Review

Electrical remodeling in ischemia and infarction

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Abstract

This is a review of the electrophysiologic changes occurring at different times following myocardial infarction, both in the infarcted region (substrate) and in areas remote from the infarct. Regulators of channel function which might contribute to re-modeling, including autocrine/paracrine factors involved in ion channel gene regulation, are discussed. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

This review concentrates on the function, molecular determinants and pharmacology of ion channels that reside in the sarcolemma and that contribute to the distinct phases of the altered transmembrane action potentials of myocytes surviving in the infarcted heart. The discussion will be divided into two general categories. First, substrate findings will be discussed and linked to the time course of arrhythmias as they are known to occur in experimental myocardial infarction models. This section will be followed by a discussion of the ion channel changes in myocytes remote from the infarct area, that is, in areas of regional hypertrophy. Finally, what is known about the molecular determinants of ion channels of myocytes from these diseased hearts is presented.

2. Myocardial infarction – arrhythmia substrate findings

Various arrhythmic phases occur after the onset of experimentally produced myocardial ischemia and infarction in animal hearts. Current hypotheses maintain that the mechanisms of some of these cardiac arrhythmias can be understood in terms of the alterations in cellular electrical activity in specific regions of the heart post myocardial infarction (MI) (see [1]). For example, in hearts of large animals, acute coronary artery occlusion results in rapid ventricular arrhythmias (ventricular tachycardias and fibrillation) (acute phase). It is unlikely that chronic or persistent changes in ion channel function underlie these acute arrhythmias. Over the following 24–48 h (subacute phase of infarction) post occlusion, delayed spontaneous arrhythmias of ventricular origin (subendocardial Purkinje) occur in experimental models and may have counterparts in humans[2–4]. During the healing (days, weeks) or healed (months) infarct phase, sustained ventricular tachycardias are inducible in both animal and human hearts suggesting that the reentrant substrate is present. The site of origin of the ventricular arrhythmias in these hearts depends on the location of the surviving cells overlying the infarcted region. In one canine model, these reentrant arrhythmias have been mapped to an area described as the epicardial border zone [1].

Numerous studies have described the specific changes in action potential (AP) configuration that occur in the canine subendocardial Purkinje fiber and the subepicardial ventricular fiber postcoronary artery occlusion. Generally, by 24–48 h after total coronary artery occlusion the APs of the subendocardial Purkinje fibers show reduced resting potentials and maximal action potential upstroke velocity ($V_{\text{max}}$) as well as an increase in total time of repolarization. On the other hand, the cells of the epicardial border zone of the canine infarction model show a reduction in

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V_{\text{max}}$, and a shortening and triangularization of the action potential by 5 days after total artery occlusion. By 14 days post occlusion further shortening of the AP occurs. Then by the time of the healed infarct (2 months), AP voltage profiles have returned to nearly normal [5] suggesting the presence of a process that might be termed ‘reverse remodeling’ (Fig. 1). In addition, changes in conduction of the impulse at various times after coronary artery occlusion as well as the altered refractoriness of the tissue in the infarcted myocardium have been documented. Electrical changes at the level of the myocyte and the role they play in providing the substrate for inducible reentrant arrhythmias have been reviewed [1].

2.1. Resting potential

2.1.1. 24–48 h post occlusion; subendocardial Purkinje myocytes

The origin of the delayed phase of spontaneous arrhythmias secondary to coronary artery occlusion in canine and porcine hearts is most likely in the depolarized and abnormally automatic subendocardial Purkinje fibers that survive. The loss of resting potential is significant and dramatic in the multicellular preparations of these fibers.

Concomitant with this dramatic loss is a reduction in intracellular K$^+$ ion concentration ($a_{K}^i$). However, a decrease in K$^+$ equilibrium potential ($E_K$) (average change 16 mV) cannot fully account for the loss in resting potential (average change 35 mV) [6].

Abnormalities in the resting potentials of subendocardial Purkinje fibers surviving in the infarcted heart persist even after they are enzymatically disaggregated and studied as single myocytes [7]. In the myocyte, a reduction in $a_{K}^i$ could not provide the basis for the reduced resting potential. Rather, Purkinje myocytes isolated from the infarcted myocardium show an increase in the ratio of the membrane permeability of Na$^+$ to K$^+$ ions ($P_{Na}/P_K$) as compared to control. The larger value of $P_{Na}/P_K$ in these cells could be due to an increase in $P_{Na}$ or a decrease in $P_K$ or both. Input resistance measurements suggest that the subendocardial Purkinje myocytes from the infarcted myocardium have higher input resistances than control cells. This is in agreement with a multicellular study on these fiber bundles [8]. Combined with the $P_{Na}/P_K$ measurements in the myocytes, it is likely that there is a net decrease in $P_K$ in the cells with reduced resting potentials. Finally, this is consistent with a decrease in the density of both the outward K$^+$ current and inward rectifying K$^+$ current, $I_{K1}$, recently described for the Purkinje myocytes surviving in the 48-h infarcted heart [9].

2.1.2. Five days post occlusion; epicardial border zone myocytes

In the multicellular preparations of the myocardium isolated from the epicardial border zone of the 5-day infarcted heart, the following abnormalities have been described: a decrease in resting potential, total AP amplitude and $V_{\text{max}}$, a reduction in AP duration at both 50 and 90% repolarization and a loss in the plateau potentials (Fig. 1) [5]. However, when the cells of the epicardial border zone are dispersed and studied as isolated myocytes in vitro [10], the resting potential is no different than control suggesting that other factors control resting membrane potential in the multicellular preparation. One likely factor may be extracellular ion accumulation, since in single cells electrical activity is studied after it is removed from the syncytium and superfused in an environment where immediate extracellular ion accumulation and depletion are not significant.

