**Lidocaine Action on Na\(^+\) Currents in Ventricular Myocytes From the Epicardial Border Zone of the Infarcted Heart**

Jielin Pu, Jeffrey R. Balser, Penelope A. Boyden

**Abstract**—Myocytes overlying a zone of infarction form the primary substrate for serious reentrant ventricular arrhythmias. In vitro and in vivo studies suggest that antiarrhythmic agents affect Na\(^+\) channels of cells from the epicardial border zone (EBZ) of the 5-day infarcted heart differently than they affect those of normal muscle. However, the mechanisms responsible for this difference remain unclear. Previous studies have revealed differences in Na\(^+\) current (\(I_{\text{Na}}\)) density and inactivation gating kinetics in myocytes dispersed from the EBZ (IZs). Since changes in inactivation gating could influence lidocaine action, we examined the effects of lidocaine on \(I_{\text{Na}}\) of IZs (\(n=38\)) and epicardial myocytes from the noninfarcted heart (NZs) (\(n=50\)) using the whole-cell variation of the patch-clamp technique. In drug-free conditions, the voltage dependence of steady-state inactivation of IZs was shifted negative to that of NZs, causing greater inactivation of IZ channels at depolarized (\(\geq-100\text{-mV}\)) holding potentials. Consistent with a high affinity for the inactivated channel conformation, lidocaine produced more tonic block in IZs than NZs at depolarized holding potentials. Additionally, in drug-free conditions, IZ \(I_{\text{Na}}\) exhibited an enhanced rate of inactivation from closed states, a delay in recovery from inactivation, and increased use-dependent reduction in amplitude during rapid (1- to 3-Hz) pulse trains. In both IZs and NZs, lidocaine (20 to 120 \(\mu\text{mol/L}\)) accelerated the rate of time-dependent loss of availability and markedly delayed recovery from availability, inducing significant use-dependent reduction of \(I_{\text{Na}}\). However, at drug concentrations \(\geq60\text{ \(\mu\text{mol/L}\)}\), the difference in use-dependent current reduction between IZs and NZs was minimized. The action of lidocaine to render Na\(^+\) channel inactivation in NZs more similar to that of IZs may be central to its (pro)antiarrhythmic effects. (*Circ Res*. 1998;83:431-440.)

**Key Words:** Na\(^+\) current • ion channel • ventricular myocyte • myocardial infarction • epicardial border zone • lidocaine

The antiarrhythmic effect of lidocaine in cardiac tissue derives from use-dependent suppression of excitability through block of voltage-gated Na\(^+\) channels. Although detailed electrophysiological studies have evaluated the mechanism of lidocaine action in healthy cardiac tissue, we sought to evaluate cells of the epicardial border zone (EBZ) of the infarcted heart, which have been reported to contribute to the formation of the substrate for serious ventricular arrhythmias.\(^1\)\(^-\)\(^3\) Abnormal electrical properties of fibers of the EBZ of the 5-day infarcted heart result partly from alterations of voltage-gated ion channels.\(^4\)\(^-\)\(^6\) \(V_{\text{max}}\) values are reduced in fibers of the multicellular EBZ preparation of the 5-day infarcted heart,\(^3\)\(^4\)\(^,\)\(^7\) suggesting that Na\(^+\) current (\(I_{\text{Na}}\)) is reduced in cells that survive in the EBZ of the healing infarcted heart (IZs). Nonetheless, infarct-induced changes in channel number or density should impose no functional change in antiarrhythmic drug action. Conversely, infarct-associated changes in Na\(^+\) channel gating may profoundly modify the use-dependent action of antiarrhythmic agents. In addition to a reduction in \(V_{\text{max}}\), a delay in recovery of \(V_{\text{max}}\) and a shift in the steady-state availability of \(V_{\text{max}}\) have been described for action potentials of IZs.\(^4\)\(^,\)\(^7\) We have reported that these changes in \(V_{\text{max}}\) are due to abnormalities in Na\(^+\) channel gating in myocytes from the EBZ.\(^6\)

Evidence from both in vitro and in vivo studies suggests that antiarrhythmic agents affect Na\(^+\) channels from the EBZ differently than they affect those from normal muscle. For instance, in noninfarcted epicardial fibers, lidocaine superfusion reduced \(V_{\text{max}}\) and increased action potential duration with little change in refractoriness\(^8\)\(^,\)\(^9\) but depressed conduction and prolonged refractoriness in EBZ fibers in vitro\(^9\)\(^,\)\(^10\) as well as in the intact dog heart after coronary occlusion.\(^11\) Studies by Coromilas et al\(^12\) and others\(^11\)\(^,\)\(^13\)\(^,\)\(^14\) have emphasized the limited efficacy of class I drugs such as lidocaine and flecainide on the ventricular tachycardias occurring in the EBZ 3 to 5 days after infarction. In fact, flecainide increased the likelihood that premature electrical impulses were blocked in the EBZ, suggesting that the proarrhythmic effect of flecainide may be due to an exceptionally prolonged drug-induced delay in conduction in fibers of the EBZ. Similarly, a prominent rate-dependent slowing of conduction observed in EBZ fibers facilitated reentry in the presence of lidocaine.\(^14\)

Received October 7, 1997; accepted June 2, 1998.

