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Dynamic remodeling of K⁺ and Ca²⁺ currents in cells that survived in the epicardial border zone of canine healed infarcted heart

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Abstract

Action potentials (APs) of the epicardial border zone (EBZ) cells from the *day 5* infarcted heart continue to be altered by *day 14* postocclusion, namely, they shortened. However, by 2 mo, EBZ APs appear “normal,” yet conduction of wave fronts remains abnormal. We hypothesize that the changes in transmembrane APs are due to a change in the distribution of ion channels in either density or function. Thus we focused on the changes in Ca²⁺ and K⁺ currents in cells isolated from the 14-day (IZ_{14d}) and 2-mo (IZ_{2m}) EBZ and compared them with those occurring in cells from the same hearts but remote (Rem) from the EBZ. Whole cell voltage-clamp techniques were used to measure and compare Ca²⁺ and K⁺ currents in cells from the different groups. Ca²⁺ current densities remain reduced in cells of the 14-day and 2-mo infarcted heart and the kinetic changes previously identified in the 5-day heart begin to, but do not recover to, cells from noninfarcted epicardium (NZ) values. Importantly, *I*_{Ca,L} in both the EBZ and Rem regions still show a slowed recovery from inactivation. Furthermore, during the remodeling process, there is an increased expression of T-type Ca²⁺ currents, but only regionally, and only within a specific time window postmyocardial infarction (MI). Regional heterogeneity in β-adrenergic responsiveness of *I*_{Ca,L} exists between EBZ and remote cells of the 14-day hearts, but this regional heterogeneity is gone in the healed infarcted heart. In IZ_{14d}, the transient outward K⁺ current (*I*_{to}) begins to reemerge and is accompanied by an upregulated tetraethylammonium-sensitive outward current. By 2-mo post-occlusion, *I*_{to} and sustained outward K⁺ current have completed the reverse remodeling process. During the healing process post-MI, canine epicardial cells downregulate the fast *I*_{to} but compensate by upregulating a K⁺ current that in normal cells is minimally functional. For recovering *I*_{Ca,L} of the 14-day and 2-mo EBZ cells, voltage-dependent processes appear to be reset, such that *I*_{Ca,L} “window” current occurs at hyperpolarized potentials. Thus dynamic changes in both Ca²⁺ and K⁺ currents contribute to the altered AP observed in 14-day fibers and may account for return of APs of 2 mo EBZ fibers.

Keywords

myocardial infarction

Sustained ventricular tachycardias (VTs) occur spontaneously and can be induced by programmed stimulation in human hearts after myocardial infarction (MI). Sustained VTs can also be induced in the canine heart 4–5 days after coronary artery occlusion (26). Cells that survive in the epicardial border zone (EBZ) of the 5-day infarcted canine heart (IZ_{5d}) show significant electrical remodeling (22). We previously reported that the basal L-type Ca²⁺ currents ($I_{Ca,L}$) are decreased in IZ_{5d} (1), as is the stimulatory effect of the β -adrenergic agonist, isoproterenol (Iso), and cAMP (2, 25). Action potentials (APs) recorded from 5-day EBZ fibers show no or reduced phase 1 consistent with the loss in the voltage-dependent transient outward current (I_{to}) (20).

Altered APs of the EBZ cells from the 5-day infarcted heart continue to be altered by day 14 postocclusion (6, 26). However, by 2 mo, EBZ cell APs appear “normal,” yet conduction of the wavefronts remains abnormal (26). This suggests that a very active process exists that restores ion channel function to these surviving epicardial cells. We hypothesize that the changes in transmembrane APs are due to a changing distribution of ion channels in density or function. Thus the goal of this study was to determine the changes in Ca²⁺ and K⁺ currents that occur in cells isolated from the 14-day (IZ_{14d}) and 2-mo (IZ_{2m}) EBZ area and compare them with changes in ion channel function occurring in cells from the same hearts but remote (Rem) from the EBZ.

METHODS

Animal Preparation

This investigation conforms to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Pub. No. 85-23, 1996).

Healthy male mongrel dogs (12–15 kg, 2–3 yr old) were used in these studies. Under isoflurane anesthesia (30 mg/kg), MI was produced by a two-step total occlusion of the left coronary artery using the Harris procedure (15). On 5 days ($N = 5$ dogs), 14 days ($N = 24$ dogs), and 2 mo ($N = 15$ dogs) after occlusion, the infarcted hearts were used for the myocyte study. Noninfarcted hearts were used for controls. Sham-operated animals were animals that had an operation but no occlusion was done. Sham animals were used for study 14 days ($N = 5$) and 2 mo ($N = 4$) post operation.

Myocyte Preparation

Single Ca²⁺-tolerant cells were dispersed from the epicardium sections with the use of a modification of our previously described method (20). The tissue was rinsed twice in a Ca²⁺-free solution that contained (in mM) 115 NaCl, 5 KCl, 35 sucrose, 10 dextrose, 10 HEPES, and 4 taurine, pH 6.95, to remove blood. The solution was then triturated in 20 ml of enzyme containing solution (0.38 mg/ml collagenase Type II from Worthington Biochemical; 0.05 mg/ml protease from Sigma; 36–37°C) for 30 min, after which the solution was decanted and discarded. The second trituration without the protease was discarded after 30 min. The next 6–7 triturations were each done for 15 min. Each time the solution was centrifuged for 3 min to collect the supernatant and dispersed cells. The resuspension solution was changed every 30 min for solutions containing increasing