While resting potentials of the 5-day myocytes are similar to control values, APs of these myocytes show changes in the repolarization during the terminal portion of phase 3. Net membrane currents are significantly different between cell groups but $I_{K1}$ appears to differ only at hyperpolarized potentials (Fig. 2).

2.1.3. Healed myocardial infarction: ventricle

Investigations of changes in the electrical function in the healing or healed infarct (weeks to months) include studies of cells overlying the infarct scar, known as the central...
There are no studies secondary to a decrease in I
V infarcted heart [15] or in epicardial border zone ®bers of the reduced negative versus control [14]. No change in resting potential remains voltage dependent [10,18].

occlusion rabbit L V were slightly but signi®cantly more course of recovery of V potentials of border zone myocytes from 8-week post- dial border zone of the 5-day infarcted heart, the time border but not infarct zone tissue [13]. In contrast, resting and voltage dependent while in myocytes from the epicar-

V border zone cells to
signi®cant reduction in resting potential (from 8-week post- dial border zone of the feline healed infarct model showed cted heart, cellular inexcitability can outlast the repolariza-

electrical of endocardial Purkinje myocytes that survive in the 24 h infarcted heart, and further reduction occurs by 48 h [16]. The loss in Ca2+ channel function could contribute to the depressed and triangular plateau phase of the APs of these arrhythmogenic Purkinje myocytes.

2.2. Upstroke velocity of phase 0 of the action potential

2.2.1. 24–48 h post coronary artery occlusion; subendocardial Purkinje myocytes

Vmax and Na+ current: By virtue of the loss in resting potential there would be a predictable change in Vmax of the subendocardial Purkinje myocytes that survive in the infarcted myocardium. As yet, there have been no voltage clamp studies that have identi®ed whether the fast Na+ current density is or is not altered in myocytes dispersed at this time period after coronary artery occlusion.

Ca2+ currents: Peak L type Ca2+ current (Ical) density is signi®cantly reduced in subendocardial Purkinje myocytes dispersed from 48 h infarcted heart as compared to control and to those from the 24 h infarcted heart [16]. Current density reduction is not accompanied by a shift in the current–voltage relationship, a change in the time course of Ical decay but by a slight shift in the inactivation curve and thus the steady-state availability of the channel. Peak T type Ca2+ current density is also decreased in subendocardial Purkinje myocytes that survive in the 24 h infarcted heart, and further reduction occurs by 48 h [16].

2.2.2. Five days post coronary artery occlusion; epicardial border zone myocytes

Vmax and Na+ current: Arrhythmias arise in the epicardial border zone region of the 5-day infarcted heart most probably because of the abnormalities in impulse conduction that occur [1]. These abnormalities may result from alterations in the fast Na+ current that is responsible for Vmax of these ®bers. While microelectrode recordings of the myocytes from the infarcted heart have shown resting potentials similar to those of control epicardial cells, the mean Vmax of these cells remains signi®cantly reduced when compared to control [5,10,17,18]. Steady-state availability relationships of Vmax in cells from the infarcted heart are shifted along the voltage axis in the hyperpolarizing direction by 10 mV [10,18]. Lazzara and Scherlag [19] have suggested that in surviving cells in the 5-day infarcted heart, cellular inexcitability can outlast the repolarization phase of the AP. The mechanism of this post repolarization refractoriness is unknown. Clearly in control noninfarcted cells, the time constant of recovery of Vmax is rapid and voltage dependent while in myocytes from the epicardial border zone of the 5-day infarcted heart, the time course of recovery of Vmax is signi®cantly prolonged but remains voltage dependent [10,18]. Whole cell voltage clamp data have now con®rmed that the reduced Vmax of cells of epicardial border zone is secondary to a decrease in INa density and altered Na+ available on function of IK1 in myocytes from the healed infarcted heart.

‘infarct zone’ cells, and those of the surrounding thin rim of cells called the lateral adjacent ‘border zone’ cells. In the feline healed infarct model, 50% of the hearts have ectopic activity [11,12]. The site of origin of these arrhythmias is not known but may be from cells within the healed border zone.

Cells overlying the healed infarct area do not show ability relationships of these cells remains signi®cantly reducedmax the current±voltage relationship, a change in the timeFig. 2. Average ‘steady-state’ net membrane current density voltage-
relations of normal (NZVM, n=28, ®lled circles) and 5-day infarct (IZVM, n=44, unfilled circles) ventricular myocytes in nisoldipine-containing Tyrode’s solution. Currents were measured at the end of a 440-ms pulse from a holding potential of ~40 mV to various test potentials (Vt). Average capacitance of cells was 123±6 pF (NZVM) and 179±7 pF (IZVM) (P<0.05). Note that the isochronal I–V curve of NZVM had a pronounced n-shape, compared to that of IZVM. The average resting potential ~87±3 mV and ~83±3 mV for NZVM and IZVM, respectively. For all IZVM studied, the average I–V differed from NZVM at test voltages ~110 to ~95 mV and at ~80 to ~30 mV. Inset shows cesum (Cs+ ~20 mM)-sensitive currents in a subset of cells (NZVM, n=12; IZVM, n=24). Cs+–sensitive currents were n-shaped in both groups, depicting the I–V of the underlying Ica (zero intercepts, NZVM= ~90±2 mV; IZVM= ~93±1 mV, P>0.05). There was a significant reduction in IZVM Ica density in the ~110 to ~90 mV range.

