Abnormal Electrical Properties of Myocytes From Chronically Infarcted Canine Heart

Alterations in $V_{\text{max}}$ and the Transient Outward Current

Wei-Ming Luc, MB, MPhil, and Penelope A. Boyden, PhD

**Background.** Reentrant ventricular arrhythmias can occur in the surviving muscle fibers of the epicardial border zone of the canine heart 5 days after coronary artery occlusion. To understand the cellular basis of these arrhythmias, we developed a method of dispersing myocytes (IZs) from the epicardial border zone.

**Methods and Results.** We compared the electrophysiological properties of IZs with those of cells dispersed from the epicardium of control noninfarcted (NZs) and of sham-operated animals (NZsham). Transmembrane action potentials of IZs are reduced in total action potential amplitude and maximum upstroke velocity compared with NZs. However, resting potential of IZs is no different from that of NZs. Action potential duration at $-10\, mV$ is significantly reduced in IZs compared with control, and IZ potentials do not show the typical "spike and dome" morphology that is evident in all NZs. Using $V_{\text{max}}$ as an indirect measure of the peak inward current available for the upstroke of the action potential, we found that the availability curve for IZs is significantly different from the NZ curve. Furthermore, the time course of recovery of $V_{\text{max}}$ after a depolarizing voltage clamp step was significantly altered in IZs. Using whole-cell voltage clamp techniques, we determined that the voltage-dependent, Ca$^{2+}$-independent, 4-aminopyridine-sensitive transient outward current ($i_{\text{hol}}$) occurred in all NZs ($n=16$) but existed in only 37% of IZs ($n=16$). There was a significant reduction in the density of $i_{\text{hol}}$ elicited by depolarizing steps in those IZs showing $i_{\text{hol}}$ compared with $i_{\text{hol}}$ density in NZs.

**Conclusions.** We have developed a single-cell model of cells that survive in the infarcted heart. Our studies indicate that there are changes in $V_{\text{max}}$ in IZs. In addition, there is no prominent phase 1 of repolarization in IZ action potentials. This is consistent with the dramatic loss in the function of the ionic channel responsible for the voltage-dependent transient outward current, $i_{\text{hol}}$. *(Circulation 1992;85:1175–1188)*

**Key Words** • myocytes • electrophysiology • ion currents • action potential duration

Several layers of muscle fibers survive on the epicardium over infarcted myocardium caused by complete occlusion of the left anterior descending artery. In the canine model of myocardial infarction, this viable layer has been called the epicardial border zone (EBZ).1–5 It has been demonstrated that ventricular tachycardias inducible in these hearts 5 days after the coronary artery occlusion are a result of reentrant excitation localized to the EBZ.1,2,4–6 Records of transmembrane potentials of fibers in isolated preparations of EBZ show certain electrical abnormalities. In fibers of the EBZ, resting potential, total action potential amplitude, and maximum upstroke velocity are reduced. In addition, the time for complete repolarization is significantly shortened.3,7,8 Whether these electrical abnormalities alone predispose the EBZ to reentrant excitation is not certain, because structural changes in the EBZ after infarction seem to increase its anisotropic conduction properties, and this change could underlie the increased susceptibility to reentrant excitation.2,3,6

To determine whether the alterations in electrical activity of these fibers occur independently of the structural changes known to occur in the heart after infarction, we have studied single myocytes dispersed from the EBZ (IZs) 5 days after occlusion and compared them with cells dispersed from the epicardium of noninfarcted hearts (NZs). Electrophysiological studies on these myocytes have provided new information on the types of alterations in sarcolemmal ionic currents that result in changes in transmembrane potentials and impulse conduction after infarction. Up to now, information about the changes in specific ionic currents of EBZ fibers has been lacking, because the complex syncytial nature of multicellular preparations of subepicardial fibers makes them difficult to voltage clamp.

Results from our initial studies show that action potentials of myocytes from the EBZ do not show either rapid repolarization during phase 1 or a notch. The absence of phase 1 in IZs may be related to a reduction or loss of the 4-aminopyridine (4-AP)–sensitive transient outward current ($i_{\text{hol}}$) that is so prominent in epicardial cells from noninfarcted hearts. Therefore, we

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examined the nature of the voltage-dependent $i_{\text{mol}}$ of IZs and compared it with $i_{\text{mol}}$ of NZs.

Methods

Mongrel dogs of either sex (weight, 12–15 kg; age, 1–2 years) were used in these studies. Under pentobarbital anesthesia (30 mg/kg i.v.), the left anterior descending coronary artery was isolated and occluded in two stages according to the Harris procedure. At the time of the second ligature, lidocaine (2 mg/kg i.v.) was given if multiple ventricular beats occurred. The chest was closed. Nineteen dogs survived this procedure and were used to provide IZs 5 days after the occlusion. In addition, hearts from 30 dogs were used to prepare control NZs. To rule out possible changes in myocyte electrophysiology that resulted from the surgical procedure rather than infarction, five dogs were subjected to the standard surgical procedure without coronary ligation. Myocytes (NZs) were dispersed from epicardial tissue sections obtained from the hearts of these sham-operated dogs 5 days after surgery.

Preparation of Cells Disaggregated From Epicardium

Five days after coronary artery occlusion, dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.). The chest was opened through the fourth intercostal space, and the heart was quickly excised. The infarcted region was visually identified on the anterior epicardial aspect of the left ventricle by its mottled appearance. A slab of muscle about 10×20 mm and 2–3 mm thick was excised from this region. Previous studies have demonstrated that these preparations contain muscle cells that survive on the epicardial surface of an apparent transmural infarction. These EBZ sections were used to prepare IZs.

To disaggregate myocytes from the sections of epicardial muscle, the following procedure (modified from Heathers et al.19) was used. The tissue was rinsed in a Ca$^{2+}$-free trituration solution (in mM: NaCl, 115; KCl, 5; sucrose, 35; dextrose, 10; HEPES, 10; taurine, 4; pH 6.95) to remove blood and then immersed in 20 ml of trituration solution bubbled with 100% oxygen and maintained at 36.5°C. For the several steps of trituration, 20 ml of solution containing both protease (type XIV, Sigma Chemical Co.) (0.35 mg/ml) and collagenase (type II, Worthington) (198 units/mg, 93 units/ml) or collagenase alone was used. After each step, the supernatant containing the dispersed myocytes was collected, and a loose pellet was resuspended in solution containing 50 μM Ca$^{2+}$ and 0.1% bovine serum albumin, pH 7.2. This resuspension solution was changed every 30 minutes for solutions containing increasing concentrations of calcium (100, 200, and 350 μM). Myocytes were kept at room temperature in the final solution (350 μM Ca$^{2+}$) and were used for experiments for up to 6 hours after completion of the procedure.