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In recent work, we showed that the inactivation gating properties of Na⁺ channels in myocytes derived from the EBZ are modified.\textsuperscript{6} I_{Na} from these myocytes inactivates at more hyperpolarized membrane potentials (negative voltage shift in steady-state inactivation) and also exhibits a prominent delay in recovery from inactivation. Recent studies with both site-directed mutations\textsuperscript{15-17} and subsidiary (β₂) subunit coexpression\textsuperscript{18,19} have shown that interventions that alter Na⁺ channel inactivation gating may profoundly influence the action of Na⁺ channel–blocking antiarrhythmic drugs. It is unclear whether and how these interventions modify lidocaine action. In theory, structural interventions that modify gating may, in turn, alter lidocaine-induced use dependence by changing the effect of the local anesthetic on inactivation gating kinetics.\textsuperscript{20,21} We examined whether Na⁺ gating may, in turn, alter lidocaine-induced use dependence.

**Experimental Conditions**

For study, an aliquot of cells was transferred onto a polylysine-coated glass coverslip placed at the bottom of a 0.5-mL tissue chamber, which had been mounted on the stage of a Nikon inverted microscope (Nikon Diaphot). Myocytes were continuously superfused (2 to 3 mL/min) with normal Tyrode’s solution containing (mmol/L) NaCl 137, NaHCO₃ 24, NaH₂PO₄ 1.8, MgCl₂ 0.5, CaCl₂ 2.0, KCl 4.0, and dextrose 5.5 (pH 7.4). The solution was bubbled with 5% CO₂/95% O₂. Temperature was continuously monitored and maintained at 19.0±0.5°C for voltage control. Patch pipettes were made from borosilicate thin wall glass (Sutter Instrument Co; outer diameter, 1.5 mm; inner diameter, 1.10 mm) using a Flaming/ Brown-type horizontal puller (model P-87, Sutter Instruments Co). Each pipette tip was pulled with a microforge (type MF-83, Narishige, Scientific Instrument Laboratory) just before use. Pipette resistances ranged between 0.6 and 0.9 MΩ when filled with an internal solution that had the following composition (mmol/L): CsOH 125, aspartic acid 125, tetraethylammonium chloride 20, HEPES 10, Mg-ATP 5, EGTA 10, and phosphocreatine 3.6 (pH 7.3 with CsOH). After the formation of the gigaohm seal, the stray capacitance was electronically nullled. The cell membrane under the pipette tip was then ruptured by a brief increase in suction, forming the whole-cell recording configuration. A period of 7 minutes was then allowed for intracellular dialysis to begin before switching to the low Na⁺ extracellular solution (mmol/L): NaCl 5, MgCl₂ 1.2, CaCl₂ 1.8, CsCl 5, tetraethylammonium chloride 125, HEPES 20, glucose 1.1, 4-aminopyridine 3, and MgCl₂ 2 (pH 7.3 with CsOH), designed for proper I_{Na}, measurements. Mn⁺ is known to affect I_{Na},\textsuperscript{24} and preliminary experiments showed that the Mn⁺ effect on I_{Na} in IZs was similar to its effect in NZs.\textsuperscript{6} With this combination of external and internal solutions, I_{Na} would be of manageable size and isolated from other possible contaminating currents.

**Materials and Methods**

**Cell Preparation**

Adult mongrel male dogs (12 to 18 kg, 1 to 2 years old) were used in these studies. Myocardial infarction was produced according to the Harris procedure.\textsuperscript{25} Under isoflurane anesthesia and sterile conditions, the left anterior descending coronary artery was isolated and completely occluded in 2 stages. Dogs were treated with lidocaine (2 mg/kg IV) if multiple ventricular beats occurred at the time of the surgical procedure. After 5 to 6 days, a cardiacotomy was performed with the dogs under sodium pentobarbital (30 mg/kg IV) anesthesia. The infarcted region of the heart was identified on gross examination as a pale white mottled area on the epicardial surface. A 432

**Voltage-Clamp and Recording Techniques**

Whole-cell I_{Na,\textsuperscript{6}} were recorded using the whole-cell patch-clamp technique. Voltage-clamp experiments were performed with an Axopatch 200A clamp amplifier (head stage, CV 201A; gain, B=1; Axon Instruments). Clamp protocols were generated with a 16-bit digital/analog converter (Digidata 1200, Axon Instruments) controlled by PCLAMP software and a Gateway 2000 computer. The currents were filtered at 10 kHz, digitized at the sampling interval 0.1 ms for whole-cell currents and 0.02 ms for capacitive transients, and stored on the computer for later analysis. The membrane capacity (in pF) of each cell was measured in the Cs⁺-rich solution by integrating the area under a capacitative transient induced by a 10-mV hyperpolarizing clamp step (from −80 to −90 mV) and dividing this area by the voltage step. Current amplitude data of each cell was then normalized to its cell capacitance (current density [pA/pF]). Averaged cell capacitances were 130±4.5 pF in NZs (n=50) and 171±8.3 pF in IZs (n=38) (P<0.05). The average time constant of decay of the capacitive transient was 0.15±0.01 ms in NZs and 0.16±0.01 ms in IZs (P>0.05). Therefore, the residual series resistance for each cell was calculated to be 1.17±0.04 MΩ in NZs and 1.0±0.04 MΩ in IZs. Thus, average steady-state voltage error resulting from series resistance was 2.1±0.11 mV for NZs and 0.9±0.08 mV for IZs.