concentrations of Ca^{2+} . With this procedure, the viable cell yield was ~30–40%. We used only rod-shaped cells with staircase ends, clear cross-striations, and surface membranes free from blebs for this study. Several groups of cells were studied. One group was cells dispersed from control noninfarcted left ventricular epicardium (NZs). Other groups comprised cells dispersed from the EBZ of the 5-day, 14-day, and 2-mo infarcted heart (IZ_{5d}, IZ_{14d}, and IZ_{2m}, respectively), cells dispersed from left ventricular epicardial sections from a region remote from the infarct (Rem) in the 14-day and 2-mo healed hearts (Rem_{14d} and Rem_{2m}, respectively). Epicardial cells from EBZ area of sham-operated animals formed two groups corresponding to the 14-day and 2-mo time points (Sham_{14d} and Sham_{2m}, respectively).

Electrophysiological Studies

For study, an aliquot of cells was transferred onto a poly-lysine-coated glass coverslip placed at the bottom of a 0.5-ml tissue chamber, which had been mounted on the stage of an inverted microscope (Nikon Diaphot; Tokyo, Japan). Myocytes were continuously superfused (2–3 ml/min) with normal Tyrode solution containing (in mM) 137 NaCl, 24 NaHCO₃, 1.8 NaH₂PO₄, 0.5 MgCl₂, 2.0 CaCl₂, 4.0 KCl, and 5.5 dextrose, pH 7.4. The solution was bubbled with 5% CO₂-95% O₂.

To measure Ca^{2+} currents, pipette resistances ranged between 1 and 2 M Ω when filled with an internal solution composed of (in mmol/l) 125 CsOH, 125 aspartic acid, 20 tetraethylammonium chloride (TEA), 10 HEPES, 5 Mg-ATP, 10 EGTA, and 3.6 phosphocreatine (pH 7.3 with CsOH). After the formation of the gigaohm seal, the stray capacitance was electronically nulled, the cell membrane under the pipette tip was ruptured, and, by a brief increase in suction, the whole cell recording configuration was then formed. A period of 10 min was then allowed for intracellular dialysis to begin before being switched to the nominal sodium-free recording solution composed of (in mmol/l) 5.0 CaCl₂, 0.5 MgCl₂, 140 TEA, 10 HEPES, 10 dextrose, and 2 4-aminopyridine (pH 7.3 with CsOH, $35.0 \pm 0.5^\circ\text{C}$).

$I_{\text{Ca,L}}$ magnitude was normalized by each cell's membrane capacitance (pF) and expressed as current density (pA/pF). Voltages were not corrected for the liquid junction potential between the bath and pipette solutions. When myocytes are dialyzed during whole cell recordings, there is the decrease of peak $I_{\text{Ca,L}}$ with time. We (1) have previously established that rundown is similar in cells of the two groups. Thus, for these studies, we started data acquisition at similar times after membrane rupture and establishment of whole cell configuration (see figures).

Peak $I_{\text{Ca,L}}$ at various test voltages (V_t) was measured as the difference between the maximal inward peak and the current level at the end of 250-ms voltage-clamp step. The time course of $I_{\text{Ca,L}}$ decay was characterized by fitting the current change between the inward peak and the current level 250 ms after depolarization (Clampfit, pCLAMP, Axon Instruments) using a biexponential function. Steady-state inactivation variables of $I_{\text{Ca,L}}$ were determined using a double-pulse protocol. For each cell, the peak current elicited at each test pulse was expressed as a fraction of the current obtained with the most negative conditioning prepulse potential (–70 mV, 1,000-ms duration) and a Boltzmann equation was used to fit data and to

obtain the half-maximum inactivation voltage ($V_{0.5}$) and slope factor (k) for each cell. Average values were used to determine the differences between cells from the different groups.

To measure K^+ currents, patch pipette resistances equaled 1–2 $M\Omega$ when filled with the internal solution composed of (in mM) 140 KCl, 1 $MgCl_2$, 10 EGTA, 5 MgATP, 5 creatine phosphate, 0.2 GTP, and 10 HEPES, pH 7.2, with KOH. Cells were superfused with a Na^+ -free solution composed of (in mM) 144 *N*-methyl-D-glucamine-Cl, 5.4 KCl, 1 $MgCl_2$, 2.5 $CaCl_2$, 0.5 $CdCl_2$, and 10 HEPES, pH 7.4, 30–31°C. Na^+ currents were suppressed via use of a Na^+ -free solution. $I_{Ca,L}$ was blocked with 0.5 mM Cd^{2+} . Membrane currents associated with Na^+/Ca^{2+} exchange were eliminated by the absence of external Na^+ . Currents were elicited by 210-ms voltage step to V_s of –50 and +60 mV from a holding potential of –60 mV at 0.1 Hz after a 10-ms prepulse to –90 mV to maximally activate I_{to} . I_{to} amplitude was determined as the difference between the peak current and that at the end of the pulse or as stated. The sustained outward K^+ current (I_{sus}) was taken as the amplitude of the current at the end of test pulse relative to zero-current level. I_{to} decay was fit with a double exponential function to estimate time constants of current decay. Steady-state inactivation relationships were determined using the following protocol: a 500-ms prepulse to various conditioning potentials between –90 and +20 mV, followed by a 210-ms test pulse to 60 mV. Because inactivation of the delayed rectifier K^+ current contaminates I_{to} inactivation at the most negative voltage (4), only data obtained from potentials positive to –55 mV were used to fit with Boltzmann equation to get $V_{0.5}$ and slope factor (k) of I_{to} for each cell. The time course of recovery from inactivation was evaluated with a paired-pulse protocol. Two identical 210-ms pulses with a holding potential of –80 to +40 mV were delivered with interpulse coupling intervals (IPI) that increased from 5 to 5,000 ms. The degree of recovery was determined by normalizing I_{to} at each IPI by the I_{to} at IPI 5,000 ms. The time course of recovery was estimated by fitting the data points to a biexponential function with the use of a simplex algorithm.