For the control noninfarcted hearts and the sham-operated hearts, the yield of rod-shaped, Ca$^{2+}$-tolerant cells was 30–40%. The yield of IZs was similar. As for any single-cell isolation procedure, there is a selection process. Therefore, with this technique, we are in no way isolating and therefore studying all cells that survive in the EBZ. Furthermore, with our methods we could in no way predict whether cell survivors were “ischemic” cells and/or cells that might have been affected by substances released as a result of the coronary artery occlusion. Nevertheless, regardless of where these cells were located in situ, the electrical changes noted in IZs are important especially when considering the substrate or underlying cell electrophysiology of the EBZ.

Recording Methods

Transmembrane potential recordings. For microelec-
trode recordings of transmembrane potentials from single cells, a drop of resuspension solution was placed on a poly-l-lysine–coated coverslip that had been placed in a chamber mounted on a Nikon inverted microscope stage. Cells were allowed to settle and then superfused with a normal Tyrode’s solution (in mM: NaCl, 137; NaHCO3, 24; MgCl2, 0.5; NaH2PO4, 1.8; KCl, 4; dextrose, 5.5; CaCl2, 2.5; bubbled with 5% CO2/95% O2; pH, 7.3) at a rate of 1.5 ml/min. Temperature was continuously monitored and maintained at 37.0±0.5°C. Fine-tipped glass microelectrodes filled with 3 M KCl (tip resistances of 30–50 MΩ) were used to record transmembrane potentials (Axoclamp-2A amplifier, Axon Instruments, Calif.). The bath was grounded through a KCl Ag–AgCl salt bridge. Cells were stimulated at basic cycle length (BCI) of 1,000 msec by injecting brief (1-msec) depolarizing current pulses. Upstroke of phase 0 of the action potential was electronically differentiated and displayed on an oscilloscope (Tektronix 565). Records of membrane current, $V_{\text{m}}$, and potential were monitored on the oscilloscope and recorded on a pen recorder (Gould). Action potentials on the oscilloscope screen were photographed. Measurement of action potential characteristics and $V_{\text{m}}$ were made directly from these photos.

Transmembrane potentials of cells from the two control groups and the infarcted group were studied. For each cell, resting potential (RP), action potential amplitude (APA), action potential duration at –10 mV (APD10), and at 50% and 100% of repolarization (APD50 and APD100), and $V_{\text{m}}$ were determined. Mean values for cells from each group were determined and compared. In some studies, discontinuous voltage clamp (dSEVC, Axoclamp2A) was used to control membrane potential. Switching frequency of clamp was adjusted so that recordings were not out of phase or distorted. A clear advantage of this technique over whole-cell patch clamp method for studies on cells from infarcted hearts is that the cell is not dialyzed.

Steady-state membrane properties. Input resistance was determined from the slope of the linear part of a “steady-state” current–voltage curve measured in normal Tyrode’s solution (4 mM [K+]). Steady-state current–voltage curves were obtained by applying a slowly depolarizing command ramp (1 mV/0.67 sec) from –120 to +20 mV using dSEVC. Mean values of the density of steady-state current were obtained for at least 43 voltage points along the ramp from current densities of each of five cells from within each group. Current density was the value of the current divided by the cell’s capacitance. Cell capacitance was estimated by first determining the dimensions of each myocyte using a calibrated eyepiece. From this we could then determine the mean two-dimensional surface area of the myocyte. In other experiments using patch pipettes in whole-cell voltage
clamp configuration (see below), capacitance of several cells in each group were determined by integrating the transient produced by a 5-mV hyperpolarizing clamp pulse from \(-85\) mV and then dividing this area by the voltage step.

\(V_{\text{max}}\) studies. An indirect assessment of Na\(^+\) channel availability was completed by determining the voltage dependence of inactivation of \(V_{\text{max}}\). For these studies, the membrane potential of the cell was clamped (dSEVC) at selected conditioning potentials for 4 seconds. Then, within 1 msec of release of the voltage clamp, the cell was stimulated by injection of a depolarizing pulse (1 msec in duration) to elicit an action potential. The strength of the current pulse was adjusted so that the latency between the end of the current pulse and the peak \(V_{\text{max}}\) was constant (1 msec) to reduce the effect of time-dependent inactivation of Na\(^+\) current. For each conditioning potential, \(V_{\text{max}}\) of the elicited action potential was determined. Each \(V_{\text{max}}\) value was normalized to the fully available \(V_{\text{max}}\) value obtained at a conditioning potential of \(-110\) mV. For each cell, these normalized \(V_{\text{max}}\) values were fit to a Boltzmann distribution equation, \(\%V_{\text{max}} = 1/[1 + \exp(V_h - V_o)/k]\), to obtain the half-maximum potential (\(V_o\)) and the slope factor (\(k\)) for that cell. The mean values of \(V_o\) and \(k\) of NZs were compared with those of IZs.

The voltage and time-dependent recovery of Na\(^+\) channel availability in normal adult cardiac cells follows a predictable time course. It may be that alterations in the recovery from inactivation of the Na\(^+\) channel occur in the cells from the EBZ, providing one mechanism by which conduction abnormalities may arise in this region of the heart after infarction. Therefore, we assessed the time course of recovery of \(V_{\text{max}}\) from voltage-dependent inactivation in cells from the experimental groups. For these protocols, a cell was first clamped to \(-90\) mV followed by a step to 0 mV for 500 msec, then to a specific repriming potential (\(V_r = -100, -90, -80\) mV) for a variable interval (1 to 500 msec). At the end of that interval, the voltage clamp was released, the cell was stimulated to elicit an action potential, and then it was reclamped to the holding potential (\(-90\) mV). For each interpulse interval (Ip), \(V_{\text{max}}\) of the elicited action potential was determined and normalized to value obtained for \(V_{\text{max}}\) of the action potential elicited at Ip=500 msec. For each cell, the time constant (\(\tau\)) of recovery from inactivation was computed by fitting the data to the exponential function \(\%V_{\text{max}} = 1 - \exp(-t + \text{delay}/\tau)\). The mean value of the time constant of recovery of \(V_{\text{max}}\) for NZ cells was compared to that of IZs.

**Patch clamp studies.** Our studies using the fine-tipped microelectrode suggested that the action potentials of IZs did not show either rapid repolarization during phase 1 or a notch. In contrast, action potentials of cells from noninfarcted epicardium typically showed a prominent phase 1 of repolarization. It is thought that the appearance of the notch is caused by activation of the large \(i_{\text{Na}}\). Therefore, voltage clamp experiments were done to identify and characterize the time- and voltage-dependent properties of the large \(i_{\text{Na}}\) in NZs and compare them with results of findings in IZs. For these studies, patch pipettes were made from borosilicate glass capillary tubes (inner diameter of 0.86 mm) according to Hamill et al. A typical electrode had a resistance of 2–4 M\(\Omega\) when filled with the internal pipette solution, which contained the following (in mM): K-aspartate, 125; KCl, 20; HEPES, 5; EGTA, 10; MgCl\(_2\), 1; ATP (Mg salt), 10; phosphocreatine, 5; and pH titrated to 7.3 with KOH. The tip liquid junction potential of this pipette solution was estimated to be \(-10\) mV. All voltages were corrected for this potential. For recording, cells were superfused with a normal Tyrode’s solution for at least 25 minutes. A gΩ seal was made in the bridge mode using Axoclamp-2a (Axon Instruments). After the seal had formed, the cell membrane was ruptured by application of gentle suction to the pipette. A constant RP of the cell was immediately recorded.