For consideration of the voltage control, we lowered the extracellular Na⁺ concentration to 5 mmol/L, maintained the temperature at 19±0.5°C, and used patch pipettes only with resistances <1 MΩ. Furthermore, we did not choose large cells. If experiments demonstrating evidence of inadequate voltage control, eg, a “threshold phenomenon” near the voltage range for Na⁺ channel activation, and/or an inappropriately steep increase in current amplitude in the negative slope region of the current-voltage relationship curve, the data were discarded. Whole-cell I_{Na,\textsuperscript{6}} was obtained by subtracting the traces elicited with comparable voltage steps containing no current (using prepulse to inactivate the Na⁺ channels) from the raw current traces. In this way, the cell capacitance and linear leakage, if present, were subtracted.
Experimental Protocols

Time-dependent changes of Na\(^+\) channel kinetics, including a shift of the availability curve (I_l\(_{max}\) curve) in the hyperpolarizing direction have been observed.\(^{20-27}\) Typically, these changes are known to occur within minutes after membrane rupture. We have previously established the degree of shift of I_l\(_{max}\) inactivation curves with time after membrane rupture under our recording conditions.\(^6\) From 20 to 50 minutes after membrane rupture, half-maximal activation voltage (V_l(0.5)) values showed a similar rate of shifting, 0.14±0.037 mV/min in NZs (n=7) and 0.11±0.029 mV/min in IZs (n=7) (P>0.05). Therefore, peak current data collected between ~20 and 50 minutes after membrane rupture were used. Furthermore, in the analysis of data from individual protocols in the absence and presence of drug, only one concentration of lidocaine was tested in each cell. Superfusion of the lidocaine-containing solution was completed for 10 minutes before data were collected. In this way, the averaged time after membrane rupture at which data were collected was well matched for the 2 cell groups.

To examine the effects of lidocaine on peak current density in cells from the 2 groups, voltage steps (50-ms duration) from a holding potential (V_h) of −100 mV were given stepwise from −70 to +5 mV (5-s intervals). Peak currents at various test voltages were plotted to obtain the current-voltage relationship curve. The maximal peak current was then divided to cell capacitance to obtain a peak current density (pA/pF) for each cell. The effect of lidocaine on the "steady-state" availability curve (I_l\(_{max}\)) was determined by using a 1000-ms conditioning pulse to various potentials as described before.\(^6\)

To determine the effect of lidocaine on time-dependent loss of availability, subthreshold prepulses to −60 mV of variable duration were applied as previously described.\(^6\) The normalized currents were plotted as a function of the prepulse duration, and a biexponential function was fitted to the data as before.\(^6,28,29\)

The time course of recovery of I_l\(_{max}\) availability was assessed using the double-pulse method as described previously.\(^6\) A biexponential function\(^6,28,30,31\) was fitted to the normalized values. With or without lidocaine treatment, many cells (especially IZs) exhibited an additional kinetic component consisting of a delay at shortest interpulse interval (Ipi) during the period of recovery from inactivation. Rather than incorporate both a time constant and amplitude for a small third component (high uncertainty), the initial delay in recovery (d) was incorporated into the 2-exponential equation as follows:

### TABLE 1. Tonic Block of I_l\(_{max}\) by Lidocaine

<table>
<thead>
<tr>
<th>V_h</th>
<th>20 μmol/L</th>
<th>60 μmol/L</th>
<th>120 μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Drug</td>
<td>Control</td>
</tr>
<tr>
<td>−90 mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZs</td>
<td>−1419±196</td>
<td>−1086±168</td>
<td>−1471±423</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>% Decrease</td>
<td>25±2.8</td>
<td>46.5±4.7*</td>
<td>50.7±2.8*</td>
</tr>
<tr>
<td>IZs</td>
<td>−717±64</td>
<td>−395±59†</td>
<td>−526±121</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>% Decrease</td>
<td>45.2±5.9</td>
<td>41.8±6.9*</td>
<td>70±3.3*</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>−100 mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZs</td>
<td>−1594±209</td>
<td>−1411±197†</td>
<td>−1513±327</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>% Decrease</td>
<td>12.4±2</td>
<td>24.6±2.9*</td>
<td>28.7±3.7*</td>
</tr>
<tr>
<td>IZs</td>
<td>−1008±113</td>
<td>−821±99†</td>
<td>−634±133</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>% Decrease</td>
<td>18.5±3.5</td>
<td>25.2±2.7</td>
<td>36±4.2*</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>−110 mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZs</td>
<td>−1600±228</td>
<td>−1435±209†</td>
<td>−1892±531</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>% Decrease</td>
<td>10.5±2</td>
<td>13.1±3.4</td>
<td>19.3±3.9</td>
</tr>
<tr>
<td>IZs</td>
<td>−1048±121</td>
<td>−918±101</td>
<td>−825±203</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>% Decrease</td>
<td>11.6±4.6</td>
<td>21.3±3</td>
<td>28.2±2.8*</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Tonic block was determined by comparing I_l\(_{max}\) from the first beat of the pace protocol with and without lidocaine. I_l\(_{max}\) was induced from different V_h levels (−90, −100, and −110 mV) to −25 mV for 40 ms. The tonic block showed a dose and V_h dependence in both cell types. Block was greater in IZs than in NZs at all V_h levels and reached significance with 120 μmol/L lidocaine at V_h of −90 and −100 mV. The greatest amount of tonic block occurred at V_h of −90 mV in both cell types, with 120 μmol/L lidocaine causing a 70% decrease in I_l\(_{max}\) in IZs and a 50% decrease in NZs. The time between membrane rupture and the beginning of data collection was 24.9±0.9 minutes for NZs and 27.1±1.1 minutes for IZs (P=NS). P value indicates the comparison between NZs vs IZs. NS indicates no statistical difference between NZs and IZs. Values are mean±SEM.

