductance for the transient outward channel; $V_{1/2}$, midpoint for activation; $k$, slope factor; $A_{\text{fast}}$ and $A_{\text{slow}}$, fast and slow fractions of recovery from inactivation; $\tau_{\text{fast}}$ and $\tau_{\text{slow}}$, fast and slow time constant of recovery from inactivation. *P < 0.05, RVW post-MI myocytes vs. SEP post-MI myocytes; †P < 0.05, RVW sham myocytes vs. RVW post-MI myocytes; ‡P < 0.05, SEP sham myocytes vs. SEP post-MI myocytes; §P < 0.05 RVW sham myocytes vs. SEP post-MI myocytes. H1162 MI ELIMINATES ELECTRICAL AND CONTRACTILE HETEROGENEITY

MI density was not different between RVW and SEP myocytes. Similar to $I_{\text{on}}$, no significant change in $I_{\text{K1}}$ density (evaluated at −130 mV) was observed in SEP myocytes, whereas $I_{\text{K1}}$ density was decreased (P < 0.05) in RVW myocytes (sham, −16.0 ± 0.6 pA/pF, n = 28, and post-MI, −12.2 ± 1.0 pA/pF, n = 20). These differences in $I_{\text{K1}}$ density assessed at −130 mV may explain the depolarized resting membrane potentials of post-MI RVW myocytes compared with RVW sham myocytes provided, of course, these differences reflect corresponding changes at more positive potentials above the equilibrium potential for K⁺. However, we cannot rule out changes in other currents, such as chloride, the electrogenic Na⁺-K⁺ pump currents, or others that can also affect resting membrane potential under our recording conditions.

$I_{\text{Ca,L}}$ density recorded at 0 mV also did not differ between RVW and SEP myocytes and did not change after MI in the RVW (sham, −6.0 ± 0.4 pA/pF, n = 15, and post-MI, −5.5 ± 0.5 pA/pF, n = 14, P = 0.59) or SEP (sham, −5.8 ± 0.4 pA/pF, n = 16, and post-MI, −5.2 ± 0.3 pA/pF, n = 16, P = 0.59). There was no difference in the steady-state or kinetic gating properties of $I_{\text{Ca,L}}$ between any of the groups (data not shown).

Action potentials and [Ca²⁺] transients. Previous studies have shown that changes in $I_{\text{to}}$ density and the corresponding changes in APD correlate with alterations in Ca²⁺ cycling. Figure 5 shows simultaneous records of action potentials and [Ca²⁺] (under current-clamp conditions) in RVW and SEP myocytes derived from sham (Fig. 5A) and post-MI (Fig. 5B) rats under Ca²⁺ buffer conditions (i.e., 75 μM fura 2). As was the case with high Ca²⁺-buffering conditions (i.e., 5 mM EGTA), there were significant differences in APD₅₀ and APD₉₀ between RVW and SEP myocytes. After MI, APD₅₀ (sham, 6.5 ± 1.4 ms, n = 17, and post-MI, 37.2 ± 5.8 ms, n = 16, P < 0.01) and APD₉₀ (sham, 72.3 ± 13.8 ms, and post-MI, 673.6 ± 89.9 ms, P < 0.05) were prolonged in RVW myocytes. As in studies with high EGTA, APD₅₀ (sham, 154.2 ± 23.4 ms and post-MI, 592.3 ± 74.3 ms, P < 0.05) was prolonged in SEP myocytes after MI without changes in APD₅₀ (sham, 24.2 ± 3.4 ms, n = 15 and post-MI, 33.3 ± 3.7 ms, n = 20, P = 0.09). It is important to note that the APD₅₀ and especially the APD₉₀ were generally prolonged in all groups when recorded in the presence of Ca²⁺ transients when low intracellular Ca²⁺ buffering was used compared to high Ca²⁺-buffering conditions.

