Remodeling in Cells From Different Regions of the Reentrant Circuit During Ventricular Tachycardia

Shigeo Baba, MD*; Wen Dun, PhD*; Candido Cabo, PhD; Penelope A. Boyden, PhD

Background—Anisotropic reentrant excitation occurs in the remodeled substrate of the epicardial border zone (EBZ) of the 5-day infarcted canine heart. Reentry is stabilized because of the formation of functional lines of block. We hypothesized that regional differences of ionic currents in cells of the EBZ form these lines of block. Therefore, we first mapped reentrant circuits of sustained tachycardias, then dispersed cells (infarct zone cells, IZs) from the central common pathway of the circuit (IZc) as well as from the other side of the line of block (outer pathway, IZO) for study.

Methods and Results—We mapped reentrant circuits in the EBZ of infarcted hearts during sustained ventricular tachycardias (>30 seconds, n = 17 episodes, cycle lengths = 218 ± 7.9 ms). I\textsubscript{Na} density was reduced in both IZc and IZO, and the kinetic properties of IZc I\textsubscript{Na} were markedly altered versus IZo. Structural remodeling of the sodium channel protein Na\textsubscript{1.5} occurred in IZs, with cell surface localization differing from normal cells. Both IZc and IZO have similar but reduced I\textsubscript{CaL}, whereas IZo showed changes in Ca\textsuperscript{2+} current kinetics with an acceleration of current decay. Computer simulations of the 2D EBZ showed that incorporating only differences between I\textsubscript{Na} in IZc and IZO prevented stability of the reentrant circuit. Incorporating only differences between I\textsubscript{CaL} in the IZc and IZO cells also prevented stability of the circuit. However, incorporating both I\textsubscript{Na} and I\textsubscript{CaL} current differences stabilized the simulated reentrant circuit, and lines of block formed between the 2 distinct regions.

Conclusions—Despite differences in I\textsubscript{Na} and I\textsubscript{CaL} properties in cells of the center and outer pathways of a reentrant circuit, the resulting changes in effective refractory periods tend to stabilize reentry in this remodeled substrate. (Circulation. 2005;112:2386-2396.)

Key Words: myocardial infarction ▪ ion channels ▪ reentry ▪ arrhythmia ▪ electrophysiology

Structural and electrical nonuniform anisotropy underlies reentrant excitation and ventricular tachycardias (VTs) in the epicardial border zone (EBZ) of 5-day infarcted canine heart.1,2 This substrate is highly remodeled compared with normal noninfarcted epicardium.3 For example, Na\textsuperscript{+}, Ca\textsuperscript{2+}, and K\textsuperscript{+} currents in cells isolated from the EBZ of 5-day infarcted heart (IZs) have altered current amplitudes as well as critical changes in kinetics.4–7 Furthermore, studies have shown a different pharmacological response of the center and outer pathway tissues, which again suggests differences in currents between cells of the center and outer pathways. Despite results from experiments in normal tissue and computer simulations8,9 that indicate that a nonuniform substrate causes drift and self-termination of reentrant waves, VTs induced in the EBZ are sustained (>-30 seconds).

To understand this paradox, we measured regional differences of ionic currents (Na\textsuperscript{+}, Ca\textsuperscript{2+} and K\textsuperscript{+}) in cells of the EBZ and studied how they contribute to the perpetuation of reentrant tachycardias. Accordingly, we first mapped reen-
We mapped reentrant circuits in the EBZ of 5-day infarcted hearts during sustained VTs (>30 seconds) (cycle lengths [CLs] = 218 ± 7.9 ms, n = 17 episodes in 17 animals) induced by programmed stimulation.11 After at least 2 sequential beats of the VT had been mapped and the location of the reentrant pathway determined (Figure 1), the location of the line of block that formed during the tachycardia was carefully marked on a transparent electrode grid. The size and location of the line of block that formed during the tachycardia was blocked and the location of the reentrant pathway determined (Figure 1). We completed 4 sets of simulations. In the first, to simulate reentry in a substrate with identical cell APs, we used our generic model of an IZc as nodes in both the center and outer regions. In the second, all ionic currents except \( I_{Na} \) as well as changes in ionic concentrations and intracellular calcium handling were formulated as reported in the model of an IN.14 In the third set, all ionic currents except \( I_{Na} \) and \( I_{Ca} \) were formulated as reported for IZc.14 In the fourth set, we combined regional changes in \( I_{Na} \) and \( I_{Ca} \) and \( K^{+} \) currents to formulate complete cell models of IZc and IZh.