*Dose-dependent effect.
†P<0.01 and †P<0.05 vs control.
I at least a 10-s interval between each train. The reduction of and B illustrate the effects of lidocaine (120 mol/L) on percent membrane rupture (at 20 μmol/L, 26.9 ± 1.1 and 49.1 ± 1.1 minutes for NZs and 25.7 ± 0.9 and 49.7 ± 2.3 minutes for IZs; at 60 μmol/L, 26.4 ± 1.3 and 47.7 ± 1.5 minutes for NZs, and 26.7 ± 1.19 and 50.9 ± 2.87 minutes for IZs; and at 120 μmol/L, 26 ± 0.66 and 44.8 ± 1.3 minutes for NZs and 25.4 ± 1.12 and 48.5 ± 1.77 minutes for IZs).

\[ f = A_1 (1-e^{-t/\tau_1}) + A_2 (1-e^{-t/\tau_2}) \]

where \( \tau_1 \) and \( \tau_2 \) are the fast and slow time constants, respectively, and \( A_1 \) and \( A_2 \) are the relative amplitudes of the fast and slow components, respectively. In this way, the time constant of recovery of availability was determined for each NZ and IZ, and the average values were compared in the absence and presence of drug.

Tonic and use-dependent block were determined using repeated pulse trains. In each train, 20 test pulses to −25 mV were given at rates of 1, 2, and 3 Hz from \( V_{th} \) at −90, −100, and −110 mV with at least a 10-s interval between each train. The reduction of \( I_{th} \) at the first pulse after lidocaine was defined as tonic block. The use-dependent block was determined by reduction of \( I_{th} \) at the end of the 20th pulse, where the reduction of \( I_{th} \) reached steady state. The data were compared between groups.

Lidocaine hydrochloride (LC Laboratories) was dissolved in water to make a stock solution (2 mmol/L) for use in the external solution. Effects of lidocaine (lido) on time-dependent loss of \( I_{th} \) were compared between groups. Panel A depicts average \( I_{th} \) values for NZs and IZs before and after lidocaine at 120 μmol/L. Panel B depicts the size of the drug-induced shift of \( V_{th} \) of \( I_{th} \) curves, which was obtained by subtracting \( V_{th} \) values before and after drug for each cell studied. Height of bar indicates average shift of \( V_{th} \) (in mV) for each of several lido concentrations indicated. Note that lido produced a greater negative shift of \( V_{th} \) in IZs versus NZs at all concentrations, reaching significance at 120 μmol/L. P values indicate comparison between NZs and IZs. A dose-dependent effect was observed in both groups (ANOVA, \( P < 0.001 \)). Data used to construct these relationships were obtained at similar times after membrane rupture (at 20 μmol/L, 26.9 ± 1.1 and 49.1 ± 1.1 minutes for NZs and 25.7 ± 0.9 and 49.7 ± 2.3 minutes for IZs; at 60 μmol/L, 26.4 ± 1.3 and 47.7 ± 1.5 minutes for NZs, and 26.7 ± 1.19 and 50.9 ± 2.87 minutes for IZs; and at 120 μmol/L, 26 ± 0.66 and 44.8 ± 1.3 minutes for NZs and 25.4 ± 1.12 and 48.5 ± 1.77 minutes for IZs).

**Figure 1.** Effects of lidocaine (lido) on \( I_{th} \) versus \( V_{th} \) inactivation relations in NZs and IZs. Inactivation of \( I_{th} \) was induced by a 1000-ms conditioning pulse from −140 to −40 mV, followed by a 40-ms test pulse from −100 to −25 mV. Panel A depicts average \( I_{th} \) values for NZs and IZs before and after lido at 120 μmol/L. Panel B depicts the size of the drug-induced shift of \( V_{th} \) of \( I_{th} \) curves, which was obtained by subtracting \( V_{th} \) values before and after drug for each cell studied. Height of bar indicates average shift of \( V_{th} \) (in mV) for each of several lido concentrations indicated. Note that lido produced a greater negative shift of \( V_{th} \) in IZs versus NZs at all concentrations, reaching significance at 120 μmol/L. P values indicate comparison between NZs and IZs. A dose-dependent effect was observed in both groups (ANOVA, \( P < 0.001 \)). Data used to construct these relationships were obtained at similar times after membrane rupture (at 20 μmol/L, 26.9 ± 1.1 and 49.1 ± 1.1 minutes for NZs and 25.7 ± 0.9 and 49.7 ± 2.3 minutes for IZs; at 60 μmol/L, 26.4 ± 1.3 and 47.7 ± 1.5 minutes for NZs, and 26.7 ± 1.19 and 50.9 ± 2.87 minutes for IZs; and at 120 μmol/L, 26 ± 0.66 and 44.8 ± 1.3 minutes for NZs and 25.4 ± 1.12 and 48.5 ± 1.77 minutes for IZs).