* indicates significance at P<0.05.
current kinetics [20]. In particular, a marked lag in recovery of \( I_{Na} \) appears to account in part for the cellular phenomenon of post repolarization refractoriness in these myocytes [20]. More recent studies have suggested that the altered inactivation gating kinetics of \( I_{Na} \) in these cells affect the cellular action of the local anesthetic lidocaine [21]. In particular, the degree of tonic block of \( I_{Na} \) is significantly increased in these border zone cells. Interestingly, while in drug free conditions there is a significant enhancement of use dependent reduction of \( I_{Na} \) in the border zone myocytes [21], differences in rates of loss and recovery of availability of \( I_{Na} \) between normal and border zone myocytes are minimized with lidocaine [21] (Fig. 3).

\( \text{Ca}^{2+} \) currents: The peak \( I_{CaL} \) density of epicardial border zone cells from the 5-day infarcted heart is significantly reduced by 36% compared to control [22]. Furthermore, this reduction is not due to a decrease in steady-state availability or a prolonged time course of recovery of \( I_{CaL} \). Further, the time course of decay of these currents is significantly faster than control. These findings may be related to a decrease in the number of functioning channels as well as an acceleration of inactivation of the remaining channels. Unlike findings in the subendocardial Purkinje myocytes studies (see above), no significant differences were found between peak density and frequency of \( T \) type \( \text{Ca}^{2+} \) currents in epicardial border zone myocytes surviving in the 5-day infarcted heart versus control myocytes [22].

Our knowledge of altered sensitivity to transmitters of the autonomic system in diseased myocytes is derived from comparisons of the effects of adrenergic agonists on specific ionic currents. Commonly, adrenergic sensitivity is assessed by the effects of the \( \beta \)-adrenergic agonist, isoproterenol, on \( I_{CaL} \). In normal ventricular myocytes, isoproterenol at low doses (\( 10^{-9} \) to \( 10^{-7} \) M) prolongs the AP; at higher concentrations (\( 10^{-6} \) M or greater), AP duration is shortened. Isoproterenol acts through depolarization of the resting membrane potential by inhibiting \( I_K \) and \( I_{Na} \) [23] and increasing \( I_{CaL} \) [24,25]. These responses to isoproterenol are blunted in epicardial border zone myocytes from the

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**Fig. 3.** Effects of lidocaine on the time course of recovery of availability of \( I_{Na} \) in normal cells (NZs) and in epicardial border zone cells of the 5-day infarct (IZs). (A) Average time course of recovery of \( I_{Na} \) availability at holding voltage \(-100\) mV in control drug-free conditions (solid symbols) and after 60 \( \mu \)M lidocaine superfusion (10 min) for both NZs (circles) and IZs (squares) for all inter-pulse intervals (Ipls) tested. (B) Data from Ipls of 2–100 ms for each group. Dotted line indicates the Ipl interval (100 ms) that was used for comparison of rate recovery of availability in the absence and presence of drug. (C and D) Actual current tracings from NZs (C) and IZs (D) during this protocol in the presence of 120 \( \mu \)M lidocaine. Note in the drug free state, \( I_{Na} \) of the epicardial border zone cells (IZs) shows a delay in the recovery phase. However, consistent with use-dependent results, lidocaine minimized the differences in the rate of recovery of availability for the two cell types. Reproduced from [21].
5-day infarcted heart and can be mimicked by forskolin and intracellular cAMP, suggesting additional defects in the β-adrenergic signaling pathway [26].

Sympathetic stimulation produces minimal AP shortening in areas overlying the infarct and the border zone, whereas in areas remote from the arrhythmia substrate pronounces AP shortening occurs [27]. Furthermore, catecholamine-induced increases of the plateau phase of APs is absent in the fibers of the epicardial border zone of the 5-day and 14-day infarct heart [17]. Similar abbreviated responses to catecholamines have been documented in the ischemic human ventricle [28] (Fig. 4). From voltage clamp data and when compared to normal cells, isoproterenol produces a smaller increase in $I_{C_{AT}}$ in cells from the 5-day- and 2-month-old infarct, independent of calcium-dependent inactivation [26,29]. This is consistent with multiple defects in components of the β-adrenergic receptor complex in epicardial border zone cells of the 5-day-old infarct, including decreases in β-adrenergic receptor density; diminished basal, guanine nucleotide, isoproterenol, forskolin and manganese-dependent adenyl cyclase activities, increases in the $EC_{50}$ for isoproterenol-dependent activation of adenylate cyclase, diminished levels of the $\alpha$-subunit of the $G_{i}$ protein and elevated levels of the $\alpha$-subunit of the $G_{i}$ protein [30].

2.2.3. Healed myocardial infarction: ventricle

In the feline healed infarct model, the surviving endocardial cells surrounding the 2-month-old infarct have characteristics different from those during acute ischemia and early infarction [12] in that cells have prolonged APs with normal resting membrane potentials and upstroke velocities. In contrast, border zone cells surviving between the scar and normal tissue have short APDs with reduced upstroke amplitudes and velocities, similar to those observed in the acute phase post MI. The duration but not the peak of the AP shortens to a greater extent in healed infarct myocytes as stimulation frequency increases [12,14]. Thus, the initial ischemic insult together with subsequent chronic healing can differentially affect select membrane functions and/or cell-to-cell coupling of border and central infarct zone cells [12].

$Na^{+}$ currents: Whole cell $Na^{+}$ currents have not been measured in cells surviving in the healed infarcted heart. Intracellular sodium activity in the infarct zone does not differ from the normal zone tissues [13], yet the increase in $Na^{+}$ activity in border zone tissue suggests a depressed $Na^{+}$-$K^{+}$ pump activity and/or increased $Na^{+}$ leak in myocytes from the feline healed infarct model. These latter changes could indirectly affect $V_{max}$ of APs of these myocytes [12].