Drugs

Isoproterenol (Iso)-containing solutions were made on the day of the experiment from stock solution (1 mg/ml). TEA (Sigma) was dissolved in double-distilled H_2O for stock solution. Flecainide (Sigma) was dissolved in DMSO at 100 mM and diluted in the external recording solution. TTX (Sigma) was dissolved in distilled H_2O for stock solution and used on the day of the experiment.

Statistics

Data are presented as means $\pm$ SE. All data were tested using ANOVA for multiple comparisons. If significant changes occurred, group means were compared with the use of Bonferroni's method. $P < 0.05$ was significant.

RESULTS

Ca²⁺ Currents in NZs and IZ_{14d} and IZ_{2m} versus Rem_{14d} and Rem_{2m}

Like data for IZ_{5d} (1), cells from IZ_{14d} and IZ_{2m} were significantly increased in cell size (by 46% and 29%, respectively) versus NZ and sham cells (Table 1). Cells from the remote regions (Rem_{14d} and Rem_{2m}) were not different from their respective IZ counterparts but were larger than NZ cells. In this dataset, as well as others, sham cells were similar to NZ cells. Therefore, here and in the remaining text, a reference is made only to differences from NZ values.

Peak $I_{Ca,L}$ densities were determined for IZ_{14d} and IZ_{2m} and found to be no different from each other but each differed significantly from NZ (Fig. 1A) as well as their respective Rem cell values at some V_s (Fig. 1B). Interestingly, we found that 87% of IZ_{14d} cells, but only 28% of IZ_{2m} cells, had significant inward currents at V_s -45 to -10 mV. These Ca²⁺ currents are most likely T-type Ca²⁺ currents ($I_{Ca,T}$) due to their rapid rate of decay and lack of sensitivity to TTX (20 μ M) ($n = 3$ cells, data not shown). Low-voltage Ca²⁺ currents were not observed in Rem_{14d} and Rem_{2m} groups (Fig. 1). Thus it appears that during the process of remodeling, there is an increased expression of $I_{Ca,T}$, but only regionally, and only within a specific time window during the healing phase post-MI.

Similar to IZ_{5d} (1), we found that, although the peak $I_{Ca,L}$ in IZ_{14d} is reduced versus NZs, the average decay of the peak current is significantly faster than that in NZs or IZ_{2m} (Table 2). Furthermore, reduced Ca²⁺ currents of IZ_{14d} and IZ_{2m} were accompanied by shifts in both activation and inactivation relations versus their respective remote values (Fig. 2 and Tables 3 and 4). As a result, a window current generated by overlap of these relations is shifted in the negative direction in both cell types. Combined with the reduction in density, these shifts could account for less $I_{Ca,L}$ at plateau voltages and thus a shortening of APD observed in IZ_{14d} cells (6, 26). However, APs of IZ_{2m} are not shortened (26), suggesting that other currents contribute to maintaining APD in IZ_{2m} (see DISCUSSION). Finally, we found there is significant slowing of recovery from inactivation of Ca²⁺ currents in both IZ_{14d} and IZ_{2m} and their remote cell counterparts (Table 2).

An important observation in our original study was that there was a reduced response of IZ_{5d} $I_{Ca,L}$ to Iso (1 μ M) (2). Thus we determined whether there was a recovery of β -adrenergic responsiveness in cells from the healed infarcted hearts. Figure 3A shows an example of Ca²⁺ currents in the absence and presence of Iso in IZ_{14d} and IZ_{2m}. Note that there is a reduced fold effect of Iso in IZ_{14d} cells versus that in Rem_{14d} cells (Fig. 3B). Importantly, Iso has a large significant effect on $I_{Ca,L}$ of IZ_{2m} cells. In fact, the responses of Iso in Rem_{2m} and IZ_{2m} cells do not differ (Fig. 3B and Tables 5 and 6). Thus the difference in the response of $I_{Ca,L}$ to Iso seen between the two regions of the 14-day infarcted heart (EBZ and remote areas) is gone by 2 mo after occlusion.

K⁺ Currents in NZs and IZ_{14d} and IZ_{2m} versus Rem_{14d} and Rem_{2m}

For these studies, we focused on I_{to} and I_{sus} induced by steps in voltage in IZ_{14d} and IZ_{2m} because in IZ_{5d} we noted that there are diminished or no transient K⁺ currents (20). We

compared $I_{Z_{14d}}$ and $I_{Z_{2m}}$ values with values obtained from cells from regions of same left ventricles but remote from the EBZ (Rem_{14d} and Rem_{2m}) and sham cells.