**Figure 2.** Effects of lidocaine (lido) on time-dependent loss of availability in NZs and IZs. The time course of loss of availability of \( I_{th} \) was obtained using a double-pulse protocol, where \( I_{th} \) was induced from \( V_{th} \) of −100 to −25 mV after a conditioning pulse to −60 mV with varied duration. Each \( I_{th} \) was normalized to maximal \( I_{th} \) (conditioning pulse, 0 ms), plotted against conditioning prepulse duration. The time course of loss of \( I_{th} \) availability was then described by biexponential function. Panels A and B illustrate the effects of lidocaine (120 μmol/L) on percent \( I_{th} \) remaining unavailable, with a prepulse of varying duration to −60 mV in NZs (solid circles) and IZs (open circles) in control solution (A) and drug-containing solution (B). Percentage of channels remaining unavailable by a 200-ms prepulse is depicted by a dotted line in both panels. See text for more detail. Each subset of data had its own control group of cells (20 μmol/L lido, n = 12 for NZs and n = 7 for IZs; 60 μmol/L lido, n = 11 for NZs and n = 12 for IZs; and 120 μmol/L lido, n = 13 for NZs and n = 6 for IZs).

**Results**

**Tonic Block of \( I_{th} \) in NZ and IZ Cells**

To examine whether IZs and NZs exhibit differences in tonic block, peak whole-cell \( I_{th} \) was measured during a depolarizing pulse to −25 mV after a 10-s period at −90 mV in the absence of drug and in 3 concentrations of lidocaine. The density of drug-free \( I_{th} \) in IZs (5.5 ± 0.53 pA/pF, n = 38, \( V_{th} = −100 \) mV) was significantly reduced compared with that of NZs (14.4 ± 0.66 pA/pF, n = 50, \( P < 0.01 \)) similar to our previous report. Furthermore, the reduction of \( I_{th} \) due to lidocaine (120 μmol/L) was significantly greater in IZs than in NZs (\( V_{th} = −90 \) mV) (Table 1) (\( P < 0.05 \)). Even at lower lidocaine concentrations, there was a consistent trend suggesting greater sensitivity of \( I_{th} \) in IZs. Nevertheless, in the absence as well as presence of lidocaine, \( I_{th} \) in IZs was significantly reduced compared with that of NZs.

When tonic block was assessed at more hyperpolarizing prepulses (−110 mV versus −90 or −100 mV, Table 1), differences in tonic lidocaine action on IZs and NZs were minimized. We have previously shown that the voltage dependence of steady-state availability is shifted in the hyperpolarizing direction for \( I_{th} \) in IZs. To determine whether differences in steady-state inactivation might explain these voltage-dependent differences in \( I_{th} \) block in IZs and NZs, we examined the availability of \( I_{th} \) over a wide range of inactivating membrane potentials (Figure 1). Under drug-free conditions, the fitted \( V_{th} \) of IZs was −83.3 ± 0.77 mV, negative to that of NZs (−79.2 ± 0.5 mV). Lidocaine (120 μmol/L) produced a greater negative shift in \( V_{th} \) in IZs than in NZs (−11.9 ± 0.82 versus −9.05 ± 0.59 mV, \( P = 0.01 \), Figure 1A). At lower drug concentrations, a similar trend suggesting a greater negative shift in IZs was observed but did not reach statistical significance (Figure 1B).
binds with high affinity to inactivated channels but also may inactivation-disabled mutants suggest that lidocaine not only available (Figure 1A), suggesting a larger fraction of I

We have previously reported accelerated development of Availability in NZs and IZs

Effects of Lidocaine on Time-Dependent Loss of Availability in NZs and IZs

The negative shift in the voltage dependence of drug-free steady-state availability for IZs may explain why the tonic block difference between IZs and NZs is more prominent at a holding potential of −90 mV than at −110 mV. In the drug-free condition, \( I_{Na} \) in NZs at −90 mV are 88.6±1.2% available (\( I/I_{max} \)), whereas that of IZs is only 78±3.5% available (Figure 1A), suggesting a larger fraction of \( I_{Na} \) in IZs is inactivated at −90 mV. Conversely, at −110 mV, both IZs and NZs are fully reprimed in the absence of lidocaine. Lidocaine binds more avidly to the inactivated state(s) of the cardiac Na\(^+\) channel than to noninactivated, rested states. Hence, IZs held at −90 mV exhibit greater tonic block of \( I_{Na} \) than do NZs, whereas at more hyperpolarized potentials where the degree of inactivation for both IZs and NZs is minimized, tonic block of \( I_{Na} \) is not significantly different.