$Ca^{2+}$ currents: Myocytes adjacent to the 8-week infarct in the rabbit heart show a significant decrease in peak $I_{C_{AT}}$ density without a change in current-voltage relations, voltage-dependence or steady-state inactivation kinetics [14]. In the 2-month feline infarct model, myocytes from the area underlying or immediately adjacent to the infarct scar also have reduced $I_{C_{AT}}$ amplitudes at most test voltages [29]. These values differ from those of controls and from those cells dispersed from the remote regions of the same infarcted heart. Reduced current density and accelerated current decay persist even when barium is used as the charge carrier, suggesting a decrease in the number of functional $Ca^{2+}$ channels at this time. Steady-state activation and recovery from inactivation remain unchanged [29]. The voltage at which $Ca^{2+}$ channels are half-maximally available (inactivation curve) is shifted by 10 mV in the hyperpolarizing direction in MI myocytes. Consequently, the availability of the $Ca^{2+}$ channels in the voltage range $-40$ to $+20$ mV is changed, resulting in smaller L type $Ca^{2+}$ inward window currents than controls. The altered $I_{C_{AT}}$ kinetic changes may partially account for decrements in the infarct zone total $I_{C_{AT}}$ density and could contribute to a reduction in voltage and duration of phase 2 of the APs of cells of the healed infarct.

2.3. Repolarization and refractoriness

2.3.1. 24–48 h post occlusion; subendocardial Purkinje myocytes

$I_{C_{AT}}$: Action potential recordings of the subendocardial Purkinje fibers that survive 24–48 h after occlusion have a small degree of rapid phase 1 of repolarization when fibers are driven at slow rates. Pacing at fast rates causes little or no change in phase 1 of repolarization in normal Purkinje
fibers, yet it has a dramatic effect on the time course of repolarization of subendocardial Purkinje myocytes surviving in the infarcted heart. In some cases, with an increase in drive rate, the rapid phase 1 of repolarization of APs in these fibers completely disappears (see Fig. 3,30 of [1]). Whole cell voltage clamp experiments have confirmed that the density and kinetics of $I_{Na}$ in these cells is reduced by 51% and that these changes are not due to alterations in steady-state availability of the channel. $I_{Na}$ currents in Purkinje myocytes from the 48-h infarcted heart also show specific kinetic changes. Notably, the time course of current decay is accelerated while the time course of reactivation of $I_{Na}$ is significantly delayed. This slowing of $I_{Na}$ recovery implies that less outward repolarizing current is available for APs occurring at high pacing rates or during closely spaced voltage clamp steps [31].

$I_K$: Subendocardial Purkinje myocytes from the 48 h infarcted heart have a significantly increased density of E4031 sensitive currents (a gain in function) compared to those of normal Purkinje myocytes [9] (Fig. 5). E4031 sensitive Purkinje myocyte currents differ from those of the normal or infarcted ventricular myocyte ($I_{Kr}$) and their molecular identity is unknown at this time. However, these data do suggest that Purkinje myocytes surviving in the infarcted heart would show an increased responsiveness to this class of antiarrhythmic drugs (methanesulfonanilide).

2.3.2. Five days post occlusion: epicardial border zone myocytes

$I_{Na}$: Action potentials recorded from the epicardial border zone cells usually show no phase 1 or reduced phase 1 of repolarization suggesting a loss in the voltage dependent transient outward current $I_{Na}$ [10]. In contrast and as predicted, APs recorded from all cells dispersed from the normal noninfarcted epicardium show a large and prominent spike and dome morphology. Voltage clamp studies confirm that the density and kinetics of the voltage dependent, non-Ca-dependent, transient outward current ($I_{Na}$) in the cells demonstrating the loss in the notch are reduced [10].

$I_K$: Similarly, densities of $I_{Kr}$ and $I_{Ks}$ (the two components of $I_K$) are reduced significantly in cells dispersed from the epicardial border zone of the 5-day canine infarcted heart [32]. These changes if occurring alone would retard AP repolarization. No kinetic changes have been found.

Fig. 5. Typical recordings of currents elicited using protocol shown in Purkinje myocytes from (A) a normal heart (NZPC) and (B) from a 48-h infarcted heart (IZPC) before and after superfusion with E-4031 (5 μM). Typical E-4031-sensitive currents are shown on the right sides of (A) and (B) (35°C, 4 mM K$_{e}$ and nisoldipine Tyrode’s). Note that for small steps, E-4031-sensitive currents are instantaneous with time dependence at larger step depolarizations. (C) Average E-4031-sensitive currents for NZPCs ($n=13$) and IZPCs ($n=10$). E-4031-sensitive currents ($I_{sens}$) were larger at all steps in IZPCs. Note the lack of strong rectification of E-4031-sensitive currents in both cell types; reproduced from [9].
Ca\textsubscript{2+} dependent outward currents: Studies using normal myocytes of some species have shown the presence of two types of transient outward currents. One is transient, voltage dependent and 4-AP sensitive, generally referred to as \( I_{\text{to}} \) (see above). The other is Ca\textsuperscript{2+} dependent \( I_{\text{to2}} \) [33–36]. It is thought that for normal myocytes that the source of intracellular Ca\textsuperscript{2+} activating \( I_{\text{to2}} \) is that which results from Ca\textsuperscript{2+} released from the sarcoplasmic reticulum. In cells from the 5-day epicardial border zone, pronounced changes in Ca\textsubscript{2+} cycling persist [37]. Furthermore, these Ca\textsubscript{2+} cycling changes are reflected in the characteristics of the Ca\textsubscript{2+} dependent outward currents \( I_{\text{to2}} \) of these cells [38]. Namely, the difference between the magnitude of \( I_{\text{to2}} \) in border zone versus normal cells is exaggerated with constant pacing. Generally, for normal cells, beat-to-beat change in \( I_{\text{to2}} \) tracked frequency dependent changes in \( I_{\text{ca}} \) and the globally assessed Ca\textsubscript{2+} transient amplitude [37]. For border zone cells, Ca\textsubscript{2+} transients varied in amplitude, as \( I_{\text{to2}} \) amplitude and \( I_{\text{ca}} \) decreased with the fast pacing rate. Thus, differences in \( I_{\text{to2}} \) observed in response to pacing in the two cell types led to heterogeneity of AP repolarization within the infarcted heart.