Figure 4, *A* and *B*, shows original current tracings for cells from the different groups. In Fig. 4, *C* and *D*, I_{to} and I_{sus} average densities are shown for cells in all groups. Note that I_{to} density in $I_{Z_{14d}}$ cells recovered to 8.97 ± 0.82 pA/pF (at +40 mV) compared with cells of $I_{Z_{5d}}$, where little or no I_{to} was observed (20). However, I_{to} in $I_{Z_{14d}}$ remained significantly reduced compared with Rem_{14d} (20 ± 2.0 pA/pF). There was no difference between sham, Rem, and NZ values. By 2 mo after occlusion, I_{to} in $I_{Z_{2m}}$ returned toward NZ/Sham values and was no different compared with Rem_{2m} (Fig. 4*C*). I_{sus} did not differ in the cells of the different groups (Fig. 4*D*).

Further analysis revealed that I_{to} of $I_{Z_{14d}}$ decayed more slowly than decays of $I_{Z_{2m}}$ and Rem cells (Fig. 5), and there was a slight but significant depolarizing shift in the inactivation relation in $I_{Z_{14d}}$ cells (Table 7). Finally, we determined that the small I_{to} of $I_{Z_{14d}}$ had a slower recovery from inactivation than that of NZ, Rem, or sham cells (Table 8).

TEA and Flecainide Sensitivity of $I_{Z_{14d}}$

If there is a change in the components of channel isoforms contributing to I_{to} and I_{sus} in $I_{Z_{14d}}$ and $I_{Z_{2m}}$, then we would predict a change in sensitivity to drugs that are selective for specific channel isoforms. Thus we determined the effects of TEA (5 mM) on the currents of $I_{Z_{14d}}$, $I_{Z_{2m}}$, and NZs. In this subset of cells, we found a small TEA-sensitive I_{to} in NZs, $I_{Z_{14d}}$, and $I_{Z_{2m}}$ (Fig. 6*A*). However, in $I_{Z_{14d}}$ with small, partially recovered I_{to} (Fig. 4), we found a significant increase in a TEA-sensitive I_{sus} component (0.3 pA/pF at +40 mV) (Fig. 6*B*). Furthermore, when testing for TEA-sensitive currents in $I_{Z_{5d}}$, we determined there was also a considerable TEA-sensitive I_{sus} in $I_{Z_{5d}}$. However, by 2 mo, TEA-sensitive currents in $I_{Z_{2m}}$ are equivalent to NZs (~ 0.04 pA/pF). Thus the recovered I_{to} of $I_{Z_{14d}}$ appears to be accompanied by sevenfold increase in a TEA-sensitive sustained current.

Finally, 30 μ M flecainide, which has been used to show block of Kv4s (9), blocked 37%, 40% of peak I_{to} of NZs and $I_{Z_{2m}}$ but showed a reduced effect on I_{to} of $I_{Z_{14d}}$ (inhibiting only 27%) (Fig. 6*C*). In sum, I_{to} in $I_{Z_{2m}}$ and Rem_{2m} do not differ from that of NZs. On the other hand, I_{to} and I_{sus} in $I_{Z_{14d}}$ show kinetic differences as well as a change in sensitivity to flecainide and TEA, suggesting that I_{to}/I_{sus} composite in $I_{Z_{14d}}$ is likely a combination of channels that are less sensitive to flecainide and more sensitive to TEA.

DISCUSSION

We report here that the changes in APs that occur in the healing EBZ of the canine heart (6, 26) are due to the changing levels of functioning Ca^{2+} and K^{+} currents (i.e., return of the voltage-dependent I_{to}). For the “new” $I_{Ca,L}$ of the 14-day and 2-mo EBZ cells, voltage-dependent processes appear to be reset, such that $I_{Ca,L}$ window current occurs at hyperpolarized potentials. Furthermore, whereas regional heterogeneity in β -adrenergic responsiveness of $I_{Ca,L}$ exists between EBZ and remote cells of the 14-day hearts, it is gone in the healed infarcted heart (2 mo). Finally, in $I_{Z_{14d}}$ cells, I_{to} begins to reemerge but it is accompanied by an upregulated TEA-sensitive outward current.

Comparison With Other Studies on Ion Channel Function in Long-Term MI Models

Ca²⁺ current changes—Cells from the EBZ of the 5-day infarcted heart have reduced Ca²⁺ current density as well as reduced responsiveness to Iso as reported earlier (1, 2). These remodeled Ca²⁺ currents were accompanied by an acceleration in peak current decay and no change in availability. However, when cells are dispersed from different regions of the reentrant circuit of the 5-day EBZ, there are regional differences in $I_{Ca,L}$ and its kinetics. Importantly, in cells from the center path of the reentrant circuit, $I_{Ca,L}$ availability is shifted negatively and in the cells from the outer pathway, the time course of recovery of $I_{Ca,L}$ from inactivation is slowed (10). The results of the current study suggest that $I_{Ca,L}$ densities remain reduced in the cells from 14-day and 2-mo infarcted heart and the kinetic changes previously identified in the 5-day heart begin, but do not recover fully, to NZ values. An important point here is the finding that the Ca²⁺ currents in the EBZ and Rem regions of the healed heart show a slowed recovery from inactivation perhaps secondary to generalized hypertrophic signals. For example, this kinetic change would be consistent with an increase in the basal level of phosphorylation of the channel. However, the precise mechanism is beyond the scope of this study. This slowed recovery could lead to augmented frequency-dependent effects on $I_{Ca,L}$ amplitude.