Effects of Lidocaine on Time-Dependent Loss of Availability in NZs and IZs

We have previously reported accelerated development of inactivation from a preopen, closed state in IZs versus NZs in drug-free conditions. Recent studies of lidocaine action in inactivation-disabled mutants suggest that lidocaine not only binds with high affinity to inactivated channels but also may accelerate the rate of inactivation. Therefore, we examined whether inactivation gating changes in IZs (Figure 2) influence lidocaine-induced changes in a time-dependent loss of availability. Loss of availability from closed states was examined using a conditioning pulse to −60 mV of varying duration. Lidocaine accelerated the rate at which channels became unavailable to open in both NZs and IZs (Figure 2B). We used the percentage of unavailable channels after a 200-ms prepulse to −60 mV as a model-independent index to compare the rate of loss of availability for both cell types in the absence and presence of lidocaine. In drug-free conditions, by 200 ms at −60 mV, 83.9±2.8% of channels were inactivated in IZs compared with only 66.5±2.9% in NZs (\( P<0.01 \)), indicating that inactivation from closed states was accelerated in IZs relative to NZs. In lidocaine (120 µmol/L), 80.8±3.1% were unavailable for opening in NZs, and the degree of availability was only slightly greater in IZs (90.5±2.8%, \( P=\text{NS} \)) (Figure 2B). Lidocaine accelerated the rate at which channels became unavailable from preopen states in both cell types. Percent change in unavailability at 200 ms induced by lidocaine (20, 60, and 120 µmol/L) in NZs (18±2%, 15±3%, and 20±2%) and IZs (8±2%, 6±1%, and 8±2%) were dissimilar and not dose dependent. Nevertheless, with drug exposure the difference between NZs and IZs was minimized.

In drug-free conditions, development of inactivation in both cell types has been described by a biexponential func-
Frequency-dependent reduction of $I_{Na}$ greater in NZs than IZs at 120 mV to 25 mV for 40 ms in drug-free conditions. We therefore fit biexponential functions to the data as a qualitative means to examine the effects of lidocaine on these kinetic components, recognizing that 2 exponentials underestimate the total number of kinetic transitions during closed-state inactivation under these conditions. In both cell types, lidocaine produced a decrease in both the fast and slow time constants ($\tau_{1}$ and $\tau_{2}$, respectively) and also decreased the relative amplitude of the fast time constant ($A_{1}$) (Table 2). The net effect of drug in the different subset of cells of both cell types was an accelerated loss of availability. The effects of lidocaine on $\tau_{1}$, $\tau_{2}$, and $A_{1}$ were dose dependent ($P=0.015$, $P=0.034$, and $P=0.026$, respectively) in NZs over a 20- to 120- $\mu$mol/L range, whereas in IZs dose-dependent effects of lidocaine did not reach statistical significance (Table 2). The lidocaine-induced reduction in $\tau_{2}$ and $A_{1}$ was significantly greater in NZs than IZs at 120 $\mu$mol/L ($P<0.05$), consistent with the qualitative effect of lidocaine to minimize the difference between the 2 cell groups.

**Use-Dependent Effects of Lidocaine on IZs and NZs**

We have reported that $I_{Na}$ in IZs exhibits a delay in recovery from inactivation relative to NZs. In drug-free conditions, such a delay in recovery should be manifested as a difference in the rate at which $I_{Na}$ diminishes during rapid trains of depolarizing pulses (use dependence). Under drug-free conditions, we examined use-dependent reduction of $I_{Na}$ in IZs and NZs at 3 stimulation frequencies. Repetitive clamp steps (40-ms duration) from a $V_{H}$ of $-100$ mV produced a frequency-dependent reduction of peak current in both cell types, but the effects in IZs were significantly greater, particularly at rapid pacing rates (2 and 3 Hz) (Figure 3A). Consistent with the slowed rate of Na$^+$ channel recovery from inactivation at depolarized membrane potentials in drug-free conditions (see below), the IZ–NZ differences in use-dependent reduction of $I_{Na}$ at 3 Hz were exaggerated at more depolarized recovery potentials ($V_{H}$, Figure 3B).

Lidocaine increased the use-dependent reduction of $I_{Na}$ in a dose-dependent manner in both NZs and IZs (3 Hz). In the drug-free condition, IZs exhibited significantly greater use-dependent reduction of $I_{Na}$ compared with NZs with the lowest lidocaine concentration (20 $\mu$mol/L) (circles, Figure 4). However, at the higher concentrations (60 and 120 $\mu$mol/L), differences between NZs and IZs were eliminated. Under drug-free conditions, $I_{Na}$ is reduced (pulse 20/pulse 1) by 10.7±1.5% in IZs but only by 6.2±0.9% in NZs (3 Hz, $V_{H}=-100$ mV, $P<0.01$ versus NZs). Conversely, in 60 $\mu$mol/L lidocaine (squares, Figure 4), there was no difference between NZs and IZs (50.9±4.5% in IZs and 47.3±3.2% in NZs, $P=NS$). Similar results were obtained in 120 $\mu$mol/L lidocaine (62.3±2.5% in IZs and 59.9±2.3% in NZs, $P=NS$) (inverted triangles, Figure 4).

Under drug-exposed conditions in which NZs and IZs exhibit similar degrees of use-dependent $I_{Na}$ reduction, similar rates of recovery of availability should also be apparent.