NaCa exchanger currents: In normal myocytes with normal Ca\textsubscript{2+} cycling, it is well established that currents generated by the NaCa exchanger can play an important role in the electrical activity of a myocyte [39]. The NaCa current is either inward (normal mode) as the transporter exchanges Ca\textsubscript{2+} for external Na\textsuperscript{+} ions, or outward (reverse mode) as the transporter causes Ca\textsuperscript{2+} influx by exchanging external Ca\textsuperscript{2+} ions for Na\textsuperscript{+}. Therefore, the time course of the exchanger current is related to the time course of Ca\textsubscript{2+} cycling.

There have been several reports of abnormal Ca\textsubscript{2+} cycling in cells that have survived in the infarcted heart [14,40–43]. In myocytes dispersed from the 5-day epicardial border zone of the infarcted heart, field stimulation [37] and voltage clamp studies [44] show that the diminished globally assessed Ca\textsubscript{2+} transient of the epicardial border cell has a slow relaxation (decay) phase. Furthermore, these cells have an altered phase 3 of their APs. These changes are consistent with changes in the NaCa exchanger current. However, under strict conditions that isolate only Ca\textsubscript{2+} and ionic current changes secondary to the NaCa exchanger, both Ca\textsuperscript{2+} entry (via the reverse exchanger) and Ni\textsuperscript{2+} sensitive currents as well as Ca\textsuperscript{2+} extrusion (via normal mode exchanger) in border zone myocytes are similar to those of normal myocytes [43]. This occurs even under conditions of different Na\textsubscript{+} loads [43]. Thus, in these cells where L type Ca\textsuperscript{2+} channels are down regulated, the NaCa exchanger has a reserve efficiency and continues to contribute current to the transmembrane AP.

Gap junction conductances: In addition to the numerous ion channels that contribute to transmembrane voltage profile, channels that form at gap junctions between two cells play a role in impulse propagation and perhaps repolarization. In the epicardial border zone of the 5-day infarcted heart, reentrant arrhythmias occur in fibers where impulse propagation shows accentuated anisotropic properties [45]. Subcellular redistribution of the gap junction protein connexin 43 (Cx43) has been observed and correlated with the likelihood of sustained ventricular tachycardias in both the healing and chronically infected myocardium [46,47]. Recently the functional consequences of changes in Cx43 proteins have been determined using cell pairs dispersed from the border zone and compared to control noninfarcted cell pairs [48]. Gap junctional (Gj) conductance between EBZ cell pairs connected side to side was reduced to 10% of its control value while Gj conductance between cell pairs connected end to end was reduced to 30% of its control value [48]. These electrical changes may be due to the cell surface redistribution of the Cx43 proteins (Fig. 6).

The Kv1.5 K\textsuperscript{+} channel has been cloned from mouse, rat, and human heart and is localized to the intercalated disc region of the normal heart [49]. In the epicardial border zone of the 5-day infarcted heart, the pattern of Kv1.5 protein cell localization begins to change. By 14 days post occlusion, the protein localization is dramatically altered from the distinct intercalated disc pattern of the noninfarcted heart to a more generalized cell surface expression [50] (Fig. 7). The functional significance of these changes remains unknown.

2.3.3. Healed myocardial infarction: ventricle

Repolarization of normal, lateral border and infarct zone cells in the heart with healed infarct is nonuniform. In some models, APs of different morphologies and varying degrees of prolongation (but never shortening) are found in almost all regions of the LV, especially adjacent to the infarct scar [11,12,15].

The duration of refractoriness in infarct zone cells is consistent with the variability of AP durations. Infarct zone and lateral border cells have functional refractory periods, which often outlast the total time course of repolarization in the healed infarcted hearts [12]. Although the initial damage to lateral border zone cells may be less than that of central ischemic area cells, electrophysiological abnormalities caused by ischemia persist in the lateral border zone cells well beyond the early phase post myocardial infarction, and appear to be more severe as assessed by 50% failure to respond to a premature stimulus (reduced response) during the late phase post infarct [12]. This may imply that post-repolarization refractoriness and impaired conduction persist in the border zone fibers of healed infarcts.

Both epicardial and endocardial tissues in hearts with healed infarcts demonstrate marked disparity in refractoriness in the border and infarct areas, especially during sympathetic stimulation [27]. The prolongation of refractoriness could be related to a slowed recovery of \( I_{\text{to2}} \) or
delay in deactivation of outward K$^+$ currents in the infarct substrate. To date however, there have been no direct measurements of these latter currents in cells overlying the healed infarct.

In rabbit 8 weeks post MI, it appears that the abnormally prolonged APs are accompanied by not only a reduction in L type Ca$^{2+}$ current activity (see above) but also an increase in NaCa exchanger current density (normal mode) [14]. Again it is not known whether NaCa exchanger kinetics are altered in these myocytes from the healed infarct hearts.