**Immunocytochemistry**

Some cells dispersed for the electrical studies described above were plated on laminin-coated glass chamber slides and then fixed with 4% paraformaldehyde for 30 minutes, rinsed with PBS (Sigma), blocked in 2% avidin-PBS and rinsed in PBS, then blocked in 2% biotin-PBS and rinsed in PBS. Cells were then stained for Na+/K+-ATPase (Sigma, St. Louis, Missouri).

**Statistics**

All values are represented as mean ± SEM. n is the number of cells, and N is the number of animals. A value of \( P < 0.05 \) was considered significant. For multiple comparisons (e.g., Table 1), ANOVA was used to determine whether sample mean values between groups were significantly different. If so, a modified t-test with Bonferroni correction was used (Sigmastat, Jandel Scientific). Fisher’s exact test was used (Sigmastat, Jandel Scientific).
significant negative shift (Table 1). Thus, a negative shift in both activation and decay and times to peak of Na were markedly altered.

**Immunocytochemistry: Na<sub>1.5</sub> in Canine Epicardial Cells**

Previous reports in mouse ventricular cells from normal hearts have suggested that the cardiac Na<sup>+</sup> channel isoform, Na<sub>1.5</sub>, localizes primarily to gap junction regions. Because other proteins also localize to this area in normal canine epicardial cells, we hypothesized that like Cx43 and K<sub>1.5</sub> proteins, the cardiac Na<sup>+</sup> channel protein may redistribute around the cell membrane (lateralize) in IZs. Thus, we completed a series of immunostaining experiments in IZc and IZo and compared their Na<sub>1.5</sub> staining pattern with that of NZs. We found that Na<sub>1.5</sub> staining in NZs was uniform, with staining all along the sarcolemma (SL) in all optical sections as well as in the gap junction region. There was no staining of T-tubular membranes. SL staining of an NZ showed a specific pattern, with staining concentrated in a small region of the SL membrane rupture (NZs, 22.3 ± 1.0 minutes). In sum, although I<sub>Na</sub> density was reduced in both IZc and IZo, the kinetic properties of IZc I<sub>Na</sub> were markedly altered.

**Results**

**Sodium Currents**

Compared with NZs, average peak I<sub>Na</sub> densities of IZc and IZo were significantly smaller, but IZo peak I<sub>Na</sub> did not differ from that of IZc (V<sub>0.5</sub> = −100 mV) (Figure 2A and B). Average current density-voltage relations of IZc and IZo also differed from that of NZs (Figure 2C). Current tracings of I<sub>Na</sub> in a NZ, IZc, and IZo are shown in Figure 2B. Note that in the IZo, the time constants of current decay and times to peak of I<sub>Na</sub> were greater than those of IZc (P<0.05) (Table 1). I<sub>Na</sub> V<sub>0.5</sub> curves of IZc showed a significant negative shift (∼8 mV) compared with that of NZs (Table 2). IZo V<sub>0.5</sub> values did not differ from the NZ value. The average activation curve of IZc also showed a significant negative shift (∼5 mV) compared with that of NZs (P<0.05). This differed significantly from IZo (Table 2). Thus, a negative shift in both activation and I<sub>Na</sub> V<sub>0.5</sub> curves was observed in IZc. I<sub>Na</sub> recovery from inactivation in IZc and IZo was slowed compared with that of NZs; however, values in IZc and IZo did not differ (Figure I, Data Supplement). Finally, clamp protocols designed to test closed-state inactivation were completed as before<sup>16</sup> and showed that the time course of closed-state inactivation was markedly accelerated in IZc (Table 3). In sum, although I<sub>Na</sub> density was reduced in both IZc and IZo, the kinetic properties of IZc I<sub>Na</sub> were markedly altered.