Myocytes adjacent to an 8-wk infarct in the rabbit heart showed a significant decrease in peak $I_{Ca,L}$ without a change in the current-voltage relationship or gating parameters (19). In cats with a healed (2–4 mo) MI and CHF, there is marked disparity in refractoriness in subendocardial border adjacent to infarcted areas and remote areas, especially during sympathetic stimulation (23), and $I_{Ca,L}$ was found to be reduced in both the hypertrophied remote and the MI area (endocardial) cells. Inactivation curves were shifted in a hyperpolarizing direction in both cell types but no differences in time course of recovery and decay were reported. Reduced β -adrenergic responsiveness of $I_{Ca,L}$ occurred in both remote and MI area cells in this study. Typically, the areas remote from the infarct scar show a tendency toward an increase in Ca²⁺ current amplitudes (23, 24); however, current densities (pA/pF) were reduced as a result of the increased cell capacitance of remote cells suggesting that channel protein expression did not exactly follow the increased plasmalemma of these hypertrophied cells. A longer-term study (3) in rats post-MI (4–6 mo) reported reduced peak Ca²⁺ current densities with no specific kinetic changes except for a slowing in peak current decay. Note that the cells studied in that report were from various regions of the infarcted ventricle and not necessarily from an EBZ or remote epicardial area.

Studies using cells dispersed from human hearts post-MI have been scarce. In a recent study (8), most human hearts were post-MI and cells were reported to have no change in basal $I_{Ca,L}$ versus cells from normal hearts. Cells used in these studies were most probably from tissues remote from the healed scar of the previous infarct. The time postocclusion was not given in this report. Interestingly, $I_{Ca,L}$ activation curves were shifted negatively in these diseased human cells much like our data for IZ_{14d} and IZ_{2m} cells. Unfortunately, other kinetic parameters such as steady-state inactivation relations and the time course of recovery from inactivation were not studied. Cells from post-MI human hearts also show a hypoadrenergic responsiveness of $I_{Ca,L}$ to Iso similar to our data and other studies using

animal models. However, in our study, we show that the regional heterogeneity in this hypoadrenergic response exists in the 14-day hearts and is gone in the healed (2 mo) heart.

$I_{Ca,T}$ —In our previous work (1, 7) in cells from the MI canine heart, we reported $I_{Ca,T}$ changes in both subendocardial cells from the 48-h heart and cells from the EBZ of the 5-day heart. Thus it is notable that there is an increase in T-type current density in IZ_{14d} cells and not in IZ_{2m} cells. In fact, we found that T-type currents existed in nearly all IZ_{14d} studied. In rat healed (3–4 wk) MI cells, the T-type current was recorded in 35% of post-MI left ventricular cells, but channel expression had increased by 158% (16). However, it is important to note that $I_{Ca,T}$ was not reported in the remodeled cells of the post-MI human heart (8). This may be because $I_{Ca,T}$ manifestation is linked only at certain times to the ongoing process of cellular remodeling. Its function may be dedicated to a specific cell function (i.e., gene expression) and/or its reexpression in pathophysiological conditions (16, 21) related to levels of angiotensin II (11) and/or endothelin-1 (12).

Depolarization-activated K^+ currents—The return of a voltage-dependent I_{to} in IZ_{14d} is striking and obviously has occurred after *day 5* postocclusion because we found no or little I_{to} in IZ_{5d} (20). The important aspects of the newly emerged I_{to} in these EBZ cells are its small current density, its positive midpoint of inactivation, and slow recovery from inactivation. All these features are reminiscent of endocardial myocytes of the noninfarcted canine heart or epicardial cells in the presence of angiotensin (28). Furthermore, the pharmacology of I_{to}/I_{sus} of IZ_{14d} suggests that a portion of the “new” depolarization activated current is less sensitive to flecainide and more sensitive to TEA (see below).

In the rat post-MI model, two long-term studies have been reported. Kaprielian et al. (18) reported that, whereas action potential duration is prolonged in 2-mo post-MI cells, I_{to} is reduced more in the right ventricle than in septal cells and density changes were not linked to differences in gating parameters. No data were reported from EBZ or epicardial cells in that study. We report here that in the 2-mo heart, I_{to} in IZs and Rem do not differ from each other or sham/normal values. Thus, even in the presence of an increase in cell size (Table 1), I_{to} in canine epicardial cells 2-mo post-MI are reasonably normal. In another study (post-MI 4–6 mo, rat), I_{to} was reduced by 50% in all cell regions studied but gating parameters did not differ (3). Epicardial cells overlying the infarct were not sampled.

Human cell studies post-MI have not been done yet. In human heart failure, where some cases of heart failure were secondary to an MI, I_{to} is downregulated in left ventricular myocytes (17). However, it is still difficult to evaluate regional changes in I_{to} in human hearts. In no previous study of ventricular cells post MI has an augmented TEA-sensitive current been reported.

In summary, it appears that in the process of ionic remodeling of canine epicardial cells, early after coronary occlusion (5 days), I_{to} disappears and a sustained TEA-sensitive current emerges. By *day 14*, I_{to} reemerges in combination with the augmented TEA-sensitive current. By 2 mo postocclusion, I_{to}/I_{sus} currents have completed a “reverse remodeling” process.

Implications of Pharmacology

As in other reports (9, 14), NZs and IZ_{2m} show a 40% block of I_{to} with flecainide, consistent with a Kv4 isoform contributing to canine I_{to} (9). In IZ_{14d}, we report here that the effect of flecainide is blunted in that only 27% of peak current was blocked. Thus it may be because there is less of a contribution of Kv4s to I_{to} in IZ_{14d}.

