Spatial alterations of Kv channels expression and K\(^+\) currents in post-MI remodeled rat heart

B. Huang, D. Qin, N. El-Sherif*

Cardiology Division, Department of Medicine, Box 1199, State University of New York Health Science Center and Veterans Affairs Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, USA

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Abstract

Objective: The hypothesis being tested in the present study is that increased anisotropic properties occurs in the remodeled post-infarction heart due to spatial alterations in Kv channels expression and K\(^+\) currents of the remodeled myocardium. Methods: Three to 4 weeks post myocardial infarction (MI) in the rat, we measured the two components of the outward K current, \(I_{\text{to-fast}}\) and \(I_{\text{to-slow}}\) in the epicardium (epi) and endocardium (endo) of noninfarcted remodeled left ventricle (LV) using patch clamp techniques. Alterations in mRNA and/or protein levels of potassium channel genes Kv1.4, Kv1.5, Kv2.1, Kv4.2 and Kv4.3 were measured in epi, midmyocardium (mid), and endo regions of LV and in the right ventricle (RV). Results: In sham operated rat heart, the density of \(I_{\text{to}}\) was 2.3 times greater in epi compared to endo myocytes. In post-MI heart, the density of \(I_{\text{to}}\) and \(I_{\text{to-slow}}\) decreased to a similar degree in LV epi and endo but the difference in \(I_{\text{to}}\) density between epi and endo persisted. The mRNA and/or protein levels of Kv1.4, Kv1.5, Kv2.1, Kv4.2 and Kv4.3 but not Kv1.5 decreased to a varying extent in different regions of LV but not in RV of post-MI heart. Conclusions: Our results suggest that regional downregulation of Kv channels expression and density of K\(^+\) currents can be a significant determinant of increased spatial electrophysiological heterogeneity and contribute to increased electrical instability of the post-MI heart. © 2001 Elsevier Science B.V.

Keywords: Gap junctions; Gene expression; Infarction; K-channel; Remodeling

1. Introduction

In recent years, the importance of ventricular remodeling after myocardial infarction (MI) on long-term survival has been better appreciated. Studies from our laboratory have shown that 3–4 weeks post-MI the noninfarcted myocardium undergoes significant hypertrophy as part of the remodeling process [1]. Electrophysiological studies indicated that the hypertrophic response of the noninfarcted remodeled myocardium engenders an arrhythmogenic substrate, including prolongation of action potential duration (APD), regional differences in APD, and increased tendency to early afterdepolarization (EAD)-triggered activity and reentrant tachyarrhythmias [1]. The prolongation of APD, 3–4 weeks after MI could be explained, in part, by downregulation of key K\(^+\) channel genes [2] resulting in decreased current densities of both components of transient outward currents; \(I_{\text{to-fast}}\) and \(I_{\text{to-slow}}\) [1]. The electrophysiological substrates for reentrant tachyarrhythmias are spatial heterogeneity of cardiac repolarization and/or conduction. The hypothesis being tested in this study is that increased anisotropic properties occurs in the remodeled post-MI ventricular myocardium. It is proposed that the changes are caused by regional alterations in the densities of different Kv channel proteins and currents resulting in regional variations in APD and hence heterogeneity of cardiac refractoriness. These changes could provide the electrophysiological substrate for reentrant excitation. Preliminary results were previously reported [3].
2. Methods

2.1. Experimental model

The experimental protocol was approved by the local institutional review board and conformed to the principles outlined in the Declaration of Helsinki (Cardiovascular Research 1997;35:2–3). Female Sprague–Dawley rats weighing 200–250 g underwent either left anterior descending coronary artery ligation or sham operation as previously described [1,4]. Briefly, after anesthesia with 35 mg/kg methohexital, i.p., and local anesthesia with 1% xylocaine, the trachea was exposed in the midline and the rats were intubated under direct vision and ventilated with room air. The chest was opened by anterolateral thoracotomy, and the pericardium was removed. The heart was retracted with an apical suture, and the left anterior descending coronary artery was occluded with a 6-O suture 1–2 mm below the left atrial appendage. Successful occlusion was confirmed by pallor of the anterior wall of the left ventricle and ST segment elevation. If neither changes were observed, the occlusion was re-attempted. The incision was closed and 100 000 U benzathine penicillin was administered intramuscularly as a prophylaxis against infection. The rats were extubated and allowed to recover in individual cages. Sham animals underwent an identical surgical procedure without coronary ligation. All rats received standard care, including ad libitum food, water, and a 12-h day/night cycle.