**Table 1. Kinetics of I<sub>Na</sub> Currents in IZc and IZo**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IZc</th>
<th>IZo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time constant of decay, ms</td>
<td>IZc</td>
<td>IZo</td>
</tr>
<tr>
<td></td>
<td>6.12±0.61</td>
<td>6.36±0.67</td>
</tr>
<tr>
<td></td>
<td>2.96±0.19</td>
<td>3.88±0.29*</td>
</tr>
<tr>
<td>Time to peak, ms</td>
<td>IZc</td>
<td>IZo</td>
</tr>
<tr>
<td></td>
<td>3.45±0.22</td>
<td>4.30±0.33*</td>
</tr>
<tr>
<td></td>
<td>2.54±0.11</td>
<td>3.35±0.22*</td>
</tr>
</tbody>
</table>

A monoexponential function was used to fit current decay at all V<sub>t</sub> (test voltage) in cells of both groups.

*P<0.05 vs IZc.

A 15-pF capacitance was used to determine the change in number of cells with specific location of Na<sup>+</sup> channel antibody (see Figure 3B below).
contrast, most cells from the EBZ (62% and 68% IZc and IZo, respectively) showed Na\textsubscript{v1.5} gap staining with variable, nonuniform SL staining (Figure 3, A and B) (P/H11021 0.05 each IZ group versus NZ). However, there was no difference in the percentage of cells with this abnormal staining pattern in IZc versus IZo.

Thus, although there were electrophysiological differences between IZc and IZo, both cell types showed abnormal cell surface staining for the Na\textsubscript{v1.5}, \(\alpha\)-subunit of the cardiac Na\textsuperscript{+} channel. This is consistent with the observed reduction in Na\textsuperscript{+} current density.

**Ca\textsuperscript{2+} Currents**
Peak L-type Ca\textsuperscript{2+} currents in IZc and IZo were reduced, similar to each other, but differed from NZs (Figure 4). Reduced Ca\textsuperscript{2+} currents in IZo and IZc occurred at most voltages (Figure 4B). However, the decays of the small peak IZc currents were faster than those in IZo and NZs (Figure 3B).

### Table 2. Steady-State Inactivation and Activation of I\textsubscript{Na} in IZc and IZo

<table>
<thead>
<tr>
<th></th>
<th>(V_{0.5}) (mV)</th>
<th>(k) (mV)</th>
<th>(E_{max}) (mV)</th>
<th>Max I\textsubscript{Na} (pA/pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Activation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZs</td>
<td>26.6±0.7</td>
<td>7.2±0.1</td>
<td>6.0±0.8</td>
<td></td>
</tr>
<tr>
<td>IZc</td>
<td>31.4±1.4*</td>
<td>7.7±0.3</td>
<td>5.8±1.5</td>
<td></td>
</tr>
<tr>
<td>IZo</td>
<td>25.4±2.2†</td>
<td>7.4±0.3</td>
<td>6.0±1.8</td>
<td></td>
</tr>
<tr>
<td><strong>Inactivation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZs</td>
<td>-73.8±0.8</td>
<td>-5.3±0.1</td>
<td>-11.0±0.8</td>
<td></td>
</tr>
<tr>
<td>IZc</td>
<td>-81.5±1.7*</td>
<td>-5.6±0.1*</td>
<td>-6.5±1.4*</td>
<td></td>
</tr>
<tr>
<td>IZo</td>
<td>-77.4±2.3</td>
<td>-5.8±0.1*</td>
<td>-4.3±1.1*</td>
<td></td>
</tr>
</tbody>
</table>

Parameters of fit derived from Boltzmann fits. \*P<0.05 vs NZs; †P<0.05 vs IZc.

### Table 3. Closed-State Inactivation

<table>
<thead>
<tr>
<th></th>
<th>(\tau_1) (ms)</th>
<th>(\tau_2) (ms)</th>
<th>(A_1) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZs (n=23)</td>
<td>70±1.3</td>
<td>273±30</td>
<td>45±4</td>
</tr>
<tr>
<td>IZc (n=8)</td>
<td>46±1.9*</td>
<td>189±24*</td>
<td>55±4</td>
</tr>
<tr>
<td>IZo (n=7)</td>
<td>66±4.5†</td>
<td>289±70†</td>
<td>55±5</td>
</tr>
</tbody>
</table>

\(\tau_1\) and \(\tau_2\) derived from fits of peak current changes with time. See online supplement for closed-state protocol. \*P<0.05 vs NZs, †P<0.05 vs IZc.