Associated with the observed differences in APD, the mean peak systolic [Ca²⁺], was higher (P < 0.01) in the SEP (687.4 ± 84.9 nmol/l, n = 15) compared with the RVW (381.8 ± 44.7 nmol/l, n = 17). After MI, peak systolic [Ca²⁺] levels were elevated (P < 0.01) more than twofold in RVW myocytes (1,056.4 ± 92.9 nmol/l, n = 16) compared with sham, with relatively smaller changes occurring in SEP (1,177.5 ± 127.5 nmol/l, n = 15) in response to MI. Remarkably, the peak systolic [Ca²⁺] was not significantly different between RVW and SEP myocytes after MI, which correlated with the abolishment of regional differences in APD and $I_{\text{to}}$. Despite these regional differences in [Ca²⁺], transient amplitudes and the changes that occur in response to MI, no differences in the time course of relaxation could be detected between the different groups (data not shown). By contrast to the [Ca²⁺], transient ampli-
tudes, diastolic \([Ca^{2+}]_i\) did not differ between the groups studied (sham RVW, 81.3 ± 5.4 nmol/l, \(n = 17\); sham SEP, 100.4 ± 8.8 nmol/l, \(n = 15\); post-MI RVW, 93.5 ± 4.5 nmol/l, \(n = 16\); post-MI SEP, 93.5 ± 10.5 nmol/l, \(n = 15\)), establishing that APD prolongation affects primarily peak \([Ca^{2+}]_i\) transient amplitude (30).

To further explore whether factors other than membrane potential contribute to differences in systolic \([Ca^{2+}]_i\) between the groups, \([Ca^{2+}]_i\) transients were measured in response to step depolarizations to +10 mV for 100 ms from a holding potential of −80 mV. Peak systolic \([Ca^{2+}]_i\) was not different in RVW and SEP myocytes derived from sham-operated (RVW, 648.9 ± 143.3 nmol/l, \(n = 10\); SEP, 841 ± 127.7 nmol/l, \(n = 10\)) or post-MI (RVW, 736.8 ± 100.8 nmol/l, \(n = 10\); SEP, 727.8 ± 124.3 nmol/l, \(n = 9\)) hearts. Similarly, diastolic \([Ca^{2+}]_i\) did not differ between the various groups (RVW-sham, 91.7 ± 3.0 nmol/l, \(n = 10\); SEP-sham, 101.9 ± 9.3 nmol/l, \(n = 10\); RVW-MI: 84.7 ± 3.2 nmol/l, \(n = 10\); SEP-MI, 82.0 ± 5.7 nmol/l, \(n = 9\)). In addition, when step depolarizations are used, no differences could be detected in the time course of the \([Ca^{2+}]_i\) transient relaxation (data not shown). These results suggest that while many differences in \(Ca^{2+}\)-handling proteins might exist between the groups, changes in action potential profile correlate with the observed alterations in \([Ca^{2+}]_i\) transients.

**DISCUSSION**

Our findings show that regional differences in APD exist in the normal rat myocardium with much shorter durations being observed in RVW versus SEP. These regional differences between RVW and SEP are very similar to those observed across the left ventricular free wall of the rat (15, 22, 48, 55). Regional gradients in APD have also been detected in other animal species (4, 18) and humans (37) and appear to be critical for orchestrating and coordinating ventricular repolarization, thereby minimizing life-threatening arrhythmias (3). The regional differences in APD across the left ventricular wall have been attributed to variations in \(I_{to}\) density (3, 5, 15, 22, 58). Our results show a strong
The correlation between APD and $I_{to}$ density in both the RVW and SEP of rat myocardium. Clearly, variations in other currents could also conceivably contribute to regional differences in APD. However, in this study, no detectable differences were observed between RVW and SEP myocytes in $I_{to}$, $I_{K1,o}$, or $I_{Ca,L}$, suggesting these currents are not responsible for APD heterogeneity as previously reported (2, 14). Nevertheless, possible differences in other currents to the regional variations in APD cannot be ruled out.