Because we were interested in establishing whether transmembrane action potentials recorded with patch pipettes were similar to action potentials recorded with microelectrodes and whether electrical abnormalities in IZs persisted when recording with a patch pipette, an action potential for each cell to be voltage clamped was recorded within 3 minutes of membrane rupture. Then the amplifier was switched to continuous voltage clamp mode. In this whole-cell configuration, the electrode resistance in series with the membrane was partially compensated electronically (up to 70%). In eight experiments, this value was 6.1±5 M\(\Omega\) before recording instabilities occurred. After the switch to clamp mode, the external Tyrode’s solution was exchanged for solutions needed to record \(i_{\text{Na}}\) under conditions that would decrease the likelihood of contamination by overlapping currents. In some experiments, this external solution contained the following (in mM): NaCl, 137; NaHCO\(_3\), 12; CaCl\(_2\), 0.5; dextrose, 5.5; MgCl\(_2\), 0.5; and KCl, 40. Interference by sodium current was largely controlled by the use of 4-AP (2 mM) in the external solution. The interference by calcium current was blocked by use of Cd\(^{2+}\) (1 mM) or Mn\(^{2+}\) (2 mM) with low [Ca\(_{\text{e}}\)] (0.05 mM) in the external solution. Interference from the Ca\(^{2+}\)-dependent transient outward current was minimized by use of EGTA (10 mM) in the internal pipette solution.

Currents were recorded in the absence and presence of 4-AP (2 mM) to obtain the 4-AP–sensitive current, which should reflect the time course and kinetics of the \(i_{\text{Na}}\). In preliminary NZ experiments, however, we found that the 4-AP–sensitive current had an inward current component (see “Results”). Therefore, in some cells, 4-AP–sensitive current was obtained when recordings were made in an external solution that was low in [Na\(^+\)] and [Ca\(^{2+}\)] (in mM: choline chloride, 145; HEPES, 5; MgCl\(_2\), 0.5; dextrose, 5.5; CaCl\(_2\), 0–0.5; KCl, 4; pH, 7.3) with Cd\(^{2+}\) (1 mM). Six experiments were done to illustrate the effects of the “sodium-free” solutions on the amplitude and kinetics of \(i_{\text{Na}}\) in NZs.

**Data acquisition and analysis.** Voltage clamp protocols were generated with a 12-bit analog–digital converter (TecMar) and IBM-AT (pClamp 4, Axon Instruments). Clamp protocols and resultant current traces were digitized (sampling interval, 0.2 msec) and stored in the computer for later analysis. Leak currents and capacitive transients were not subtracted electronically. The amplitude of \(i_{\text{Na}}\) was measured as the difference between the peak of the 4-AP–sensitive current and the baseline extrapolated from the current recorded between 140 and 160 msec after the beginning of the clamp step. The contamination of \(i_{\text{Na}}\) by other outward currents was minimal because the delayed rectifier
current ($i_k$) and the ATP-dependent K current, if present, were avoided by use of this method. Each whole-cell current value was normalized to the capacitance value of each cell.

For curve fitting of the digitized current data, the following general method (Clampfit) was adopted. We first chose the length of data to be fit. Then, by curve-fitting routines whereby the best fit is achieved according to the least-squares principle, a monoeponential fit was tested. The same data were then fit by use of a double exponential function. The function that fit the data better was accepted. Curve fitting of normalized data points to such functions as the Boltzmann distribution function or the recovery time course function was done with a simplex algorithm. For the latter case, determination of best fit to either a single or double exponential function was done by analysis of mean residual squares data.

Data are presented as mean±SD unless otherwise noted. In all cases, a statistical difference was evaluated first by ANOVA test or a nested ANOVA test on the groups of data (e.g., $\tau$ versus $V_{in}$, time to peak [TTP] versus $V_{test}$). The variance within groups was analyzed by $F$ test. Comparison of mean values obtained for the data from the experimental groups was done with an unpaired Student’s $t$ test for populations of unequal or equal variances. Values of $p<0.05$ were considered significant. In some experiments, paired $t$ test was used to determine differences between paired observations.

**Results**

**Morphology**

When viewed by light microscopy (×350, modulation contrast), the surface membranes of the healthy NZs were similar to those described previously for canine myocytes obtained from a transmural preparation (Figure 1A). Ca$^{2+}$-tolerant NZs had smooth surface membranes and were rectangular (Table 1). NZs possessed staircase configuration at their ends. IZs were similar in shape, and there was no obvious change in the staircase configuration at the disk area. Just as for NZs, IZs were selected as those that had refractive surface membranes and in which striations were apparent and regular. In most IZs, the membrane was “bumpy,” that is, it appeared ruffled (Figure 1B). IZs were slightly larger than NZs, but this difference was not significant ($p>0.05$) (Table 1). There was a significant increase in the average IZ cell capacitance compared with control ($p<0.05$). In addition, there was a significant increase in average IZ cell input resistance compared with NZ average value ($p<0.05$).

**Electrophysiology of Cells: Microelectrode Studies**

As we have previously described for an intracellular recording from a single Purkinje cell, a “good” cell is one that quickly seals after the entry of the fine-tipped microelectrode. “Sealing” means that no holding current is needed to maintain an RP. The RP had to be stable for at least 2 minutes before any measurements were made. In addition, we recorded transmembrane action potentials from cells with a stable RP. Transmembrane action potential data were collected only from cells that had been maintained at a steady driven rate (BCI=1,000 msec) for at least 2 minutes.

**Table 1. Cell Parameters**

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<tr>
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<th>NZs</th>
<th>IZs</th>
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<tr>
<td>Length ($\mu$m)</td>
<td>129±18 (26)</td>
<td>135±14 (21)</td>
</tr>
<tr>
<td>Width ($\mu$m)</td>
<td>26±6</td>
<td>28±5.5</td>
</tr>
<tr>
<td>Capacitance (pF)</td>
<td>77±28 (31)</td>
<td>92±31* (36)</td>
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<tr>
<td>Input resistance (Mf1)</td>
<td>38±8.3 (43)</td>
<td>42.8±7.7* (24)</td>
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NZs, cells dispersed from epicardium of noninfarcted hearts; IZs, monocytes dispersed from the epicardial border zone.