Thus, although there were electrophysiological differences between IZc and IZo, both cell types showed abnormal cell surface staining for the Na\textsubscript{v1.5}, \(\alpha\)-subunit of the cardiac Na\textsuperscript{+} channel. This is consistent with the observed reduction in Na\textsuperscript{+} current density.
This is interesting, considering the well-known current dependence of Ca\(^{2+}\) channel inactivation. Activation curves were similar in all 3 cell types. However, there were differences in steady-state availability curves between IZc and IZo, with there being a significant negative shift in IZc versus NZs (Table 4). Although there is a significant slowing in recovery in both IZc and IZo versus NZs, there is no difference between the 2 IZ cell groups (Table 5).

In a subset of cells from each group, we tested whether we could augment peak Ca\(^{2+}\) currents with \(\beta\)-adrenergic stimu-
loration as described by Aggarwal and Boyden. Figure II (Data Supplement) shows that compared with NZs, in which isoproterenol 1 \(\mu\)mol/L increases peak current \(2.72 \pm 0.05\)-fold (n = 10), it had a similar effect in IZc (2.13 \(\pm\) 0.2-fold, n = 10) and a diminished effect in IZo (1.79 \(\pm\) 0.13-fold) (n = 14) (P \(<\) 0.05 versus NZ).

**K**+ **Currents**

As reported previously, most IZ cells have no transient outward current that can be elicited with depolarizing clamp steps. In both IZc and IZo, this is also true. Only 5 of 11 IZc and 2 of 11 IZo cells had a measurable \(I_{\text{Na}}\) in this study (Figure III, Data Supplement). However, the densities of these transient currents in both cell groups did not differ from each other and were small compared with NZs (20 pA/pF at 40 mV). Furthermore, there were no differences in \(I_{\text{Ks}}\) or Cs-sensitive currents \(I_{\text{K1}}\) in cells of the 2 IZ groups.

In sum, IZc and IZo have reduced \(I_{\text{CaL}}\). However, there was no difference in the peak \(I_{\text{CaL}}\) in IZc and IZo. IZc cells showed changes in Ca\(^{2+}\) current kinetics: an acceleration of current decay, a shift in availability, and a slowing in recovery from inactivation. The small \(I_{\text{CaL}}\) of IZc responded normally to \(-\alpha\)-adrenergic stimulation, whereas IZo \(I_{\text{CaL}}\) did not. There are similar changes in K currents in both IZc and IZo.
Computer Simulations

Reentry in a Uniform Model of the EBZ
To simulate reentry in a substrate with identically remodeled cells (same APs), we used our generic IZ cell model\textsuperscript{14} for both the center and outer regions. Reentry was stable for the duration of the simulation (5 seconds), with a CL=$100\text{ ms}$ (Movie I, Data Supplement). Although it is not unexpected to obtain stable reentry in such a medium, experimental results reported above show that there are significant differences in $I_{\text{Na}}$ and $I_{\text{Ca,L}}$ between IZc and IZo. Therefore, to understand the stability of reentrant circuits in the EBZ, we need to understand the conditions under which EBZ circuits are stable in this remodeled substrate.

Effect of $I_{\text{Na}}$ Remodeling on Reentry
Therefore, we adapted our generic IZ cell AP model\textsuperscript{14} to generate a cell model of an IZc and an IZo by incorporating the $I_{\text{Na}}$ data (for changes in rate constants/equations used, see Data Supplement). Figure 5 shows the calculated $I_{\text{Na}}$ density-voltage curves, activation and inactivation curves, and simulated APs of the IZc (solid line) and IZo (dotted line). Except for a small reduction in AP plateau, there were no differences in APD$\text{90}$ (Figure 5D). Notably, there were differences in the maximum depolarization rate and refractory period as measured in a 1D cable using a S1-S2 protocol.\textsuperscript{14} As a result, conduction velocity was decreased by $\approx10\%$ in the IZc cable. Both cell types exhibited postrepolarization refractoriness, with IZc exhibiting a longer refractory period ($215\text{ ms}$) compared with that of IZo ($198\text{ ms}$).

To understand whether differences only between $I_{\text{Na}}$ in the IZc and IZo cells promote or prevent stability of the reentrant circuit, we performed the computer simulations illustrated in Figure 6A. Note that the reentrant wave was not stationary terminating after 6 rotations ($\approx1400\text{ ms}$) (Movie II, Data Supplement). In sum, although the tip trajectory and the lines of functional block during the 6 beats stayed within 5 mm of the boundary of the central and outer regions (Figure 6A,
right), differences in refractoriness between cells of the central and outer regions produced by the changes in \( I_{\text{Na}} \) in these cells caused the spiral to drift into the IZc region. Eventually, it is extinguished by colliding with the top boundary of the preparation and refractory tissue of the IZo cells.