The rats were studied 3–4 weeks post-MI when compensated hypertrophy has peaked [5]. Post-MI and sham hearts were collected under deep anesthesia with 50 mg/kg sodium pentobarbital (i.p.).

2.2. Voltage-clamp recording and current analysis

Two distinct depolarization-activated K⁺ currents were described in adult rat ventricular myocytes [6]. The characteristics of the fast component is similar to the 4-aminopyridine-sensitive Iᵩᵩ and is termed Iᵩᵩ. The slow component has been termed Iᵩᵩ [6] or Iᵩᵩ [1,7]. Epicardial (epi) and endocardial (endo) tissues were obtained from the free wall of sham-operated rat heart and from the noninfarcted left ventricular (LV) wall of post-MI heart for recording of Iᵩᵩ and Iᵩᵩ in epi and endo myocytes. Details of cell isolation have been previously reported [1]. The composition of external and internal solutions for recording Iᵩᵩ and Iᵩᵩ was previously published [1]. Membrane capacitance and series resistance were compensated [1]. The currents were digitally recorded at room temperature (24°C) and analyzed using pCLAMP software (pCLAMP version 6.02, Axon Instrument Inc., Foster City, CA, USA). Clampfit software was used to measure amplitudes and time constants of ionic currents. To analyze the two outward K⁺ currents the cell was depolarized from a holding potential of −100 mV for 5 s to potentials ranging from −50 to +60 mV in steps of 10 mV. The current decay was fitted by two exponentials. The weights and time constants τᵣ and τᵣ correspond to Iᵩᵩ and Iᵩᵩ, respectively [1,6]. Current density of both Iᵩᵩ and Iᵩᵩ and their voltage dependence were analyzed as previously reported [1].

2.3. K⁺ channel RNA expression

Details of preparation of RNA from ventricular myocardium, RNase protection assay (RPA) and western blot analysis have been previously reported [2]. In both sham and post-MI experimental groups, the left and right atrial appendages were carefully excised. For the post-MI experimental group the infarct region was carefully separated from the hypertrophied LV including the septum under a dissecting microscope. To obtain tissue from epi, the mid-myocardium (mid), and endo of LV, the free wall of LV was dissected free from the rest of the heart and laid out as a sheet between two glass cover-slips. The two largest papillary muscles were then dissected from the bulk of the muscle wall. After being quickly frozen, the inner endo layer, approximately one third depth of the wall, was first dissected out with a surgical blade, and then the remaining sheet was split in two to give the mid and epi regions. The tissues were rinsed in saline to remove excess blood, snap-frozen in liquid nitrogen, and stored at −70°C. Total RNA was extracted from the LV as well as the right ventricle (RV) using the standard protocol of Chomczynski and Sacchi [8] of homogenization in acid guanidinium thiocyanate followed by phenol–chloroform extraction and ethanol precipitation. The amount of RNA recovered in each sample was determined spectrophotometrically at a wavelength of 260 nm and the integrity of each sample confirmed by analysis on a denaturing agarose gel. For Western blot analysis, the membranes were incubated with Kv1.4, Kv1.5, Kv2.1 (Upstate Biotechnology, Lake Placid, NY, USA) and Kv4.2/Kv4.3 (that recognizes both Kv4.2 and Kv4.3) and was generously provided by Dixon et al., SUNY HSC, Stony Brook, NY).