Length and width, cell dimensions as measured with calibrated eyepiece; capacitance, cell capacitance determined by integration of transient; input resistance determined at resting potential.

Numbers in parentheses indicate number of observations used to obtain average value.

*p<0.05.
Mean RPs from two cell types (NZs and IZs) were compared (Table 2, lower panel). Mean RP of IZs did not differ significantly from that of NZs ($p>0.05$). However, mean APA and $V_{\text{max}}$ of IZs were significantly reduced with respect to NZ values ($p<0.001$ and $p<0.001$, respectively). The configuration of transmembrane action potentials of IZs differed from that of NZs. Representative action potentials are shown in Figure 2. In the right-hand column, voltage recordings from an NZ and an IZ are superimposed to demonstrate the altered time course of repolarization of the IZ cell. The action potentials of IZs were more triangular than those of NZs. In fact, APD measured at the $-10$ mV level was significantly shorter in IZs than in NZs ($p<0.001$). Although there was a trend for mean APD$_{50}$ and APD$_{100}$ to be different in the IZ cell group, the difference was significant ($p<0.05$) only for APD$_{100}$ values (Table 2, lower panel). In addition, for all IZs, the large notch seen between phase 1 and phase 2 of the action potentials of NZs was not evident.

**Steady-State Properties**

The steady-state total membrane current–voltage relations of five NZs and five IZs were obtained with the discontinuous voltage clamp technique. The action potentials of these cells were similar to those described in Table 2. Density of current at several voltages was determined for each cell. Then, values from individual cells within each group were averaged to obtain average current–voltage relations for NZs and for IZs (Figure 3). At the RP (zero current level), the input resistance of NZs ($42\pm3.35 \, M\Omega, n=5$) was less ($p<0.05$) than that of IZs ($50.8\pm7.22 \, M\Omega, n=5$) although the resting potentials of the cells did not differ (NZs, RPs $= -90\pm2.4 \, mV$; IZs, RPs $= -87\pm2.6 \, mV$). The current–voltage relation for NZs was n-shaped, with two zero current levels ($-90$ and $-33 \, mV$). In addition, there was a prominent region of negative slope conductance. For IZs, the shape of the
curve was also n-shaped, and the two zero current levels were −87 and −27 mV. In IZs, there was a third zero current level (at −46 mV). In the voltage range −82 to −48 mV, the density of steady-state membrane current in NZs was significantly larger (p<0.05) than that measured in IZs. The densities of steady-state currents at voltages more depolarized than −46 mV were identical in the two cell types (p>0.05).

**Properties of \( V_{\text{max}} \) in NZs and IZs**

IZs show a significant reduction in \( V_{\text{max}} \) and APA, with no significant change in RP. Therefore, we can conclude that the decrease in \( V_{\text{max}} \) seen in IZs was not secondary to a decrease in membrane RP. We wished to determine, however, whether \( V_{\text{max}} \) values in IZs would recover to levels more similar to those seen in control cells. Therefore, in a subset of cells from each experimental group, we indirectly assessed and compared the voltage dependence of steady-state inactivation of the fast \( Na^+ \) current (see "Methods"). In this way, an "apparent" \( V \)-curve (availability curve) was constructed for each cell.

In these subsets of IZs and NZs, there was no significant difference in mean RP between the two groups (NZ, RP=−88±5 mV, \( n=21 \); IZ, RP=−88±4 mV, \( n=12 \)). Furthermore, the action potentials for these cells (Table 3) were similar to those described in Table 2. However, the IZ availability curve differed from the NZ availability curve. There was a significant change in mean \( V_{\text{0.5}} \) in IZ cells compared with control \( V_{\text{0.5}} \) value (Table 3). In addition, there was an increase in mean slope factor value in IZ cells, but this was not significant (p>0.05). Availability curves are shifted to more negative potentials in IZs (Figure 4). Finally, and importantly, hyperpolarization of IZs did not restore \( V_{\text{max}} \) values to values similar to control. That is, the mean fully available \( V_{\text{max}} \) of IZs was significantly reduced (p<0.001) with respect to the mean fully available \( V_{\text{max}} \) of NZs (Table 3).

Using \( V_{\text{max}} \) as an index of the available sodium conductance, we indirectly assessed the recovery from \( Na^+ \) channel inactivation after a voltage clamp step in IZs and compared it with the same recovery process in NZs. In NZs (RP, \( x=89 \pm 5 \); \( n=12 \); APA, \( V_{\text{max}}=118 \pm 8 \) mV, \( k=230 \pm 13 \) V/sec, \( n=5 \)), the time course of recovery of \( V_{\text{max}} \) was rapid and depended on the value of the \( V \) (Figure 5A). And, for all \( V \), a single exponential function best fit the data points. At depolarized \( V \), the time constant of this process in NZs was increased (Figure 5C). In IZs (RP, \( x=86 \pm 2 \) mV, APA=\( n=12 \); APA, \( V_{\text{max}}=130 \pm 21 \) V/sec, \( n=5 \)), the time course of recovery of \( V_{\text{max}} \) also depended on \( V \) (Figure 5A). At all \( V \), tested, however, this was a significant increase in the mean \( \tau \) of this process in IZs compared with control (p<<0.01) (Figure 5C). In some IZ cells, we were unable to elicit an action potential with intracellular stimulation at very short coupling intervals (e.g., note the several points along the zero line on the \( x \) axis for the IZ in Figure 5A). In this cell and others, data were fit to include this delay. The duration of this delay was 1−7 msec in NZ cells but ranged from 7 to 40 msec in IZs. The amount of delay depended on the value of the repriming voltage, with the longer delays being associated with more depolarized \( V_r \).

**Table 3. Properties of \( V_{\text{max}} \) in NZs and IZs**

<table>
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<tr>
<th></th>
<th>( V_{\text{max}} ) (mV)</th>
<th>( V_{\text{max}} ) (V/sec)</th>
<th>( V_{\text{max}} ) (mV)</th>
<th>( V_{\text{max}} ) (V/sec)</th>
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<tbody>
<tr>
<td>NZs (( n=21 ))</td>
<td>-88±5</td>
<td>121±7</td>
<td>-63.9±5</td>
<td>2.9±0.68</td>
</tr>
<tr>
<td>IZs (( n=12 ))</td>
<td>-88±5*</td>
<td>113±6.5*</td>
<td>-73.5±5*</td>
<td>3.67±0.59</td>
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RP, resting membrane potential recorded using 3 M KCl microelectrodes; APA, total action potential amplitude; \( V_{\text{max}} \), maximum upstroke velocity of phase 0; \( V_{\text{0.5}} \), half maximum potential; \( k \), slope factor; peak \( V_{\text{max}} \), measured when conditioning potential is −110 mV; NZs, cells dispensed from control noninfarcted epicardium; IZs, cells dispensed from epicardial border zone 5 days after coronary artery occlusion.