**Figure 3.** Frequency-dependent and $V_{H}$-dependent reduction of $I_{Na}$ in drug-free conditions. A, $I_{Na}$ was elicited by repetitive clamp steps from $V_{H}$ at $-100$ to $-25$ mV for 40 ms in drug-free conditions at 1-, 2-, and 3-Hz pacing rates. The average beat-to-beat reduction of $I_{Na}$ during the 20-pulse train is shown. Frequency-dependent reduction of $I_{Na}$ was seen in both NZs (solid symbols) and IZs (open symbols). However, the reduction at the 20th beat in IZs was significantly greater than that in NZs at 2 Hz (squares) and 3 Hz (triangles) (see $P$ values). B, $I_{Na}$ was elicited by repetitive clamp steps from $V_{H}$ at $-90$, $-100$, and $-110$ mV to $-25$ mV for 40 ms in drug-free conditions at 3 Hz. $I_{Na}$ during the pulse train for each cell group at the different $V_{H}$ is shown. The reduction of $I_{Na}$ was $V_{H}$ dependent in both cell types; however, $I_{Na}$ reduction was significantly greater in IZs (see $P$ values).

**Figure 4.** Use-dependent block of $I_{Na}$ by lidocaine (lido) at different concentrations in NZs and IZs. For these data, $I_{Na}$ was elicited in separate subsets of cells from $V_{H}$ at $-100$ mV to $-25$ mV for 40 ms at 3 Hz before and after lidocaine. Each subset of cells had its own control. $I_{Na}$ for each beat was normalized to $I_{Na}$ of the first beat ($I/I_{Na}$) in the presence of drug concentration indicated. The degree of lidocaine use-dependent block of $I_{Na}$ was dependent on pacing rate, $V_{H}$, and drug concentration. At each drug concentration, this fast pacing rate produced more block in both cell groups. However, at 20 $\mu$mol/L lidocaine, IZs showed a greater use-dependent block compared with NZs. $P$ values are for comparison between NZs and IZs.
Figure 5 examines recovery of availability as a function of time at –100 mV after a 1000-ms conditioning pulse to –25 mV. As shown previously, in the absence of drug a delay in recovery from inactivation occurs in IZs compared with NZs. As a model-independent means of comparing the rate recovery of availability in the absence and presence of drug, we measured fractional recovery of availability by 100 ms at –100 mV. Fractional recovery by 1 Pl at 100 ms was 76.1 ± 2.5% for NZs but only 63.7 ± 2.8% for IZs (P < 0.01) under drug-free conditions (Figure 5B). Drug exposure substantially increased the delay in recovery for both cell types; in lidocaine (20, 60, and 120 μmol/L), the respective percent change induced by 1 Pl at 100 ms was 37 ± 2%, 61 ± 4%, and 77 ± 0.8% for NZs and 44 ± 3%, 61 ± 3%, and 77 ± 2% for IZs (P < 0.001 for both cell groups). Consistent with the use-dependent results, lidocaine minimized the difference in the rate of recovery of availability for the 2 cell types (Figure 5).

To assess the effect of lidocaine on fast and slow components of recovery of availability, we fit the recovery data to a biexponential expression (τ1 and τ2). A delay (d) was incorporated to account for a third exponential component seen in NZs exposed to lidocaine (see Materials and Methods). Lidocaine increased both time constants of recovery, the delay, and the amplitude of the slow (A2) component in both cell types (Table 3). Furthermore, the differential effects of lidocaine on the individual kinetic components reported in Table 3 were consistent with the overall action to reduce predrug differences in recovery of excitability.

Discussion

Experimental Considerations

The experimental conditions were highly unphysiological, yet they were chosen for the following reasons: Voltage-clamp control of the cell membrane was considered. Under physiological conditions, INa is large, and the kinetics are so rapid that successful voltage-clamp control is challenging, even in single-cell preparations. Some investigators have been successful with a macropatch technique. For these reasons, the usual way to study INa in the whole-cell recording configuration is to reduce experimental temperature and lower the Na+ gradient. Under our conditions, INa in 91% of NZs was reduced to <3 nA, with the largest INa being 4.16 nA. These values are similar to other reported values under similar conditions.

Although a time-dependent negative shift of the steady-state availability curve has been described during whole-cell INa recordings, we think it is unlikely that this phenomenon has influenced our results. First, we found time-dependent changes to be similar in cells of the 2 groups, and currents of the 2 groups were measured at similar times after membrane rupture. Second, as we have discussed previously, it is unlikely that the current reduction and alteration of Na+ channel kinetics are due to the surgical procedure, cell isolation process, or criteria used for cell selection. A contamination of outward currents present only in IZs cannot account for the INa reduction, since the composition of intracellular and extracellular solutions was chosen to minimize contaminating currents. An increase of the cell capacitance or membrane surface area without change in the absolute channel number and a negative shift of the INa curve can only be responsible for a portion of the current reduction measured, since the total amplitude of INa was also reduced in IZs versus NZs, and the maximally available INa in drug-free conditions remained significantly different in IZs versus NZs.

Lidocaine Minimizes Gating Differences Between IZs and NZs

We have previously shown that IZs and NZs exhibit marked differences in inactivation gating. Recent studies have shown that mutations and subunit interactions that modify Na+ channel gating can in turn influence lidocaine action. Therefore, we
tested the hypothesis that the Na\(^+\) channel gating changes induced in cells that survive in the infarcted heart also influence the effects of lidocaine on the whole-cell \(I_{\text{Na}}\).