2.4. RNase protection assay (RPA)

RPAs were performed through concomitant measurement of cyclophilin genes expression (internal standard) [2]. RPA was modified from the method described by Kerig and Melton [9]. Previously described templates [2] were used to prepare a 32P-UTP antisense-radiolabeled cRNA probes (MAXIscript™, Ambion, Austin, TX, USA). To differentiate between the specifically protected region of the probe and any remaining undigested probe, all probes contain regions of plasmid sequence at one end of the transcript. Yeast tRNA (10 μg) was used as a negative control to test for the presence of probe self-complementa-
tion by intramolecular hybridization, resulting in smaller than expected protected bands. To account for the relatively greater abundance of internal control mRNA compared to potassium ion channel mRNA in cardiac tissues and to avoid saturation of autoradiography in hybridizations, the reaction was carried out in the presence of excess cold UTP (200 µM for cyclophilin), rendering a probe with less specific activity. To obtain full-length transcripts and lengthen the shelf life of the cRNA probes for all the potassium ion channels, transcription was done in the presence of 25 µM cold UTP. All cRNA probes were purified prior to use over 5% polyacrylamide/8 M urea gel. Concomitant hybridization of the two probes (1×10⁴ cpm ionic channel cRNA and 1×10⁵ cpm cyclophilin cRNA per 10 µg total RNA sample) were carried out at 50°C for 18 h followed by digestion with RNase A (250 U/ml) and T1 (10 000 U/ml) [Ambion] at 37°C for 30 min. The reaction was terminated by the addition of SDS and proteinase K followed by phenol–chloroform extraction and ethanol precipitation. The protected fragments were visualized by autoradiography after electrophoresis on a 5% polyacrylamide/8 mol/l urea gel. Quantitative evaluation was carried out using scanning densitometric analysis. For comparisons between sham and post-MI myocardium the arbitrary densitometric units were normalized to the value of the cyclophilin gene.

2.5. Western blot analysis

Cardiac cell membrane preparation and Western blot analysis were performed as described previously by Barry et al. [10] Sham and post-MI rats cardiac cell membranes were prepared and protein was fractionated on a 10% polyacrylamide–SDS gel. Because of anticipated lower immunoreactive protein level, 200 µg of protein was used for the Kv1.4 western blot compared to 85 µg for the other Kv genes. After electrophoretic transfer to polyvinylidilfluoride (Bio-Rad Lab., Hercules, CA, USA), the membranes were incubated with antisera against Kv1.4, Kv1.5, Kv2.1, and Kv4.2/Kv4.3, at dilutions of 1:1000, 1:500, and 1:250, respectively. Bound primary antibody was detected with a 1:10 000 dilution of alkaline phosphatase-conjugated goat anti-rabbit IgG and the Western Light chemiluminescent protein detection kit according to the manufacturer’s protocol (Tropix Inc., Bedford, MA, USA). Quantitative immunoreactivity was determined by densitometric analysis of the developed film that was in the linear ranges with respect to film exposure. Linearity between amounts of protein and immunoreactive signals were proved for each Kv channel subunit protein by plotting different amounts of protein at varying exposure times against corresponding densitometric units. Quantitative densitometric analysis was performed (Jandel Scientific, San Rafael, CA, USA).

2.6. Statistical analysis

For electrophysiological studies, data are presented as mean±S.E.M. Current amplitude, current density, and time constants were determined at different test potentials. When the data could be fit to a physiologically appropriate model with r²>0.95, the parameters for that model were tested for differences between the sham-operated and post-MI groups. In experiments in which the response pattern could not be fit, repeated measures ANOVA were used to test for differences between groups. Only linear models fit the criteria for acceptance, and for these models, the slope and intercept differences between groups were tested using multiple regression. In all other cases, localization of differences between groups was determined using t-tests, with the Bonferroni correction applied to yield experimental α level of P≤0.05.

For comparisons of mRNA expression between sham and post-MI myocardium the arbitrary densitometric units were normalized to the value of the cyclophilin gene and were statistically compared by one-way ANOVA. The results were reproducible in two independent determinations, i.e. every sample pair had consistent changes in level of mRNA expression in the post-MI relative to sham experimental groups. Differences in level of mRNA expression and immunoreactive protein levels were considered significant at P<0.05 and dispersion from the mean was noted as mean±S.E.M.

3. Results

Electrophysiological data were obtained from eight sham-operated animals and ten post-MI animals. Cell membrane capacitance was 135±6 pF in epi cells (n=36) and 127±7 pF endo cells (n=24) and increased in post-MI group to 228±10 pF in epi cells (n=74) and 192±9 pF in endo cells (n=42) (P<0.001).