In addition, and different from a previous report (14), we observed TEA-sensitive transients in NZs, IZ_{14d}, and IZ_{2m}. Furthermore, and differing from the observed effects in NZs, we saw an 18% inhibition of I_{sus} with TEA in both IZ_{14d} and IZ_{5d}. Large TEA-sensitive currents were not seen in Rem or IZ_{2m} cells. It is unlikely that these TEA-sensitive currents are due to Kv1.4 currents because the latter is not TEA sensitive. Rather, the emergence of augmented TEA-sensitive currents in these remodeled cells, where epicardial I_{to} has been downregulated, resembles the regional upregulation of TEA-sensitive Kv2.1 currents in ventricular cells of KvDN mice where fast I_{to} is suppressed and a slow I_{to} is induced (5, 13, 29). Interestingly, phorbol ester-induced hypertrophy in a cell monolayer system is associated with a downregulation of Kv4 subunits (and I_{to}) and an enhancement of Kv2.1 subunits (and TEA-sensitive I_{sus}) (27). The changes in K^+ current function in post-MI cells may be related to a robust PKC stimulation but only in cells of the EBZ of the 5- and 14-day hearts. Thus, during the healing process post-MI, canine epicardial cells downregulate the fast I_{to} , but compensate by upregulating a K^+ current that in normal cells is minimally functional. In IZ_{14d}, this augmented K^+ current in the absence of a robust Ca^{2+} current would contribute to the shortened APs of these cells (6, 26).

Our results are surprising. First, despite the return of a normal-appearing AP in 2-mo EBZ fibers (26), Ca^{2+} currents remain abnormal, whereas I_{to}/I_{sus} has regained full NZ like function. Perhaps these subtle differences could contribute to the restored AP plateau of 2-mo IZs. Cellular modeling of these changes is underway to test such. Second, the reverse remodeling process of the I_{to}/I_{sus} currents is dynamic with changes in 5-day EBZ cells differing from changes in 14-day cells differing from changes in 2-mo cells and the lost and/or markedly inhibited I_{to} currents in the 5- and 14-day cells are accompanied by augmented TEA-sensitive currents.

Limitations

The changes in the fast/slow Na^+ currents in EBZ cells post-MI are not included in this study. Changes in these currents could contribute to the abnormal impulse propagation in the EBZ of the healed infarcted heart as well as the AP changes of 2-mo EBZ fibers. Furthermore, we have not identified changes in Ca^{2+} and K^+ currents in all regions of the remodeled heart but rather have focused on the changes in the substrate (the EBZ) where reentrant tachycardias have been mapped.

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Fig. 1.

A: original tracings of L-type Ca^{2+} currents ($I_{\text{Ca,L}}$) in cells from noninfarcted left ventricular epicardium (NZ), isolated cells from day 14 epicardial border zone ($\text{IZ}_{14\text{d}}$), and 2-mo epicardial border zone ($\text{IZ}_{2\text{m}}$) under conditions of these experiments: 5 mM Ca^{2+} ; 10 mM EGTA; holding voltage, -70 mV to various test voltages as shown. Arrows indicate zero current. B, left: average peak I_{Ca} density-voltage relations in NZ ($n = 22$ cells, $N = 9$ animals), $\text{IZ}_{14\text{d}}$ ($n = 23$, $N = 14$) and $\text{IZ}_{2\text{m}}$ ($n = 18$, $N = 12$). Data were collected at similar times after whole cell membrane rupture (NZs: 24.9 ± 1.5 , $\text{IZ}_{14\text{d}}$: 24.7 ± 1.1 , $\text{IZ}_{2\text{m}}$: 24.4 ± 1.1 , $\text{Rem}_{14\text{d}}$: 24.0 ± 1.3 , $\text{Rem}_{2\text{m}}$: 22.6 ± 3.0 min; $P > 0.05$). Middle, average peak I_{Ca} density voltage relations in $\text{IZ}_{14\text{d}}$ and $\text{Rem}_{14\text{d}}$ ($N = 11$). Note there were no or small Ca^{2+} currents activated in negative voltages (V_t : -45 to -10 mV) in $\text{Rem}_{14\text{d}}$ vs. $\text{IZ}_{14\text{d}}$. Right, average peak I_{Ca} density voltage relations in $\text{IZ}_{2\text{m}}$ and $\text{Rem}_{2\text{m}}$ ($n = 9$, $N = 7$).

Fig. 2.

A: activation curves the half-maximal voltage ($V_{0.5}$) and slope factor k were calculated from current-voltage (I - V) protocol and reversal potential (E_{rev}) and are shown in Table 3. IZ_{14d} ($n = 21$, $N = 14$) and IZ_{2M} ($n = 18$, $N = 12$) showed significant negative shifts of activation curves vs. Rem cells. B: steady-state inactivation $V_{0.5}$ in both IZ groups was shifted negatively vs. Rem values. All data were collected same time after whole cell rupture (IZ_{14d}: 28.0 ± 0.6 , IZ_{2M}: 26.8 ± 1.2 , Rem_{14d}: 28.8 ± 0.7 , Rem_{2M}: 24.8 ± 1.5 min, $P > 0.05$). $G/G_{\max}$, fractional conductance; V_t , test voltage; V_c , conditioning prepulse voltage.

Fig. 3.

A: effect of isoproterenol (Iso) on $I_{Ca,L}$ was diminished in IZ_{14d} ($n = 9$, $N = 6$) and IZ_{2m} ($n = 6$, $N = 6$). Solid lines indicate control peak $I_{Ca,L}$ and dashed lines indicate peak $I_{Ca,L}$ in the presence of 1 μM Iso. B: Iso (1 μM) increased peak $I_{Ca,L}$ in both IZ groups but fold effect was significantly smaller in IZ groups (1.5- and 2.0-fold in IZ_{14d} and IZ_{2m}).