**Effect of \( I_{\text{CaL}} \) Remodeling on Reentry**

To understand how the kinetic differences in \( I_{\text{CaL}} \) seen in IZc and IZo would affect the AP, we incorporated the averaged data into our cell model. While these simulations showed differences in APs (the ERP was 188 ms in the IZc and 224 ms in the IZo) between IZc and IZo, only a nonsustained tachycardia could be induced in the 2D array (Movie III, Data Supplement). The large difference in ERP (36 ms) caused a drift in the reentrant circuit along the boundary.\(^8,9\) Thus, differences only in \( I_{\text{CaL}} \) in the different regions of the reentrant circuit did not promote a stable line of functional block.

**Effects of Remodeling of Both \( I_{\text{Na}} \) and \( I_{\text{CaL}} \) on Reentry**

We next produced cell models of IZc and IZo that incorporated both \( I_{\text{Na}} \) and \( I_{\text{CaL}} \) data. Results from our simulations are shown in Figure 5, E and F. The combination of the regional differences of both inward currents produced an IZo AP (and ERP) that was longer than IZc AP, but the ERP difference (13 ms) was smaller than when \( I_{\text{Na}} \) (17 ms) or \( I_{\text{CaL}} \) (36 ms) differences were considered alone. This is because remodeling in \( I_{\text{Na}} \) and \( I_{\text{CaL}} \) affects the ERP in the central and outer pathway in opposite directions: remodeling of \( I_{\text{Na}} \) makes the ERP longer in IZc than in the IZo; remodeling of \( I_{\text{CaL}} \) makes the ERP longer in IZo than in IZc. Overall, when the remodeling of both currents is taken into account, differences in ERP decrease. Notably, although ERP was longer in IZo, velocity was increased over IZc (Figure 5F).

To understand whether together, \( I_{\text{Na}} \) and \( I_{\text{CaL}} \) differences in IZc and IZo promote or prevent stability of the reentrant circuit, we performed the simulations illustrated in Figure 6, C and D. The circulating wave was stationary, forming a line of functional block between the 2 regions (Figure 6C) for 9 rotations, after which it terminated (Movie IV, Data Supplement). Figure 6C shows isochronal maps of the last 5 beats of the reentrant wave. During beats 5, 6, and 7, the reentrant circuit is stable at the boundary between IZc and IZo (Figure 6C). During beats 5 and 6, sites g–a (Figure 6C) activate transversely without delay. However, the wave of beat 7 cannot propagate from f to e because of postrepolarization refractoriness of site e (Figure 6D, asterisk, small hump in e). Site e is eventually activated after the wave turns around a longer line of functional block (Figure 6C). The premature activation of IZc cells during beat 7 causes a decrease in APD. Figure 7 illustrates the dynamics of \( I_{\text{Na}} \) (Figure 7B) and \( I_{\text{CaL}} \) (Figure 7C) during reentry at 2 sites, one in the central pathway (solid line) and another in the outer pathway (dashed line) and shows that the decrease in APD in the central pathway is the result of a reduced \( I_{\text{CaL}} \) (Figure 7C) that results from the acceleration in current decay and its delay in recovery (Table 2). Because IZc APD is shortened, the reentering wave in beat 8 propagates readily across the boundary between the central and outer pathway, causing a further reduction in IZc APD (note the decrease in APD during beats 6 to 7–8 at sites b, c, and d in Figure 6B), thus allowing the reentering wave to rotate inside the center region.

Figure 8 summarizes our findings. Incorporating only differences between \( I_{\text{Na}} \) in the central and outer pathway cells prevented stability of the reentrant circuit. Incorporating only differences between \( I_{\text{CaL}} \) in the central and outer pathway cells also prevented stability of the reentrant circuit. However,
incorporating both $I_{\text{Na}}$ and $I_{\text{Ca,L}}$, current differences in the central and outer pathway cells stabilized the circuit, and lines of block formed between the 2 distinct regions.