Fig. 1 illustrates outward K⁺ currents recorded from a sham myocyte (top) and a 3-week-old post-MI myocyte (bottom) from an epi (A) and an endo cell (B). The amplitude of K⁺ current is smaller in endo compared to epi cells both in sham and post-MI myocytes. Although the post-MI cells have larger membrane capacitance, the K⁺ current amplitude was not significantly different between both cells. Fig. 2 compares the current density of I_{Po} and I_{Po,4} between sham and 3–4 week-old post-MI epi and endo myocytes, respectively. The densities of I_{Po}, I_{Po,4} of both epi and endo post-MI myocytes were significantly reduced compared to sham cells. In both sham and post-MI myocytes the kinetics of inactivation of the outward K⁺ current required two exponentials for adequate fit. The inactivation kinetics of both I_{po} and I_{po,4} were faster at LV epi compared to LV endo. Although the density of I_{po} and
I_{o-s} decreased in post-MI myocytes at both LV epi and LV endo, the inactivation kinetics of both currents were not significantly changed. The data are summarized in Table 1.

3.1. Regional changes in Kv gene expression and protein levels in the 3–4 week-old post-MI rat ventricle

There are relatively minor differences in the abundance of the Kv1.5, Kv2.1 and Kv4.3 transcripts across the ventricular free wall [11,12]. By contrast, Kv4.2 mRNA was found to be expressed in a steep gradient across the LV wall [11]. There was no change in the expression of Kv1.5 mRNA (Fig. 3) or protein level (Fig. 4) across the LV wall in post MI compared to sham-operated rats. On the other hand, compared to sham, the mRNA level of Kv2.1 was significantly decreased in post-MI LV by 22, 39 and 38% in epi, mid, and endo zones, respectively (P<0.05, n=6) (Fig. 5). Similarly, the protein levels of Kv2.1 in post-MI LV were reduced by 21, 26 and 30% in epi, mid and endo zones, respectively (P<0.05, Fig. 6). In sham-operated rat heart, the mRNA level of Kv4.2 in the epi region was 2.9 times higher than in the endo region of the LV free wall (Fig. 7). Significant changes in the mRNA level between sham-operated and post-MI rats across the LV free wall were seen in Kv4.2 and Kv4.3 channel subunits expression. Expression of Kv4.2 channel message was decreased by 37, 28 and 46% in the epi, mid and endo zone of post-MI LV, respectively, compared to sham (all values are significant at P<0.05, n=6) (Fig. 7). Similarly, expression of Kv4.3 channel message was decreased by 31.4, 41 and 21.8% in the epi, mid and endo zones of post-MI LV, respectively, compared to sham (all values are significant at P<0.05, n=6) (Fig. 7). Fig. 9 shows that the protein levels of Kv4.2/4.3 in post-MI LV wall were decreased by 49, 53 and 51% in epi, mid and endo zones, respectively compared to the sham group (P<0.01). There was no significant change in the protein levels of Kv1.5, Kv2.1, Kv4.2/4.3 between RV of sham and post-MI rats (Figs 4, 6 and 9).

We have previously reported that the mRNA level of Kv1.4 is reduced in the 3–4 week post-MI rat LV [2]. In the previously reported study we did not evaluate changes in the protein level of Kv1.4 since in a report by Barry et al. [10] only a trace ~97 kDa protein could be detected using the anti-Kv1.4 antibody. In the present study we investigated the protein level of Kv1.4 in the RV, LV endo and LV epi zones in sham and post-MI rat heart as well as in the rat brain using a commercial anti-Kv1.4 antibody (Upstate Biotechnology) (Fig. 10). The Kv1.4 protein level was much higher in the rat brain compared to heart. In the sham rat heart Kv1.4 protein was detected in RV, LV endo and LV epi. However, the Kv1.4 protein level was 2.3 times higher in LV endo compared to LV epi and 2.1 times higher compared to RV. In the post-MI heart the Kv1.4
protein level showed no significant change in RV but significantly decreased by 52 and 48% in LV endo and LV epi, respectively ($P<0.01$).