*Significant when mean values are compared. \( V_{\text{0.5}} \) and \( k \) values were obtained by best fitting the data from each cell to a Boltzmann function and then averaging these values.

\[ i_{\text{iol}} \] in Cells From Noninfarcted Hearts: Effect of Removing External \( Na^+ \)

\[ i_{\text{iol}} \] was recorded in NZs by using a holding potential \( (V_h) \) of either −85 or −55 mV and applying depolarizing steps for 180 msec to various test potentials \( (V_i) \) (−40 to +80 mV) at 0.1 Hz. Preliminary studies in a \( Na^+ \)-containing Tyrode's solution showed that a large \( i_{\text{iol}} \) was recorded with steps to potentials positive to −20 mV. This \( i_{\text{iol}} \) was blocked by externally applied 4-AP.

**Figure 4.** Curves depict \( Na^+ \) current availability in myocytes dispersed from epicardium of noninfarcted hearts (NZs) and myocytes from epicardial border zone of heart 5 days after coronary artery occlusion (IZs) as assessed with \( V_{\text{max}} \) measurements. Fractional \( V_{\text{max}} \) (\( V_{\text{max}} \) of elicited action potential normalized to its maximal value) vs. conditioning potential is illustrated for cells representing the group results (three IZs [solid symbols] and four NZs [open symbols]). In addition, the solid line is drawn according to the Boltzmann equation using average NZ values of \( V_{\text{0.5}}=−64 \) mV and \( k=3 \) mV (\( n=21 \)). Dashed line is drawn according to the Boltzmann equation using average IZ values of \( V_{\text{0.5}}=−73 \) mV and \( k=3.67 \) mV (\( n=12 \)). See text for more detail.
FIGURE 5. Graphs show recovery of peak inward current using $V_{\text{pp}}$. Fractional $V_{\text{pp}}$ (normalized to $V_{\text{pp}}$ at the longest interval) vs. interpulse interval ($I_{\text{pp}}$). Panel A: Time course of recovery of $V_{\text{pp}}$ in one myocyte dispersed from epicardium of noninfarcted hearts (NZ) (open symbols) and one myocyte from epicardial border zone of heart 5 days after coronary artery occlusion (IZ) (solid symbols) after a depolarizing clamp pulse to 0 mV for 500 msec. This pulse should inactivate all Na$^+$ channels. Individual data points are shown. Repriming voltage was $-80$ mV (triangles). Panel B: Recovery of $V_{\text{pp}}$ depends on the value of the repriming voltage in both NZs (open symbols) and IZs (solid symbols). For this graph, semilog representation of data shown in panel A is on y axis. In addition, data obtained when repriming voltage = $-100$ mV (circles) are illustrated for each cell. Panel C: Summary of the effects of the repriming voltage on the recovery time course (tau) of $V_{\text{pp}}$ in NZs and IZs. Each point represents mean ± SD of data obtained from the number of cells shown in parentheses. Asterisks denote significant differences between IZ and NZ values (p<0.05).

(1–2 mM) (not shown) and therefore was identified as the 4-AP-sensitive $i_{\text{h01}}$ (Figure 6A). What is evident, however, is that for certain test voltages (e.g., to $-40$ mV), an inward current component in addition to an outward component was observed. It is unlikely that this inward current component resulted from Na$^+$ flowing through TTX-sensitive Na$^+$ channels because TTX (30 μM) did not diminish it. It is unlikely that this inward current component was secondary to activation of a Ca$^{2+}$ current because Mn (2 mM) did not diminish it.

To remove this overlapping inward current, we substituted Na$^+$ ions in the external solution with choline ions. In 12 NZs, the 4-AP-sensitive current in the Na$^+$-free solution no longer showed this inward current transient (Figure 6C). A comparison of the current–voltage relation of peak $i_{\text{h01}}$ in the same cell obtained in the presence and the absence of external sodium is illustrated in Figure 6B. In the absence of sodium, there is a slight increase in peak $i_{\text{h01}}$ amplitude at test voltages between $-10$ and $+30$ mV. There was no significant difference in the time course of inactivation ($\tau$) or the time to peak (TTP) of $i_{\text{h01}}$ when sodium was removed from the external solution (Figure 6D).

In conclusion, we used “sodium-free” Tyrode’s solution to further remove contaminating inward currents apparent in 4-AP–sensitive currents. Thus, it is unlikely that we underestimated the density of $i_{\text{h01}}$ in NZs and IZs.

Voltage Dependence of Activation of $i_{\text{h01}}$ in NZs and IZs

As has been described previously, $i_{\text{h01}}$ in canine myocytes dispersed from normal hearts rises to a peak amplitude and then decays even though the depolarizing step is maintained. In NZs ($n=16$), a small $i_{\text{h01}}$ was sometimes elicited with clamp steps to a test voltage of $-20$ mV. The amplitude of $i_{\text{h01}}$ then increased with the increasingly positive depolarizing steps (Figure 7A). In addition, we found no evidence of “run-down” of $i_{\text{h01}}$.

We found that the transmembrane potential changes noted in our microelectrode studies using IZs (Table 2) were conserved when recording through the patch electrode (Table 4). In particular, there was no notch evident in transmembrane action potentials of IZs. In addition and in marked contrast to our findings for NZs, $i_{\text{h01}}$ was very difficult to elicit with depolarizing steps in IZs. In I6 IZs in which the voltage clamp protocol was completed, only six (37%) showed a small outward current transient at test potentials positive to $-20$ mV. When $i_{\text{h01}}$ was elicited in an IZ, its amplitude increased only slightly with more positive depolarizing steps (Figure 7B). The density of $i_{\text{h01}}$ in IZs was significantly reduced compared with that of NZs at each test voltage positive to 0 mV (Figure 7C). To determine whether there was an alteration in the voltage dependence of
activation of this current in IZs, the depolarizing step protocol was repeated from at least two other holding potentials ($V_h = -100$ and $V_h = -55$ mV). In IZs in which $i_{hol}$ was absent at $V_h = -85$ mV, a negative shift in the holding potential to $-100$ mV did not restore $i_{hol}$. Finally, very strong depolarizing steps (to $+80$ mV) were applied to some IZs showing no $i_{hol}$ to test for the presence of $i_{hol}$. These steps to very positive potentials did not induce detectable $i_{hol}$.

An indirect measure of $i_{hol}$ activation time course was obtained by determining the TTP current. TTP was the measured time from the start of the depolarizing step to the peak $i_{hol}$ and may underestimate the true value of TTP because of our recording conditions. Like the findings of others, we found that the TTP of $i_{hol}$ in NZs decreased with more positive test potentials (data not shown).$^{11,16}$ TTP values of the small $i_{hol}$ currents elicited in IZs were compared with those values obtained in NZs for a $V_{test}$ where $I_Z$ $i_{hol}$ amplitudes were sufficiently large to be measured accurately. There was a slight but significant increase in TTP values for $V_{test}$ of $+30$ mV in IZs ($12.1\pm5$ msec, $n=6$) compared with the NZ value ($4.2\pm1.1$ msec, $n=8$). Furthermore, in IZs where $i_{hol}$ was measurable, there was also a slight but significant increase in the time constant of decay (NZs, $V_{test}= +30$ mV; $\tau = 14.3\pm7$ msec; $n=4$) (Figure 8).