3. Regions remote from the infarct – regional hypertrophy changes

Structural remodeling of the left ventricle after myocardial infarction involves the regions of necrosis and infarct scar, the central and border zone areas as described above (substrate), as well as the noninfarcted myocardium remote from the arrhythmic infarct substrate. In many models, the noninfarcted myocardium shows gradual morphological changes indicative of hypertrophy as it adapts to the increased workload of the compromised heart both during the subacute or healing (3 days, weeks) or chronic, healed (2-month) stages.

Regional hypertrophy that accompanies ventricular remodeling is of interest since left ventricular hypertrophy is
a strong risk factor for ventricular arrhythmias [51]. Remote areas include noninfarcted LV or RV tissue. Large
(40% of LV) infarcts tend to show greater increases in cell size in the non-infarcted remote areas, compared to smaller
infarcts (<15%) [52]. Non-transmural infarcts show increases in cell diameter adjacent to the infarct, but not in
the remote areas; conversely, transmural infarcts, all cell diameters increase [52]. In some experimental models
increases in myocyte length but not width have been described [15].

3.1. Subacute and healing (3-day, 3–4 weeks) myocardial infarction – remote

3.1.1. Resting potential

$\dot{\mathcal{I}}_{K_1}$ is reduced in epicardial but not endocardial LV myocytes of 3-day-old rat infarcted heart. Minor changes
in $\dot{\mathcal{I}}_{K_1}$ density occur in RV myocytes remote from the infarcted region of the 3 day rat heart [53]. It is unlikely
that these changes would be accompanied by a change in resting potential of the myocytes. No change in resting
potential is seen in myocytes dispersed from the 3-week infarcted rat heart [54].

3.1.2. Upstroke velocity of phase 0 of the action potential

Differences in AP amplitude, and $V_{\text{max}}$ between post-MI and sham cells, both those from the endocardial and
epicardial regions are related to the type of MI model studied. $V_{\text{max}}$ of epicardial cells is lower than that of
diastolic cells in the post-MI remodeled LV [54].

Hypertrophied LV myocytes remote from a 3 week MI scar show a significant increase in $\mathcal{I}_{\text{Cat}}$ amplitude but when normalized to cell capacitance changes, no significant change in $\dot{\mathcal{I}}_{\text{Cat}}$ density is observed [54]. A reemergence of the T type Ca$^{2+}$ current has been reported to occur in myocytes of the hypertrophied rat ventricles [55].

3.1.3. Repolarization and refactoriness

Marked variations in APD configuration can also be seen in single cells obtained from the remodeled LV wall,
remote from the infarct [54]. Outward K$^+$ currents in rat remodeled LV decay with two phases. The characteristics of the fast component are similar to $\mathcal{I}_{\text{no}}$, while the slow component is termed $\mathcal{I}_K$. A difference in the ratio of the relative densities of $\mathcal{I}_{\text{no}}$ to $\mathcal{I}_K$ can explain the difference between APs of epicardial and endocardial myocytes. Noninfarcted RV myocytes from the 3-day-old rat infarct show no reduction in $\mathcal{I}_{\text{no}}$ or $\mathcal{I}_K$ density compared to myocytes from sham-operated hearts, however, both K$^+$ channel densities are increased relative to control RV myocytes, possibly as a result of surgical trauma [53].

For the rat LV myocytes not overlying the 3-day infarct but adjacent to it, $\mathcal{I}_{\text{no}}$ densities are reduced with more severe reductions occurring in myocytes from the epicardial versus the endocardial layers. Furthermore, $\mathcal{I}_K$ is reduced in density evenly across the myocardial wall in surviving cells remote from the infarct [53]. By 3 weeks post MI in the rat, the hypertrophied LV myocytes continue to show reduced $\mathcal{I}_{\text{no}}$ and $\mathcal{I}_K$ densities with little change reported for channel kinetics [54]. These latter studies are consistent with the persistent AP prolongation in these myocytes.

In studies of 3-week post MI myocytes isolated from regions remote from the arrhythmogenic substrate, reverse NaCa exchanger current density is reduced [40]. No kinetics of NaCa exchanger function were examined. Reduced NaCa exchanger current density would be consistent with less outward and inward currents during AP repolarization in these myocytes. Whether APs of these post MI myocytes had voltage profiles consistent with reduced NaCa exchanger density is not known.

3.2. Healed (2–6 months) myocardial infarction – remote

3.2.1. Resting potential

In general, there are no differences in resting potentials in myocytes from epicardial and endocardial areas that are remote from the 2–6-month-old infarcts [15,29,56].

3.2.2. Upstroke velocity of phase 0 of the action potential

Myocytes from the LV free wall remote from 2-month-old infarcts in rats have a significantly reduced $V_{\text{max}}$ of phase 0 [15]. No studies to date have identified whether there are persistent changes in the density or function of $\mathcal{I}_{\text{no}}$ in these cells.

Ca$^{2+}$ currents: Typically, areas remote from the infarct scar show a tendency toward an increase in Ca$^{2+}$ current magnitude [15,29], regardless of the holding voltage. Current–voltage curves have the typical bell-shaped configuration, with similar peak potential ranges in both post-infarct and sham or control cells. However, peak $\mathcal{I}_{\text{Cat}}$ density is reduced as a result of an increase in capacitance of the remote area myocyte [15,29]. When barium is used as the charge carrier, inward current in remote cells is enhanced compared to control cells, suggesting that voltage-dependent inactivation is decreased, but more importantly, that calcium-dependent inactivation may be increased. Time course of decay of calcium currents is biexponential, voltage-dependent and unchanged in remote cells [15,29]. Steady-state availability curves may be unchanged [15], or shifted to more negative potentials [29], while restitution of $\mathcal{I}_{\text{Cat}}$ remains normal.