**Discussion**

Location of the central isthmus of a reentrant circuit or the area of slowed conduction of VTs has been used as an approach for ablation of these tachycardias. Although this has been useful, sometimes the lines of block and therefore the isthmus are difficult to locate, and the reentry is nonsustained or not inducible. Thus, we have approached this problem by determining the ionic determinants of the lines of functional block that form during a reentrant tachycardia. For these studies, we used the well-studied VTs that occur in the canine epicardium 5 days after coronary occlusion. In our studies, we mapped reentrant circuits with CLs similar to those previously described in this model. Our maps showed that lines of block formed between the center and outer regions. Using tissue slices obtained at each side of the line of block, we describe here the differences in ionic current function in the cells from these 2 regions.

Similar to our previous findings using somewhat larger tissue slices from the EBZ (and for which tachycardias had not been mapped), we found that IZc and IZo cells have a reduced $I_{\text{Na}}$ density. However, we found that there are significant differences between the kinetic parameters of $I_{\text{Na}}$ in IZc versus IZo. IZc $I_{\text{Na}}$ tended to enter inactivation from a closed state more rapidly, displaying shifts in both availability and activation curves. These differences are not a result of differences in experimental techniques, because we sampled both IZc and IZo, controlling for time after membrane rupture (see figure legends).

The mechanism of these differences is unknown at this time. With the immunocytochemistry experiments presented here, we show that there is a loss of the protein of the $\alpha$-subunit Na,1.5 in cells from the EBZ (Figure 3). Thus, there is marked structural remodeling of this protein in IZs. However, quite different from the structural remodeling of other proteins in cells of the EBZ, we find that there is a loss of Na,1.5 protein at the cell surface in both IZc and IZo, consistent with reduced peak $I_{\text{Na}}$. This staining differs from the staining pattern we observed in NZs, which showed significant SL, non–T-tubular staining of Na,1.5 protein with some gap junctional staining and from the Na,1.5 pattern seen in mouse ventricle by our laboratory (data not shown) and by others. Our findings in normal canine ventricle cells are consistent with the findings of several patch-clamp reports in which investigators have studied single Na channels in cardiac cells (see, eg, Berman et al). In none of these reports was it stated that single sodium channel records could be obtained only if the investigator clamped regions of the gap junction. In both IZc and IZo, we found that surface staining for Na,1.5 was nonuniform, non–T-tubular, and gap junctional. In fact, in some IZs, gap junctional staining was the only staining observed. The change in the location of Na,1.5 proteins may have little effect on the cell electrophysiology but may affect propagation.

**Ionic Current Heterogeneity and Stability of Reentrant Circuits in the EBZ: Pharmacological Implications**

One of the main results of our study is that despite differences in $I_{\text{Na}}$ and $I_{\text{Ca,L}}$ channel properties in the IZc and IZo, the resulting change in refractory period tends to stabilize reentry in the EBZ. An important consequence of this is that the stability of the reentrant circuit is not a result of the remodeling of a single current (ie, $I_{\text{Na}}$ or $I_{\text{Ca,L}}$) but rather is determined by how any combination of remodeled currents affects the refractory period. This insight has important pharmacological implications. We suggest that one therapeutic strategy for converting a sustained VT to a nonsustained VT would be to make the refractory periods of the central and outer pathway as different as possible. Such a difference in ERPs would cause the reentrant wave to drift and eventually stop. For example, if we chose to use an $I_{\text{Ca,L}}$ agonist to decrease the differences in $I_{\text{Ca,L}}$ between IZc and IZo, we might predict that the circuit would become unstable and terminate, because regional differences in $I_{\text{Na}}$ alone show unstable circuits (Figure 8). In fact, this has been reported for this model. Conversely, if we chose to normalize $I_{\text{Na}}$ differences between IZc and IZo (eg, by using a drug that would relieve closed-state inactivation of IZc cells), we might expect the circuit to be unstable and tachycardias to terminate. This idea is not in conflict with the idea that pharmacological manipulation of anisotropy could lead to destabilization of reentry in this model (but this was not tested here). What we show here is that manipulation of anisotropy is not the only way to destabilize reentry. From our computer simulations, we suggest that the possibility of destabilizing anisotropic reentry can be done by creating gradients of refractory period in the highly remodeled center/outer regions. All that this requires is sufficient quantitative knowledge of the ion channels that are critical (as opposed to bystanders) in the remodeling process.