Our findings demonstrate high mRNA and protein expression of Kv4.2 in the RVW compared with the SEP, mirroring closely the $I_{to}$ densities. Similar correlations between Kv4.x genes and $I_{to}$ have been documented in humans (17, 28, 52) and other animal species (16, 17, 21, 25, 58, 59). By contrast, no regional differences in Kv4.3 expression were observed. These results suggest that varied expression of Kv4.2 channels might underlie the electrical heterogeneity observed in rat myocardium, as suggested previously (58). mRNA and protein expression of Kv1.4 were higher in the RVW versus SEP. The signifi

caption: Fig. 4. Representative comparison of candidate K^+ channel α-subunits encoding the transient outward current in the RVW and SEP after MI. Each blot shows side-by-side Western blots for Kv4.2 (A), Kv4.3 (B), and Kv1.4 (C) in the RVW and SEP derived from sham and post-MI rats. The bars show mean changes in Kvα subunits in tissues derived from RVW sham (solid bars), SEP sham (open bars), RVW post-MI (gray bars), and SEP post-MI (hatched bars). Values have been normalized to sham RVW samples and are expressed as percentage units. †P < 0.05 for RVW sham myocytes vs. RVW post-MI myocytes; ‡P < 0.05 SEP sham myocytes vs. SEP post-MI myocytes; §P < 0.05 RVW sham myocytes vs. SEP sham myocytes.
force generated and larger contribution to pressure development by endocardial versus epicardial regions of the left ventricle (19, 54). Differences in peak magnitudes of the \([\text{Ca}^{2+}]_i\) transients between the groups might also conceivably be related to variations in \(I_{\text{Ca,L}}\) density independent of changes in APD. However, \(I_{\text{Ca,L}}\) and \([\text{Ca}^{2+}]_i\) transients did not differ between SEP and RVW in voltage-clamp experiments. Nevertheless, it is possible that regional differences in other proteins modulating \(\text{Ca}^{2+}\) handling might also vary between the different regions we studied.

**Electrical and molecular changes after MI.** Prolongation of APD, reductions in \(I_{\text{to}}\) density, decreased expression of Kv4.2 and increases in \([\text{Ca}^{2+}]_i\), transient amplitudes have been reported previously in the rat myocardium for a number of models of cardiac hypertrophy (6, 9, 10, 30, 50). Although alterations in any number of currents could explain the changes in APD after infarction, only reductions in \(I_{\text{to}}\) without changes in \(I_{\text{Ca,L}}, I_{\text{sus}},\) or \(I_{K1}\) were observed in the two regions studied. We found that reductions in \(I_{\text{to}}\) density, the maximal conductance of \(I_{\text{to}}\) (\(G_{\text{max}}\)) and Kv4.2 expression, as well as the degree of APD prolongation, were far larger in RVW myocytes after MI compared to SEP myocytes. These differential regional effects of MI eliminated the normal pattern of Kv4.2 expression and APD50 while reducing substantially the \(I_{\text{to}}\) density and \(G_{\text{max}}\) between RVW and SEP. Similar effects on the normal regional electrical heterogeneity in rat hearts have been reported after MI (55) and other disease models (11, 22, 49). On the other hand, more uniform changes in the electrical properties between different regions in heart have also been reported previously (5, 13). These published discrepancies in the regional response of the myocardium may be related to differences in the type, severity or duration of the disease model under investigation.

One important feature of the electrical changes that occur after MI in our studies is the convergence of frequency distribution of APD and \(I_{\text{to}}\) densities between RVW and SEP myocytes. This suggests that myocytes become more uniform in their electrical properties after MI. This coincided with a complete loss of heterogeneity of the \([\text{Ca}^{2+}]_i\) transients and the level of Kv4.2 expression between these regions. It will be interesting to see whether other cellular and biochemical properties of the myocardium also become more uniform after MI.