No reemerging T-type Ca$^{2+}$ currents have been recorded in the grossly hypertrophied myocytes of the 2-month feline healed infarct model [Pinto et al., unpublished data]. Thus several characteristics including decreased $\mathcal{I}_{\text{Cat}}$ density and altered Ca$^{2+}$ current inactivation potentially contribute to persistent AP duration prolongation of hy-
pertrofied myocytes of the remote regions of the healed infarct.

3.2.3. Repolarization and refractoriness

AP duration is typically prolonged in remote areas. The extent of AP prolongation appears to depend on the age of the infarct; myocytes from older infarcts (6–11 months) show greater prolongation compared to younger infarcts (1–2 months) [15]. Action potential durations at all phases of repolarization appear to be increased to a greater extent than in the infarct zone [15]; this may vary depending on whether cells are epicardial or endocardial in origin.

There are no data on function or density of $I_{Ks}$ in myocytes from areas remote to the healed infarct.

$I_{Ks}$: In non-infarcted subendocardial ventricular myocytes adjacent to the healed infarct, $I_{Ks}$ density but not amplitude is significantly decreased, similar to findings in global left ventricular hypertrophy [57–59], reflecting cell enlargement under both conditions. Current and current–voltage relationships of $I_{Ks}$ tails are unchanged in remote area myocytes; however, tail current density is reduced at positive potentials and showed strong inward rectification. Voltage dependence of activation of $I_{Ks}$ tail is shifted to more positive potentials in myocytes from the remote area of the infarct (compared to normal myocytes), but there were no differences between the slopes of the activation and deactivation curves [56].

4. Possible molecular mechanisms underlying the change in ion channel function post myocardial infarction

It is obvious, from the examples cited above, that disease states can and do alter ion channel function. Observed changes in macroscopic currents can be the result of an acquired (versus genetic) change in structure and function of normally expressed channels, a change in the number of functional channels, or a combination of both.

A change in the number of functional channels can be determined at both the single channel and molecular levels. From a molecular standpoint, a change in the number of functional channels could be due to changes in the levels of expressed protein or to alterations in channel protein incorporation into the membrane.

A change in expression of functional channel proteins is most likely due to a change in gene transcription, translation, or post translational modification. Transcription, the initial step in protein expression, involves the production of an mRNA copy from a DNA template.

4.1. Changes in mRNAs with disease

Recent studies suggest that transcription of different ion channel proteins is greatly affected by disease state. For instance, experimental myocardial hypertrophy, and treatments to prolong AP duration, result in a substantial increase in the $K^+$ channel Kv1.4 mRNA levels in cultured rat myocytes [60] (for review of nomenclature, see [61]). This gene regulation is reversed by normalization of hypertrophy [62]. There is no report yet, as to whether this enhanced transcription results in an enhancement of functional channel proteins in the hypertrophied cell. Tseng and her colleagues [32] have studied the effects of myocardial infarction (at 2–5 days post occlusion) on $K^+$ channel expression using RNase protection assays. In the course of so doing they found that while the molecular marker, $\beta$-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels are homogeneous in the normal ventricle, they are severely reduced in the infarcted canine ventricle making them unsuitable measures for internal controls. Therefore, using 28S rRNA as internal controls, they have recently reported on both the suppressed transcription and function of the native delayed rectifying $K^+$ currents in myocytes surviving in the infarcted heart. In particular, by 48 h post MI, mRNAs for dKvLQT1, dIsK and dERG are all reduced. By day 5, dKvLQT1 transcripts have recovered, but dIsK and dERG remain reduced [32]. These findings are consistent with loss in function of $I_{Ks}$ and $I_{K1}$ in the 5-day border zone myocytes (see above). In the rat model by 3 days post MI there is a significant reduction in Kv4.2 channel protein levels in noninfarcted but regionally hypertrophied tissues with no changes in Kv2.1 or Kv1.5 levels [53]. By 3–4 weeks post MI in the same model, mRNAs of Kv1.4, Kv2.1 and Kv4.2 all appear to be significantly decreased, with no changes detected in either Kv1.2 or Kv1.5 levels [63]. Quantitative analysis of mRNA levels in normal and failing human ventricles (some post myocardial infarction) show that steady state mRNAs for Kv4.3 and HERG are decreased while no mRNA changes occur for Kv1.2, 1.4, 1.5, 2.1 or $I_{K1}$ proteins [64].

In regional hypertrophied myocardium 3 weeks post MI where L type Ca$^{2+}$ currents are relatively normal, a brief report has suggested that mRNA level of the fetal isoform of the L type Ca$^{2+}$ channel has reemerged suggesting new functioning L type Ca$^{2+}$ channels may be more fetal-like in phenotype [65].

4.2. Cause of altered mRNAs

Changes in intracellular Ca$^{2+}$ and hormonally induced changes in cAMP levels have both been linked to altered transcription of mRNAs encoding different ion channel proteins [66,67]. In some studies, increases in intracellular Ca$^{2+}$ produced by pacing can cause a fall in mRNA as well as the functional density of rat skeletal muscle Na$^+$ channel proteins [66,68] or decreased density of the fast cardiac Na$^+$ current [69]. On the other hand, forskolin induced changes in cAMP stimulate Na$^-$ channel mRNAs. Additionally, cAMP presumably through a protein kinase
system, decreases the rate of transcription of a K⁺ channel gene (Kv1.5) [70]. In contrast, cellular mRNA from primary neonatal atrial cells depolarized with KCl for 30 min demonstrates a dramatic increase in Kv1.5 transcript [70]. In PC12 cells exposed to prolonged hypoxia (18 h), Kv1.2 gene but not Kv1.3, Kv2.1, Kv3.1 or Kv3.2 is upregulated [71]. This is accompanied by an enhanced K⁺ current inhibition produced by hypoxia. Interestingly, expression of two K⁺ channel mRNAs is reciprocally controlled by cell-to-cell contact in adult rat cells. Kv1.5 mRNAs appear to downregulate as Kv4.2 mRNAs upregulate while L type Ca²⁺ channel α subunit or KvLQT1 channel mRNAs do not change [72].