**Voltage Dependence of Inactivation of $i_{hol}$ in NZs and IZs**

The voltage dependence of inactivation of $i_{hol}$ was studied by clamping the cell to different conditioning potentials ($V_c$) for $500$ msec, returning to $V_h$ for $10$ msec, and then applying a test pulse to $+40$ mV for $180$ msec. The $V_h$ was $-85$ mV, and the interval between each test pulse protocol was $8$ seconds. The peak amplitude of $i_{hol}$ was measured as the difference in current between the current recorded at each $V_c$ and the current recorded at $V_c = +30$ mV. The peak amplitude of $i_{hol}$ obtained with each test pulse was normalized to the maximum amplitude of $i_{hol}$ obtained with $V_c = -85$ mV. For each cell tested, the normalized values were fit to a Boltzmann distribution equation to determine the
half maximum inactivation voltage \( (V_{0.5}) \) and slope factor \( (k) \).

The steady-state voltage dependence of inactivation of \( i_{I01} \) in NZs was similar to that described for \( i_{I01} \) in other myocytes.\(^{11,15,16} \) For NZs, the mean voltage at half maximal inactivation was \(-38\pm6.3 \, \text{mV}\), and the slope factor was \(3.5\pm0.5 \, \text{mV}\). Inactivation of \( i_{I01} \) was fully developed at potentials positive to \(-10 \, \text{mV}\). The steady-state voltage dependence of inactivation of \( i_{I01} \) in four of the six IZs in which the protocol was completed exhibited a relation similar to that seen in NZs. In these IZs, the peak \( i_{I01} \) \((219\pm82 \, \text{pA})\) elicited at positive potentials was significantly reduced \((p<0.05)\) compared with the peak in NZs \((1,242\pm550 \, \text{pA})\). This corresponds to the absence of a notch in the transmembrane action potentials of these IZ cells. In IZs, the voltage at which half maximal inactivation \((-36\pm4.5 \, \text{mV})\) occurred was not significantly different when the means of the two groups were compared. Because of the small size of the IZ group, however, the physiological significance of this finding is unclear at this time. What is clear from the present observations is that the residual \( i_{I01} \) currents in IZs do not differ greatly from NZs.

**Recovery From Inactivation**

The time-dependent nature of the recovery of \( i_{I01} \) is an important feature of this current, and in normal cells...
it has important physiological implications. Furthermore, our inability to record large amplitude $i_{o1}$ currents in some IZs may be related to an altered mechanism of recovery from inactivation. Therefore, the time course of recovery of $i_{o1}$ from inactivation was studied in IZs and compared with that determined for NZs. For these studies, the following double-pulse protocol was used. Two depolarizing clamp pulses (180 msec in duration, step to +40 mV) with varying $I_{pl}$s were applied every 8 seconds from a $V_h$ of −85 mV. The $I_{pl}$s ranged from 10 to 2,000 msec. The peak amplitude of $i_{o1}$ was measured as the difference between the current recorded at each $I_{pl}$ and the current recorded at $I_{pl}=10$ msec (we assumed that there was no reactivation of $i_{o1}$ at $I_{pl}=10$ msec). The peak current amplitude obtained at each $I_{pl}$ was normalized to the peak amplitude obtained. For the five NZs studied (mean peak $i_{o1}$, 1,122 pA), $i_{o1}$ recovered along a single exponential time course (average $\tau$, 60±30 msec) with a majority of $i_{o1}$ amplitude recovering within a reasonably short coupling interval (Figure 9, upper panel). The time course of recovery of $i_{o1}$ was determined in four IZs with peak $i_{o1}<300$ pA (Figure 9, lower panel). Note that for IZs, the small residual $i_{o1}$ currents also recovered reasonably rapidly (range of $\tau$, 35–59 msec). For example, by $I_{pl}=1,000$ msec, currents had nearly reached almost peak amplitude. Therefore, it is unlikely that a dramatic alteration in the kinetics of recovery from inactivation can account for the reduced or absent $i_{o1}$ in IZs.

In summary, the voltage-dependent $i_{o1}$ is either absent or markedly reduced in cells dispersed from the EBZ. The reduction or loss in $i_{o1}$ occurred in IZs that showed no prominent early phase of repolarization (phase 1) in the transmembrane action potential. In IZs with reduced $i_{o1}$, the average TTP values and the time course of inactivation of $i_{o1}$ during depolarizing pulses were slightly greater than control. Furthermore, the voltage dependence of inactivation or an altered recovery of residual $i_{o1}$ currents after inactivation cannot account for the reduced density of $i_{o1}$ in IZs.

**Discussion**

We have successfully developed a preparation of myocytes dispersed from the EBZ fibers that survive in the canine heart 5 days after left anterior descending coronary artery ligation. This preparation allows us to identify electrical abnormalities that persist in the myocytes even after they have been dispersed from the myocardium and studied in vitro. In addition, it allows us to compare our findings with data reported for transmembrane potentials of EBZ fibers in large multicellular preparations.

**NZs Versus Normal Epicardial Tissue**

We must first discuss our normal epicardial cell preparation and its characteristics in terms of data obtained from fibers of normal noninfarcted canine epicardium. Mean RP and APA values of our NZ cells (−88 mV and 120 mV, respectively) are somewhat lower than mean values reported for canine epicardial fibers (e.g., −82 and 91 mV, −84 and 103 mV) studied under similar conditions but are similar to values obtained by others using dog ventricular cells. Mean RP and APA values of NZ cells fell very close to predicted $E_K$ (−89.4 mV) for the normal epicardial canine fiber, suggesting that our disaggregation procedure did not alter intracellular K+ ion activity. In addition, $V_{max}$ values of our NZs are

**FIGURE 8.** Graphs show time course of inactivation of $i_{o1}$ in myocytes dispersed from epicardium of noninfarcted hearts (NZ) (upper panel) and a myocyte from epicardial border zone of heart 5 days after coronary artery occlusion (IZ) (lower panel). For both cell types, the relaxation of the 4-aminopyridine-sensitive current (dots) during a maintained depolarizing step to +30 mV is illustrated. Superimposed on each current trace is a solid curve best describing the decay time course (NZ $\tau=9.56$ msec and IZ $\tau=18.2$ msec). Vertical calibration bars are 300 pA (upper panel) and 30 pA (lower panel). Horizontal calibration bars are 8 msec.