### 4. Discussion

We have investigated the well-established model of post-MI remodeling in the rat heart and showed that the remodeling process is associated with compensatory hypertrophy of the noninfarcted portion of the LV. The hypertrophic response engenders distinct molecular and electrophysiological alterations that are potentially deleterious. The changes include downregulation of $K^+$ channel genes with consequent decrease of outward $K^+$ currents contributing to prolongation of APD of hypertrophied ventricular myocytes. We have previously shown that the alterations in duration and configuration of APD is not the same in epi and endo regions of the LV thus resulting in increased heterogeneity of repolarization, an important substrate for reentrant tachyarrhythmias [1]. Spontaneous and/or induced ventricular tachyarrhythmias have been reported to occur in the rat model in the late post-MI phase [1,13,14]. The findings in the present study provide, in part, the molecular and electrophysiological basis of the arrhythmogenicity of post-MI heart.

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**Fig. 3.** Representative comparison of cardiac Kv1.5 gene expression across LV free wall and in RV in 3–4-week-old post-MI and sham-operated rats by RPA. (A) In these experiments, the samples from LV free wall and RV contained 10 μg total RNA, and the negative sample contained 10 μg of yeast RNA. (B) Bar graphs of Kv1.5 mRNA across LV free wall (epi, mid, and end), and in RV of sham-operated and post-MI rats. There was no significant change in the mRNA level of Kv1.5 across LV free wall or in RV of the 3-week post-MI group compared to the sham-operated group ($n=6$ for each group).

**Fig. 4.** Western blot analysis of Kv1.5 channel subunit immunoreactive protein across LV free wall (epi, mid, and end) and in RV in post-MI and sham hearts (A) Data from 3 to 4-week-old post-MI ($n=6$) and sham operated ($n=6$) rats. (B) Bar graph showing Kv1.5 immunoreactivity by measuring the signal for the protein by densitometry. Columns represent the mean values, with error bars indicating S.E.M. There was no significant change in the Kv1.5 immunoreactive protein level across LV free wall, and also no significant change in the Kv1.5 protein level in RV between the two experimental groups.
Fig. 6. Western blot analysis of Kv2.1 channel subunit immunoreactive protein across LV free wall (epi, mid, and end) and in RV in post-MI and sham hearts. (A) Data from 3 to 4-week-old post-MI ($n=6$) and sham-operated ($n=6$) rats. (B) Bar graph showing Kv2.1 immunoreactivity by measuring the signal for the protein by densitometry. Columns represent the mean values, with error bars indicating S.E.M. Immunoreactivity of Kv2.1 protein across LV free wall (epi, mid, and end) was significantly decreased in post-MI rats compared to sham-operated rats ($P<0.05$). There was no significant change in the Kv2.1 immunoreactive protein level in RV between the two groups.

Fig. 5. Representative comparison of cardiac Kv2.1 gene expression across LV free wall (epi, mid, and end) and in RV in post-MI and sham hearts. (A) Data from 3 to 4-week-old post-MI ($n=6$) and sham-operated rats ($n=6$) by RPA. In these experiments, the samples from LV free wall and RV contained 10 µg total RNA, and the negative sample contained 10 µg of yeast RNA. (B) Bar graph of Kv2.1 mRNA across LV free wall (epi, mid, and end), and in RV of sham-operated and post-MI rats. There was no significant change in the mRNA level of Kv2.1 in RV of the 3–4-week-old post-MI group compared to the sham-operated group. However, there were significant decreases in the mRNA levels of Kv2.1 across the LV free wall (epi, mid, and end) of the post-MI group compared to the sham-operated group ($P<0.05$). Values are mean±S.E.M.