Along with the regional differences in \(I_{\text{to}}\) density, the recovery kinetics of \(I_{\text{to}}\) were also measurably slowed after MI (30) with a greater change in RVW myocytes versus SEP myocytes (Fig. 3). The significance of this
observation is unclear. At the present time, the relative contribution of Kv1.4 versus Kv4.2 and Kv4.3 protein to adult I_{to} in cardiac myocytes remains unclear (36). However, as already mentioned, a series of recent studies has concluded that the fast inactivating/recovering component of I_{to} is produced by Kv4.2 and Kv4.3 genes while the slow inactivating/recovering portion of I_{to} is produced by Kv1.4 genes (20, 25, 27, 56, 58, 59). These conclusions suggest that the combination of marked downregulation of Kv4.2 coupled with a significant increase in Kv1.4 expression probably explain the emergence of a slowly recovering component of I_{to} after MI.

The loss of regional heterogeneity after MI has several important implications on the electrical and contractile properties of the whole heart. Previous studies have demonstrated that, as heart disease progresses toward heart failure, the amplitude of the [Ca^{2+}]; decreases as a result of any number of potential molecular mechanisms, such as decreases in sarcoplasmic reticulum Ca^{2+}-ATPase activity or expression (1, 6), uncoupling between I_{Ca,L} and the ryanodine receptors (23) or increases in Na^{+}/Ca^{2+} exchange function (41). However, before the development of heart failure in rodents, [Ca^{2+}]; transient amplitudes can actually be increased in hypertrophic heart disease as a result of APD prolongation (10, 30), thereby increasing contractility of the mechanically challenged heart. Our results demonstrate that after MI the [Ca^{2+}]; transient amplitudes are increased far more in RVW myocytes than SEP myocytes mirroring precisely the alterations in APD, I_{to} density, and Kv4.2 expression. These observations suggest that the changes in electrical properties convert RVW myocytes to become more like the strongly contracting SEP myocytes (19, 54). This change in contractility of the RVW myocytes is expected to functionally compensate for the elevated work loads placed on the right heart after MI (30).

The normal electrical heterogeneity within the heart is expected to reduce the propensity of heart to develop (global) reentry type arrhythmias by synchronizing repolarization. Therefore, one possible consequence of the loss of the electrical heterogeneity between different regions of the heart, as observed in our study, might be a disturbance of the normal repolarization process, thereby favoring the onset of global reentry circuits in the myocardium. Whereas our experiments were limited to cellular studies, an earlier study (43) using the rat infarct model showed evidence for increased propensity of arrhythmias in this model. Action potential prolongation might also conceivably promote enhanced susceptibility to certain Ca^{2+}-dependent triggered arrhythmias. Future studies will be necessary to assess whether and how this loss of electrical heterogeneity and action potential prolongation contributes to arrhythmias in this and other models of heart disease.

The extent to which our results are applicable to other regions of the heart or to other species remains uncertain. However, in the rat heart, the I_{to} density is identical between RVW myocytes and left ventricular epicardial myocytes (R. Kaprielian and P. H. Backx, unpublished observations), as reported previously (14), whereas the I_{to} density in SEP myocytes is similar to that observed in left ventricular endocardial myocytes (11, 49, 55, 61). Moreover, a recent study (55) in the rat aortic stenosis model showed a similar pattern of electrical changes between the epicardium and endocardium of the left ventricle between the epicardial RVV and the endocardial septum.

In conclusion, our studies demonstrate that differences in the expression of Kv4.2 channel protein between RVW myocytes and SEP myocytes in normal and diseased hearts account for regional changes in I_{to} density, APD, and [Ca^{2+}];. Transients. Our studies further suggest that the differences in Kv4.2 channel expression play a role in normal regional differences in myocardial contractility and changes in Kv4.2 expression might lead to changes in the regional differences in contractility and increase the susceptibility of the heart to arrhythmias.

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