Fig. 4.

A and B: representative current recordings in myocytes isolated from IZ_{14d}, Rem_{14d}, Sham_{14d}, IZ_{2M}, Rem_{2M}, and Sham_{2M} dogs. The data were obtained with a prepulse potential of -90 mV from a holding potential of -60 mV with 10-mV depolarizing steps to voltages from -50 to $+60$ mV. Arrows denote zero current level. C: I - V relation for I_{to} as a function of test potential NZ ($n = 83$, $N = 42$), IZ_{14d} ($n = 45$, $N = 24$), Rem_{14d} ($n = 15$, $N = 12$), Sham_{14d} ($n = 14$, $N = 5$), IZ_{2M} ($n = 25$, $N = 15$), Rem_{2M} ($n = 14$, $N = 13$), and Sham_{2M} ($n =$

7, $N = 4$). *D*: summary of sustained outward K^+ current (I_{sus}) at +40 mV. Values are means $\pm$ SE. * $P < 0.05$ vs. NZ; ‡ $P < 0.05$ vs. $I_{Z_{2m}}$.

Fig. 5.

Average time constants (τ) of decay of I_{to} at various V_t potentials (V_t s) in NZ ($n = 66$, $N = 42$), IZ_{14d} ($n = 40$, $N = 24$), and IZ_{2m} ($n = 20$, $N = 15$). Average τ -values were plotted as a function of V_t . The protocol was the same as in Fig. 4. Values are means $\pm$ SE. * $P < 0.05$ vs. NZ.

Fig. 6.

Effects of 5 mM tetraethylammonium (TEA) on I_{to} and I_{sus} at +40 mV. **A:** responses of I_{to} to TEA in NZ ($n = 10$, $N = 9$), IZ_{14d} ($n = 10$, $N = 7$) and IZ_{2m} ($n = 4$, $N = 3$). **B:** comparison of the responses of I_{sus} to TEA between NZ, IZ_{5d} and IZ_{14d}, IZ_{2m}. **C:** effects of flecainide 30 μ M on I_{to} . Responses of I_{to} at +40 mV to flecainide in NZ ($n = 18$, $N = 11$), IZ_{14d} ($n = 10$, $N = 8$), and IZ_{2m} ($n = 10$, $N = 9$). Values are means $\pm$ SE. * $P < 0.05$ vs. NZ.

Table 1

Cell capacitance

Groups	<i>n</i>	<i>N</i>	Capacitance, pF
NZ	22	12	140±5.0
IZ _{14d}	30	17	208±8.0 ^{*†}
Rem _{14d}	11	11	170±8.9 [*]
Sham _{14d}	9	4	162±16
IZ _{2m}	26	15	188±9.8 ^{*†}
Rem _{2m}	9	7	176±13 [*]
Sham _{2m}	8	3	140±12

Values are means ± SE; *n*, no. of cells; *N*, no. of animals. IZ_{14d} and IZ_{2m}, cells from epicardial border zone (EBZ) of *day 14* and 2-mo infarcted hearts; Rem_{14d} and Rem_{2m}, cells from remote area of 14-day and 2-mo infarcted hearts; Sham_{14d} and sham_{2m}, cells from EBZ of *day 14* and 2-mo sham-operated hearts.

^{*}*P* < 0.05 vs. NZ;

[†]*P* < 0.05 vs. sham.

Table 2Peak $I_{Ca,L}$

Groups	<i>n</i>	τ_{fast} , ms	τ_{slow} , ms	A_{fast}/A_{total}
Decay				
NZ	22	16.2±0.3	92.9±7.4	0.90±0.01
IZ _{14d}	22	13.0±0.6 [*]	63.6±6.8 [*]	0.84±0.02 [*]
Rem _{14d}	13	17.4±0.7	151.9±22.2	0.87±0.009
Sham _{14d}	9	16.9±0.7	96.7±12.4	0.91±0.01
IZ _{2m}	18	14.9±0.7 [†]	72.4±7.4	0.84±0.02 [*]
Rem _{2m}	9	16.7±0.7	68.6±8.0	0.88±0.03
Sham _{2m}	9	16.0±0.6	105.1±23.0	0.86±0.02
Recovery from inactivation				
NZ	21	44±3.6	186±23	0.61±0.04
IZ _{14d}	12	62±5.3 [*]	459±122 [*]	0.71±0.05
Rem _{14d}	10	70±10 [*]	357±96 [*]	0.75±0.05 [*]
Sham _{14d}	8	49±4.3	228±35	0.68±0.03
IZ _{2m}	8	68±10 [*]	294±51 [*]	0.66±0.02
Rem _{2m}	6	59±5.1	366±82 [*]	0.83±0.02 [*]
Sham _{2m}	2	51±12	207±71	0.72±0.12

Values are means ± SE; *n*, no. of cells. $I_{Ca,L}$, L-type Ca^{2+} current; A_{fast}/A_{total} , fast amplitude/total amplitude; τ_{fast} , fast time decay constant; τ_{slow} , slow time decay constant.

^{*} $P < 0.05$ vs. NZ;

[†] $P < 0.05$ vs. IZ_{14d}.