The presence of catecholamines appears to regulate the level of L type Ca²⁺ channel expression in cardiac cells. In particular, alpha adrenergic agonists decrease, while beta agonists increase, L type Ca²⁺ channel mRNA and function [73]. In these latter studies, the change in the level of mRNA is correlated with a change in functional channel density. More direct contact of sympathetic neurons with cardiocytes also increases Ca²⁺ channel expression [74]. Interestingly, nonsarcolemmal ion channels also are regulated by cAMP. When neonatal cardiac cells are incubated with cAMP for 24 h, there is enhanced conduction as well as increased expression of Cx43 and Cx45 gap junction proteins [75].

In the setting of myocardial infarction, several autocrine/paracrine factors are activated and may also become important regulators of ion channel function. For instance, as a consequence of immune-mediated myocardial injury and subsequent leukocyte activation, cytokines and oxygen-derived free radicals are released, complement is activated and there is an increased expression of endothelial adhesion molecules (EAMs) [76].

Elevated concentrations of proinflammatory cytokines, including tumor necrosis factor-α (TNFα) interleukin-1β (ILβ), interleukin-2 (IL2), interferon-γ (IFNγ), macrophage colony stimulating factor and granulocyte colony stimulating factor are associated with ischemic heart disease [77–81]. In a rat model of MI, gene expression of TNFa, IL1β and IL6 in the infarcted region peaks at 1 week and decreases rapidly thereafter [82]. In contrast, by 20 weeks post infarction, cytokine gene expression levels remain significantly higher in the noninfarcted versus infarcted zone and correlate well with the increased LV end-diastolic diameter [82] (Fig. 8). In this model, ILβ expression was highest, and its level correlated with collagen deposition in the regions of the noninfarcted myocardium [82].

To date, these cytokines have been shown to adversely regulate the expression of proteins important in cardiac excitation–contraction coupling, including calcium regulatory proteins (SERCA2), calcium releasing channel (CRC), and L type Ca²⁺ channel [83], and inducible nitric oxide synthases (iNOS) through regulation of gene expression at the transcript level [84,85]. Elevated ILβ levels can cause negative regulation of skeletal α-actin and β-myosin heavy chain gene expression [86], induction of cardiac myocyte hypertrophy through induction/reexpression of fetal genes ANF and β-MHC [83], induction of vasodilator C-type natriuretic peptide which exerts its actions by binding to guanylate cyclase [87] and upregulation of cyclooxygenase/prostaglandin and protein kinase C signaling pathways [88]. An IL1β responsive gene, Eph-related receptor tyrosine kinase, r-EphA3 participates in cytokine signal processing including rapid tyrosine phosphorylation of signaling molecules through tyrosine kinases [89]. Taken together cytokine elevation results in an

Fig. 8. Relationship between end-diastolic diameter (EDD) and gene expression levels of TNFα, IL1β and IL6 in the noninfarcted zone at 8 and 20 weeks after coronary ligation in rat hearts. The expression of each cytokine correlated positively with EDD; reproduced from [82].
immune mediated cascade of events involving upregulation of various signaling pathways that ultimately can contribute to myocardial remodeling post coronary artery occlusion.

Few studies have focused on either the long or short term effects of the activated immune system on ion channel function. Activated neutrophils in the presence of normal cardiac myocytes can lead to arrhythmogenesis (within minutes) through marked AP prolongation, decreases in AP amplitude and resting potential and development of early afterdepolarizations via release of platelet activating factor [90]. The short term cytokine IL1 exposure (seconds) alone can significantly prolong APD_{90} by changes in conductance of calcium channels via lipid second messenger generated by cyclooxygenase and lipoxygenase pathways [88]. Modulation of membrane currents at plateau potentials is via increases in conductance of calcium channels but it remains to be determined whether other currents (I_{Ks}, I_{K1}) are altered as well [88]. Long term effects of these agents on ion channel function remain unknown.

Once the mRNA has been transcribed from the DNA template, it must be translated into the polypeptide product. It is possible that disease could affect the ability of the cell to translate the mRNA into a polypeptide, perhaps by altering the ability of the mRNA to interact with ribosomes. A breakdown in translation may be a manifestation of a disease state, rather than an adaptation of the cells to disease. As yet, there is no documented evidence of a disease-related breakdown in translation of mRNA into ion channel proteins.

After mRNA has been translated into a polypeptide, it undergoes post-translational modifications leading to incorporation into the cell membrane. Post-translational modifications include glycosylation, phosphorylation, and alternate splicing. In the case of potassium channels, post-translational modifications also include the assembly of channel α subunits into a functional homo- or heterotetramer. For sodium, calcium and some potassium channels, post-translational aligning with specific subunits is required for appropriate ion channel function (for review see [91]). An alteration in subunit availability (due to changes in translation), and/or tetramer assembly could then greatly affect channel function [92].

Although much research has been done to understand the mechanisms which promote myocardial remodeling, still more work needs to be done to reverse the remodeling via pharmacological means or gene therapy and prevent life-threatening arrhythmias associated with myocardial infarction.

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References

[16] Boyd PA, Pinto JMB. Reduced calcium currents in subendocardial Purkinje myocytes that survive in the 24 and 48 h infarcted heart. Circulation 1994;89:2747–2759.