**FIGURE 9.** The time course of recovery of $i_{o1}$ in myocytes dispersed from epicardium of noninfarcted hearts (NZs) and myocytes from epicardial border zone of heart 5 days after coronary artery occlusion (IZs). In both the upper panel (NZs) and lower panel (IZs), fractional $i_{o1}$ ($i_{o1 \text{ test}}/i_{o1 \text{ max}}$) is plotted against the value of the interpulse interval ($I_{pl}$). Holding potential = −85 mV. Data from several cells in each group are illustrated by the different symbols.
similar to other myocyte values.19,20 Finally, cells dispersed from the sham-operated hearts appeared to be no different from control. Therefore, the surgical procedure itself cannot account for the observed changes in electrical activity of IZs.

**NZs Versus IZs**

The IZs were slightly larger than the NZs. Therefore, the calculated two-dimensional surface area of the average IZ is approximately 13% larger than that of NZs. The average cell capacitance of IZs was approximately 19% greater than that measured for NZs. Therefore, the increase in two-dimensional IZ cell size could account for much of the measured increase in IZ cell capacitance. Other variables, however, such as indexes of membrane infolding (or scalloping of membrane), swelling, or even cell hypertrophy were not measured in this study and may well contribute to the increased IZ cell capacitance.

Mean RPs in NZs and IZs were not significantly different. Studies on multicellular preparations of the EBZ have shown that there is a significant loss in RP of EBZ fibers of the 5-day infarcted hearts.3,7 However, RP values ranged from −99 to −50 mV in these fibers, with a majority of cells (65%) having RPs between −80 and −99 mV.3 Other studies7,25 have described a loss in RP in some fibers in the EBZ, but a frequency distribution analysis of measured values was not done.

Thus, it is apparent that after the cells are dispersed from the infarcted heart and studied individually, the membrane depolarization observed in the multicellular preparation is no longer evident. This could be for one or several reasons. First, inherent in all single-cell studies is a selection process, and our study is no exception. It may be that after the cell dispersion process, only the cells with “normal” RPs survive. This is unlikely, because we might have expected at least some of the 62 cells from which we have recorded intracellular potentials to have shown some loss in RP. However, we did not. Even though the cells dispersed from the EBZ showed no significant change in RP compared with NZs, these myocytes showed other electrical abnormalities (see below).

We suggest that a fraction of the membrane depolarization observed in fibers in the EBZ multicellular preparation is secondary to other factors known to affect potentials of fibers in a multicellular preparation. One factor could be extracellular ion accumulation. In the isolated cell, the electrical activity of the cell is studied after it is removed from the synctium and superfused in an environment where ion accumulation and depletion are not significant problems. The most likely ion to accumulate in a large multicellular preparation is the K⁺ ion. If K⁺ accumulation is significant in multicellular EBZ preparations, then we would also expect [K⁺]i accumulation to affect the electrical activity of fiber in the multicellular noninfarcted control preparations. In fact, as mentioned above, mean RP and APA values of our NZ cells are somewhat higher than mean values reported for canine epicardial fibers studied under similar conditions.22,23

In spite of a normal mean RP of IZs, there was a significant decrease in Vmax and APA in IZs. This finding is in agreement with the studies of these fibers in the multicellular preparation. Ursell et al3 found that there was a significant decrease in Vmax (approximate decrease, 31%) in fibers in the EBZ compared with control. But in these studies, RP of fibers studied varied from −50 to −99 mV. Others have reported that fibers in the EBZ have a nearly normal RP but demonstrated a significant decrease in Vmax.29 At the time of these multicellular studies, it was impossible to determine whether this decrease was an intrinsic decrease in Vmax of the action potential of an individual myocyte or was secondary to the observed membrane depolarization of the fibers from the EBZ. We have now demonstrated that when individual cells are isolated and studied in vitro, there is a significant decrease in maximum upstroke velocity of phase 0 and APA in IZs that have RPs within a normal range.

By maximally hyperpolarizing IZs to potentials at which, in NZs, fully available Vmax values were attained, however, we were unable to “restore” Vmax. Furthermore, by indirectly assessing the availability of the Na⁺ channel for upstroke of an action potential, we found that voltage dependence of availability was different in IZs compared with NZs. We think it is unlikely that the shift in the IZ availability curve results from our recording techniques. Several studies using normal cardiac cells or fibers have determined availability curves using Vmax and have reported Vrs ranging from −55 to −103 mV.20,26–29 One Vmax study completed on dialyzed guinea pig myocytes at 37°C reported Vrs = −57 mV and k = 4.6 mV.30 Our studies were completed on nondialyzed cells. Under these conditions, we obtained availability curves for NZs where Vrs = −63.9 mV and k = 2.9 mV.

The shift in the mean IZ availability curve may be a result of the reduction in the fully available Vmax in IZ cells. Vmax studies of Cohen et al26 have shown that when extracellular Na⁺ was reduced (from 155 mM [Na]o to 35 mM [Na]o), there was a consequent shift in the availability curve. However, the magnitude of the shift seen with this decrease in driving force was only 5–6 mV. We measured an average shift of 10 mV (range, 10–15 mV). Therefore, part of the shift observed in IZs may be a result of the overall decrease in Vmax but a reduction in Vmax in IZs cannot account for all of the shift observed.

We have also demonstrated that there is a significant slowing in the recovery of Vmax from inactivation in IZs. This depression in the recovery from inactivation is somewhat similar to the effects described in a study by Kodama et al,20 in which ischemic conditions were mimicked by superfusing fibers with a high K⁺ and hypoxic solution. Under these conditions, the recovery kinetics of Vmax were slowed. In our study, acute “ischemic” conditions were not the cause of the marked slowing in recovery kinetics of Vmax in IZs, because myocytes were studied in normal Tyrode’s solution (4 mM [K+]o). Therefore, the changes observed were a result of a chronic change in the membrane current(s) underlying the recovery process of Vmax in the myocyte. In our study, the ability to elicit an action potential was a necessary condition to measure a partially or fully recovered Vmax. We did not measure iNa directly. Thus, it is possible that the delay in the onset of recovery of Vmax could result from several factors. First, the delay in IZ may have been because there was a reduction in available Na⁺ channels for the upstroke. Second, the cell
may have remained inexitable because, although the fast Na+ current had fully recovered from inactivation, the inability to elicit an action potential was a result of the existence of a large, slowly decaying outward current. It has been hypothesized that iK can alter excitability of a normal guinea pig myocyte. Either of these changes may help to explain the nonzero x-intercept observed in some IZ experiments. Nevertheless, once an IZ cell was able to elicit an action potential, Vmax of that action potential was reduced compared with control and remained far from its maximum value for a much longer time in IZs than in NZs.