Table 3 $I_{Ca,L}$ activation

Groups	<i>n</i>	$V_{0.5}$, mV	<i>k</i>	E_{rev} , mV
NZ	22	11±0.6	6.8±0.1	59.5±0.7
IZ _{14d}	21	7.3±1.0*	6.9±0.2	60.8±0.6
Rem _{14d}	11	12±1.3	6.7±0.1	61.2±1.2
Sham _{14d}	7	13±0.7	6.7±0.1	61.8±1.5
IZ _{2m}	18	4.1±1.3*	6.4±0.1	57.6±0.9
Rem _{2m}	9	11±1.6	6.6±0.2	62.4±1.3
Sham _{2m}	6	9.0±0.7	6.2±0.1	58.2±0.7

Values are means ± SE; *n*, no. of cells. $V_{0.5}$, half-maximal voltage; *k*, slope; E_{rev} , reversal potential.

* $P < 0.05$ vs. NZ.

Table 4**I_{Ca,L}** inactivation

Groups	<i>n</i>	<i>V</i> _{0.5} , mV	<i>k</i>	Maximum <i>I</i> _{Ca,L} (<i>V</i> _c = -70 mV, pA/pF)
NZ	21	-11.0±0.8	5.0±0.4	-7.1±0.4
IZ _{14d}	21	-17.4±1.3 *	5.4±0.1	-5.1±0.3 *
Rem _{14d}	12	-9.9±1.6	4.8±0.1	-6.9±1.2
Sham _{14d}	10	-10.3±0.9	4.9±0.1	-5.9±0.5
IZ _{2m}	20	-16.8±1.3 *	5.0±0.1	-4.8±0.3 *
Rem _{2m}	7	-10.0±1.5	4.7±0.1	-7.1±0.9
Sham _{2m}	8	-12.2±1.4	5.1±0.1	-5.7±0.5

Values are means ± SE; *n*, no. of cells. *V*_c, conditioning prepulse potential.

* *P* < 0.05 vs. NZ.

Table 5Effect of Iso on $I_{Ca,L}$ peak density

Group	<i>n</i>	Control Peak Density, pA/pF	+ Iso (1 μ M)
IZ _{14d}	9	-4.7 ± 0.3	$-6.7 \pm 0.5^*$
Rem _{14d}	10	-6.5 ± 1.3	$-16.9 \pm 3.2^*$
IZ _{2m}	6	-5.8 ± 0.9	$-12.1 \pm 2.3^{*\dagger}$
Rem _{2m}	7	-7.5 ± 0.9	$-15.7 \pm 2.7^*$

Values are means $\pm$ SE; *n*, no. of cells. Iso, isoproterenol.

* $P < 0.05$ vs. predrug.

$^\dagger P < 0.05$ vs. IZ_{14d} in the presence of Iso.

Table 6Effect of Iso on steady-state inactivation $V_{0.5}$

	<i>n</i>	Control	Iso (1 μ M)
IZ _{14d}	8	-14.6 $\pm$ 1.7	-17.1 $\pm$ 1.2*
Rem _{14d}	9	-9.4 $\pm$ 2.2	-11.1 $\pm$ 2.1*
IZ _{2m}	6	-15.0 $\pm$ 2.0	-18.7 $\pm$ 1.7*
Rem _{2m}	6	-11.1 $\pm$ 2.2	-12.5 $\pm$ 1.5*

Values are means $\pm$ SE (in mV); *n*, no. of cells.* $P < 0.05$ vs. predrug.

Table 7Steady-state inactivation characteristics of I_{to}

Group	<i>n</i>	$V_{0.5}$, mV	<i>k</i> , mV	$I_{to,max}$ at -55 mV
NZ	54	-35.4±0.6	4.1±0.1	22.4±1.3
IZ _{14d}	30	-30.5±1.3*	5.3±0.3*	10.0±0.3*
Rem _{14d}	14	-34.6±1.1	3.9±0.2	21.5±2.0
Sham _{14d}	11	-35.9±1.7	4.1±0.1	17.4±1.9
IZ _{2m}	16	-33.0±1.0	4.0±0.1	15.4±0.1*†
Rem _{2m}	12	-34.6±2.4	3.1±1.1	19.1±2.6
Sham _{2m}	5	-35.0±1.4	4.4±0.4	18.7±2.5

Values are means ± SE; *n*, no. of cells. I_{to} , transient outward current; $I_{to,max}$, maximum I_{to} .

* $P < 0.05$ vs. NZ;

† $P < 0.05$ vs. IZ_{14d}.

Table 8

Characteristics of recovery from inactivation of I_{to}

	<i>n</i>	τ_{fast} , ms	τ_{slow} , ms	A_{fast} , %	$I_{to,max}$, pA/pF
NZ	22	74.9±5.2	393±33	53.6±2.1	17.0±2.3
IZ _{14d}	13	84.0±11.8	628±115*	60.2±4.8	8.7±1.3*
Rem _{14d}	10	60.0±6.5	585±128	56.0±3.1	17.8±2.6
Sham _{14d}	8	63.5±8.0	355±53	53.0±4.0	13.2±1.4
IZ _{2m}	13	99.2±19.6	630±147	54.2±3.1	19.2±1.8 [†]
Rem _{2m}	7	84.0±16.1	541±130	47.5±4.5	19.7±2.8
Sham _{2m}	4	81.2±29.4	519±241	59.5±9.3	22.4±0.7

Values are mean ± SE; *n*, no. of cells.

* $P < 0.05$ vs. NZ;

[†] $P < 0.05$ vs. IZ_{14d}.