4.1. Spatial heterogeneity in the expression of Kv channel subunits and $I_{\text{to}}$ density in the post-MI rat ventricle

The main consistent electrophysiological abnormality associated with cardiac hypertrophy is prolongation of APD [15]. In the remodeled post-MI myocardium, it was important to ascertain whether the enlargement in myocyte size is accompanied by proportional or disproportional changes in key sarcolemmal ion channels that could contribute to the changes in APD. In this regard, we have previously reported that the prolongation of APD 3–4 weeks post-MI was not related to alterations in the density or kinetics of the L-type Ca$^{2+}$ current which were not significantly different from control [1]. The electrophysiological observations were consistent with our report of no change in the mRNA level of the adult isoform of the $\alpha_1$ subunit of the L-type Ca$^{2+}$ channel [16]. Several evidences suggest that changes in repolarizing K$^+$ currents $I_{\text{to-f}}$ and $I_{\text{to-s}}$ are major factors in prolongation of APD of post-MI hypertrophied myocytes [1,15,17]. Dixon and McKinnon [11] have shown that there is a large gradient of Kv4.2 expression across the rat ventricular wall. Kv4.2 expression in epi muscle was more than eight times higher than in papillary muscle. However, the difference between Kv4.2 expression between epi muscle and endo muscle from the free wall of the LV was approximately 3 to 1. This is similar to the findings in the present study where the expression of Kv4.2 in sham animals between epi and endo myocardium, the later was obtained from the noninfarcted free wall of the LV, was 2.9 to 1. These data also correlate with our finding that the density of $I_{\text{to-f}}$ in epi myocytes from sham animals is 2.3 times higher than that in endo myocytes. In an early study, Clark et al. [18] showed that peak $I_{\text{to-f}}$ in rat myocytes obtained from epi and endo free wall was 2.24 versus 0.59 nA, respectively, at +50 mV depolarization.

Our electrophysiological studies have shown that although $I_{\text{to-f}}$ density in post-MI LV is decreased to a similar degree across the LV wall significant differences in density of $I_{\text{to-f}}$ continue to exist between LV epi and endo. The role of the different K$^+$ currents and their relative influence in contributing to action potential profile is different in different regions of the heart. This can explain the difference in the action potential profile of LV epi and endo myocytes that was previously reported [1]. Our observa-
Fig. 7. Representative comparison of cardiac Kv4.2 gene expression across LV free wall (epi, mid, and end) and in RV in post-MI and sham hearts. (A) Data from 3 to 4-week-old post-MI (n=6) and sham-operated rats (n=6) by RPA. In these experiments, the samples from LV free wall and RV contained 10 μg total RNA, and the negative sample contained 10 μg of yeast RNA. (B) Bar graph of Kv4.2 mRNA across LV free wall (epi, mid, and end), and in RV of sham-operated and post-MI rats. There was no significant change in the mRNA level of Kv4.2 in RV of the post-MI group compared to the sham-operated group. However, there were significant decreases in the mRNA levels of Kv4.2 across the LV free wall (epi, mid, and end) of the post-MI group compared to the sham-operated group (P<0.05). Values are mean±S.E.M.

Fig. 8. Representative comparison of cardiac Kv4.3 gene expression across LV free wall (epi, mid, and end) and in RV in post-MI and sham hearts. (A) Data from 3 to 4-week-old post-MI (n=6) and sham-operated rats (n=6) by RPA. In these experiments, the samples from LV free wall and RV contained 10 μg total RNA, and the negative sample contained 10 μg of yeast RNA. (B) Bar graph of Kv4.3 mRNA across LV free wall (epi, mid, and end), and in RV of sham-operated and post-MI rats. There was no significant change in the mRNA level of Kv4.3 in RV of the post-MI group compared to the sham-operated group. However, there were significant decreases in the mRNA levels of Kv4.3 across the LV free wall (epi, mid, and end) of the post-MI group compared to the sham-operated group (P<0.05). Values are mean±S.E.M.