**Time Course of Remodeling and Inducibility of VT**

Our results here may also explain why in this experimental model, in which there is a time course in the inducibility of the VT, a sustained VT is induced in some animals but not in others. The remodeling of ion channels is a dynamic process, as we know from both ion channel studies on cells from acute and more healed infarcts and from this study, in which the dynamics of the ion channel changes in different regions of a circuit affect the stability of the circuit. Each ionic current contributing to the AP waveform has its own time course of remodeling and reversal. Therefore, if the time course of remodeling in cells in each region for each current were known, we should be able to predict the occurrence of sustained VT in the substrate in question. Sustained VTs would occur only when the sum of all ionic current remodeling results in center/outer regions with similar ERPs. In fact, if the time course of remodeling of one inward current
were altered, we predict that we would never have a substrate that would sustain a reentrant wave.

**Ion Channel Remodeling, Gap Junction Remodeling, and Stability of Reentrant Circuits**

In this model of reentry, called anisotropic reentry, lines of block form in regions of slow transverse propagation associated with the nonuniform anisotropic nature of this substrate. Peters et al. suggested a relationship between the location of the stable lines of block and the microanatomy of the EBZ as well as the distribution of abnormal connexin43 staining in EBZ cells. A limitation of our in silico representation of the EBZ is that it is one cell deep and has no structural components (macro or micro). It is clear from several of our previous studies, as well as those of others, that the electrical remodeling occurring in the 5-day EBZ is not limited to SL ionic channels. Gap junction distribution in the central pathway of reentrant circuits and gap junction conductance between side-to-side coupled cell pairs (direction transverse to the fiber orientation) are also remodeled. Although the functional effects of gap junction remodeling on anisotropy are still unknown (and currently under investigation), it is possible that changes in cell coupling and anisotropy contribute to further stabilization of reentry in the EBZ. This is supported by the fact that even though the incorporation of the remodeling of $I_{Na}$ and $I_{CaL}$ in the models of IZo and IZc tends to stabilize reentry, the simulated reentry is not as sustained as it is in the experimental model. Again, we do not believe that the findings we report here invalidate the concept that anisotropy is important for stabilizing reentry. Rather, we contend that any mechanism of reentry in the EBZ should take into account all new findings, such as those presented here, where cells of the center and outer pathways show differences in ionic current function.

**Limitations**

We have shown that when we incorporate in a computer model ionic currents measured in IZc and IZo, reentrant circuits tend to stabilize. However, in the in situ infarcted hearts from which the IZc and IZo cells were harvested, reentrant tachycardias were stable for more than 30 seconds. Thus, it is possible that the remodeling of SL currents alone is insufficient for stabilization of reentrant circuits for longer periods. Other factors like gap junction remodeling, anchoring to anatomic obstacles, or the presence of areas of discontinuities may contribute to the stability of reentrant circuits.

**Acknowledgment**

This study was supported by National Institutes of Health grants HL-66140 and HL-30557.

**References**


**CLINICAL PERSPECTIVE**

Ventricular tachycardia caused by stable reentry in the absence of an anatomic obstacle can be induced in 5-day-old infarcts in the canine heart. Extensive structural remodeling with altered cellular coupling exaggerates directional differences in conduction (anisotropy) and promotes formation of lines of functional block that stabilize reentry, known as anisotropic reentry. We hypothesized that regional differences of ionic currents in cells of the epicardial border zone may also be important. After the circuits are mapped, cells from different parts of reentry circuits were removed and dispersed for study. Voltage-clamp techniques demonstrated regional differences in sodium and calcium currents. Computer simulations of the epicardial border zone were created. Simulations incorporating the sodium current or calcium current changes individually did not stabilize reentry. However, incorporating both sodium and calcium current changes stabilized reentry, with formation of lines of block. These findings have potential implications for pharmacological treatment of postinfarction ventricular tachycardia.
Remodeling in Cells From Different Regions of the Reentrant Circuit During Ventricular Tachycardia
Shigeo Baba, Wen Dun, Candido Cabo and Penelope A. Boyden

_Circulation._ 2005;112:2386-2396; originally published online October 3, 2005;
doi: 10.1161/CIRCULATIONAHA.105.534784

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/112/16/2386

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2005/09/19/CIRCULATIONAHA.105.534784.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/