Implications of Vmax Findings

A change in Vmax and APA of individual myocytes of the EBZ might be expected to alter the velocity of a conducting impulse in this region of the heart. Several investigators have measured the conduction velocity of an impulse in the noninfarcted normal dog epicardium. These values are much greater than average conduction velocities determined in the isolated multicellular EBZ preparation (25 cm/sec [Reference 3]; 0.01 mm/sec [Reference 7]) but somewhat equal to the average longitudinal conduction velocities measured from the EBZ in the in situ heart. Nevertheless, the changes in Vmax and APA observed in IZs certainly are not consistent with the very slow conduction velocities that have been reported for EBZ fibers. In conclusion, the significant reduction in Vmax and APA in IZs could account for a slight reduction in the conduction velocity of an impulse in EBZ, suggesting that other factors, for example, cell-to-cell coupling, play an important role in conduction of the impulse in the EBZ.

The recovery kinetics of Vmax are slowed in IZs compared with NZs. This finding may help to explain the reduction in conduction velocity of premature impulses elicited late in diastole in EBZ fibers. It is in contrast to what would be expected to occur with premature impulses in fibers within normal epicardium. In this latter case, premature impulses elicited late in diastole should conduct reasonably well, and only premature impulses closely coupled to the basic driven impulse should conduct slowly. The slow recovery of Vmax in IZs may also contribute to the diminished excitability of EBZ fibers after repolarization that has been noted by some.

At this time, we do not know the mechanism of the decrease in Vmax, the shift in the apparent availability curve, or the altered kinetics of recovery of Vmax in IZs. It may be that an altered intracellular milieu has contributed to our findings, because all Vmax studies were completed in nondialyzed myocytes. For instance, both hydrogen and calcium ions are known to accumulate within the myocyte during acute ischemic interventions. Recently, an increase in Ca2+ associated with acute ischemia has been implicated as a contributor to the membrane RP-independent depression of Vmax. The effects of altering pH on iK have been studied in nerve, but studies on the effects of intracellular pH or pCa on the cardiac sodium channel are limited. On the basis of recent studies investigating the effects of pH on sodium channel availability and its recovery kinetics, however, one might predict that because of a surface charge effect, both a decrease in pH, and an increase in pCa, would shift the availability curve in a hyperpolarizing direction and would delay the recovery of the sodium channel from inactivation. Alone or together, these changes could underlie our observed effects in IZs.

Repolarization Abnormalities

Our third major finding is that the time course of repolarization of IZ potentials differed from that of NZs. From our steady-state current–voltage curves, we observed that although the inwardly rectifying regions of the curves of the two cell types differ, the steady-state current densities at more depolarized potentials are the same. This suggests that large outward currents (e.g., the ATP-dependent K current) probably do not contribute to the triangularization of the IZ action potential.

One of the most striking differences observed was the absence of a significant phase 1 in IZ action potentials. We used the patch pipette technique in the whole-cell configuration to study the iK in myocytes dispersed from the noninfarcted and infarcted heart. In most IZs, no iK could be recorded; in the small percentage of cells that did show an iK, however, the current was linearly dependent on the step voltage, similar to NZs. In NZs, the loss in iK or the significant reduction in the density of iK in IZs correlates well with the disappearance of the rapid phase 1 of repolarization and the loss of the notch in potentials recorded from IZs before clamp protocols. The function of the small 4-AP-sensitive outward currents in the few IZs in which the current was recorded was determined, and slight but statistically significant differences were noted. The physiological significance of these latter findings is unclear at this time. It may be that the size of the iK remaining in some IZs is so small that it would have minimal impact on the time course of the action potential. Nevertheless, iK, when it occurred in IZs, still recovered from inactivation relatively quickly and also decayed reasonably fast during depolarizing steps, much like the large iK current in normal epicardial myocytes. Because this residual iK current appears to be only slightly altered compared with the normal iK current, it may be that the major effect of the 5-day period of ischemia is only to reduce the number of available iK channels.

It is possible that the loss in ion channel function in IZs results from our disaggregation procedure. We think this is unlikely for the following reasons. First, unlike the preparation of myocytes obtained from the midmyocardium of the left ventricle, all the cells from epicardial preparation of our noninfarcted hearts showed a large and prominent iK. This always coincided with a large notch in the transmembrane action potential of the cell. This is consistent with Litovsky and Antzelevitch’s observation that a rapid repolarization phase is obvious in potentials from all fibers in the canine epicardial preparation.

Second, even though loss of iK or a change in its function has not been defined in studies determining the effects of acute ischemia on cell electrophysiology, several laboratories have published illustrations of transmembrane potentials recorded from fibers in multicellular epicardial preparations that are consistent with ischemic or simulated ischemic conditions altering the rapid repolarization phase of potentials of cat and...
dog epicardial fibers. Presumably, these potential changes resulted from a change in the function of \( i_{\text{Na}} \).

The mechanism of the reduced function or loss of this ion channel in the infarcted heart is not known. Several laboratories, however, have suggested that \( i_{\text{Na}} \) of cardiac cells can be modified by agents that are known to promote channel phosphorylation. Apkon and Nebbons have shown that \( \alpha \)-adrenergic receptor stimulation can markedly reduce the amplitude of \( i_{\text{Na}} \) in myocytes. These latter effects could be mimicked by phospholipid esters, known activators of protein kinase C. Diacyl monoxide, an agent having "phosphatase-like" activity, clearly affects this current. Diacyl monoxide enhances the plateau height and APD of rat fibers and depresses the peak transient outward current. These studies suggest that phosphorylation of the cardiac channel responsible for \( i_{\text{Na}} \) can alter its amplitude. It may be that the mechanism of channel phosphorylation/dephosphorylation is altered in cells surviving in the infarcted heart.

In conclusion, the results of this study suggest that some of the long-term electrophysiologic abnormalities resulting from myocardial infarction are probably secondary to alterations in normal ion channel function. In particular, conduction velocity abnormalities in the EBZ may be related to the abnormalities in \( V_{\text{Na}} \) and its recovery. In addition, early repolarization abnormalities in action potentials of cells surviving on the epicardial surface of the 5-day infarcted heart are secondary to a reduced or absent \( i_{\text{Na}} \). The absence of \( i_{\text{Na}} \), however, cannot account for all the observed repolarization abnormalities of the Iz potentials. In fact, in normal canine endocardial fibers, no notch in the action potential is associated with a significant increase in APD and APD_{90} compared with potentials of epicardial fibers. This is not the case for IZs. We found that there was a significant shortening of APD of Iz at the APD_{90} level compared with NZs. These data suggest that the disease may have altered other membrane currents important to the repolarization process of the epicardial myocyte. One such current could be the L-type \( Ca^{2+} \) current. We have already completed experiments where we indirectly assessed the \( Ca^{2+} \) current in the EBZ fibers. Our findings suggested that the L-type \( Ca^{2+} \) current is altered in EBZ fibers. Studies are under way to further characterize these differences.

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