4.2. Molecular basis of K⁺ channels

Several attempts have been made to correlate channel subunit genes expression to native K⁺ currents. For example, heterologous expression of Kv4.2 and Kv4.3 mRNA generates channels with kinetics and pharmacological properties similar to I_{to-f} in cardiac myocytes [12]. Moreover, Kv4.2 mRNA is expressed in a gradient across LV wall, which correlates well with the gradient of I_{to-f} density across the wall [11]. On the other hand, Kv4.3 is homogeneously expressed across the LV wall [12]. Kv4.2 homomeric and/or Kv4.2-Kv4.3 heteromeric channels are likely to contribute to the difference of I_{to-f} in different parts of the rat ventricle. This can possibly explain the somewhat slower inactivation kinetics of I_{to-f} in LV endo in the present study. Some investigators have shown that recovery from inactivation was slower for Kv4.3 compared to Kv4.2 based currents expressed in tsA-201 cells [19]. We have demonstrated that the protein levels of Kv4.2/Kv4.3 were significantly decreased in epi and endo of post-MI remodeled myocardium by 49 and 51%, respectively, compared to sham. These findings provide the molecular basis of our electrophysiological observations of decreased density of I_{to-f} by 54 and 61% in myocytes obtained from epi and endo, respectively of post-MI rat heart. The decreased density of I_{to-f} is a major contributor to changes in action potential profile and duration [20].

In contrast to I_{to-f}, the molecular basis of I_{to-s} in the rat has not been well established. In a study by Barry et al. [10], the authors showed that heterologous expression of Kv2.1 revealed a slowly activating TEA-sensitive K⁺ current similar to I_{to-s} in adult rat ventricular myocytes and therefore seemed to be the likely candidate for this current.
Fig. 9. Western blot analysis of Kv4.2/4.3 channel subunit immuno-reactive proteins across LV free wall (epi, mid, and end) and in RV in post-MI and sham hearts. (A) Data from 3 to 4-week-old post-MI ($n=6$) and sham operated ($n=6$) rats. (B) Bar graph showing Kv4.2/4.3 combined immunoreactivity by measuring the signal for the proteins by densitometry. Columns represent the mean values with error bars indicating S.E.M. Immunoreactivity of Kv4.2/4.3 across LV free wall (epi, mid, and end) was significantly decreased in post-MI rats compared to sham-operated rats ($P<0.05$). There was no significant change in the Kv4.2/4.3 immunoreactive protein level in RV between the two experimental groups.

Other investigators have suggested that Kv1.4 underlies the slowly recovering component of $I_{to}$, especially in endo myocytes [19,21,22]. The present study shows that Kv1.4 protein is present in adult rat LV, RV endo and RV epi, and that the density is at least twice as high in LV endo compared to RV and LV epi. Furthermore, the inactivation kinetics of $I_{to}$ in LV endo was slower than that in LV epi, which may suggest a larger contribution of Kv1.4 to $I_{to}$ in LV endo. However, the presence of a relatively small amount of Kv1.4 protein does not constitute enough evidence for its contribution to $I_{to}$ in the LV.

### 4.3. Study limitations

We have previously shown regional variations in APD in the 3–4 week post-MI rat heart [1]. The present study is an attempt to provide the molecular and ionic basis of these changes. It should be emphasized, however, that conclusions regarding the correlation between action potential configuration and current densities remain speculative in the absence of reconstruction of simulated action potential that incorporates complete data on the density, time course, and voltage dependence of all currents that contribute to the repolarization phase [1]. The electrophysiological characteristics of RV myocytes were not investigated. However, we found no significant changes in Kv subunits expression in right ventricular myocardium obtained from 3 to 4-week-old post-MI rat heart. Finally, the possibility of gender difference in post-MI remodeling has not been addressed in the present study. Female rats usually have been utilized as a model for post-MI remodeling [1–5]. The first quantitative analysis of K+ channel genes in Sprague–Dawley rats also was done in young adult female animals [11]. Female sex has long been associated with a slower rate of cardiac depolarization based usually on analysis of surface ECG QT interval and with a higher risk of developing drug-induced torsades de pointes arrhythmias [23]. Several studies have suggested that gender difference in the expression and/or modulation of cardiac K+ channel genes and currents in some animal models may underlie these changes [24,25]. There is no data, however, on gender difference in the downregulation
of $K^+$ channel genes and currents and/or the degree of electrophysiological alteration in the rat post-MI model. Further investigations are required to address these issues.

5. Conclusion

Regional variations in the downregulation of Kv channels expression and density of $K^+$ currents can be a significant determinant of increased spatial electrophysiological heterogeneity and contribute to increased electrical instability of the post-MI heart.

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