Although \(I_{\text{Na}}\) density in IZs was significantly lower than in NZs, we found no statistical relationship between \(I_{\text{Na}}\) density and the degree of tonic block (data not shown). Nonetheless, tonic block by lidocaine is significantly increased in IZs when the resting membrane potential is partly depolarized, and differences were attenuated when the membrane was hyperpolarized. These effects are readily explained by differences in the voltage dependence of drug-free steady-state inactivation relations for IZs and NZs (Figure 1). Since the voltage dependence of IZs is shifted negative to that of NZs, a larger proportion of Na\(^+\) channels in IZs is inactivated at relatively depolarized \(V_{\text{es}}\). Recent studies examining the greater tonic block effects of lidocaine in cardiac versus skeletal muscle Na\(^+\) channels have proposed an identical mechanism based on the negative shift in the voltage dependence of steady-state inactivation in the cardiac isofrom.\(^{43}\) Similarly, developmental changes in Na\(^+\) channel blocking properties of lidocaine in the postnatal rat heart have been attributed to changes in inactivation gating.\(^{43}\)

<table>
<thead>
<tr>
<th></th>
<th>20 (\mu)mol/L Control</th>
<th>20 (\mu)mol/L Drug</th>
<th>60 (\mu)mol/L Control</th>
<th>60 (\mu)mol/L Drug</th>
<th>120 (\mu)mol/L Control</th>
<th>120 (\mu)mol/L Drug</th>
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<tbody>
<tr>
<td>(n)</td>
<td>15</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>% Increase</td>
<td>53±7</td>
<td>111±19*</td>
<td>498±80*</td>
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<tr>
<td>NZs</td>
<td>40±3.4</td>
<td>59±4.2†</td>
<td>46±3.9</td>
<td>95±12.6†</td>
<td>35±3.1</td>
<td>196±27†</td>
</tr>
<tr>
<td>(A_1)</td>
<td>78±2.6</td>
<td>54±1.4†</td>
<td>72±4.4</td>
<td>33±3.0</td>
<td>82±1.3</td>
<td>26±2.6†</td>
</tr>
<tr>
<td>IZs</td>
<td>67±8.6</td>
<td>110±21.3†</td>
<td>66±5.8</td>
<td>127±20.7†</td>
<td>47±6.4</td>
<td>146±17.6†</td>
</tr>
<tr>
<td>(A_1)</td>
<td>81±1.9</td>
<td>54±2.6†</td>
<td>68±5.1</td>
<td>33±3.4†</td>
<td>82±1.5</td>
<td>22±3.2†</td>
</tr>
<tr>
<td>% Increase</td>
<td>59±11</td>
<td>90±23*</td>
<td>241±59*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(P)</td>
<td>NS</td>
<td>&lt;0.05</td>
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Lidocaine produced an increase in the fast recovery time constant \(\tau_1\), which was accompanied by decreasing the relative amplitude of the \(\tau_1\) component (\(A_1\)) and increasing the \(\tau_2\) component (\(A_2\)) dose-dependently in both cell types. This effect was greater in NZs than in IZs at 120 \(\mu\)mol/L. Lidocaine also increased \(\gamma_1\) in NZs but not in IZs; this difference reached significance at 60 and 120 \(\mu\)mol/L. Data used to construct these relationships were obtained at similar times after membrane rupture (at 20 \(\mu\)mol/L, 27±1.1 and 51±1.6 minutes for NZs and 30±1.3 and 55±2.9 minutes for IZs; at 60 \(\mu\)mol/L, 28±0.9 and 55±1.8 minutes for NZs and 31±0.8 and 53±2.3 minutes for IZs; and at 120 \(\mu\)mol/L, 27±0.8 and 48±2.1 minutes for NZs and 27±0.9 and 49±1.1 minutes for IZs). % Increase refers to change in \(t\) values. \(P\) value indicates the comparison of % increase in \(\tau_1\) or \(\tau_2\) between NZs and IZs. NS indicates no statistical difference between NZs and IZs. Values are mean±SEM.

*Dose-dependent effect.

†\(P<0.01\) and ‡\(P<0.05\) vs control drug-free conditions.

The negative shift in the drug-free steady-state availability curve in IZs relative to NZs suggests that one or more inactivated states are energetically stable in IZs. Consistent with this, the drug-free rate of development of inactivation from closed states is accelerated in IZs (Figure 2), and the drug-free rate of recovery from inactivation is delayed (Figure 5), causing a significant enhancement of drug-free use-dependent reduction of \(I_{\text{Na}}\) in IZs relative to NZs. In both cell types, lidocaine accelerated the rate at which channels became unavailable to open (Figure 2), delayed the recovery of availability (Figure 5), and enhanced the use-dependent reduction of \(I_{\text{Na}}\) during rapid trains of stimuli in a dose-dependent manner (Figure 4). Most notably, differences in the rates of loss and recovery of availability in the 2 cell types were eliminated with higher concentrations of lidocaine. Functional manifestation of this effect is best illustrated in Figure 4, where lidocaine eliminated the drug-free differences between IZs and NZs in the use-dependent reduction of \(I_{\text{Na}}\).

Many features of use-dependent drug action are explained by the modulated receptor model, which suggests that lidocaine affinity for the Na\(^+\) channel is highest in the inactivated conformational state. However, recent studies using mutant
channels with modified inactivation properties indicate that lidocaine not only binds with highest affinity to the inactivated state but can also accelerate the inactivation process, suggesting that local anesthetic drugs may function under more general paradigms applicable to allosteric effector molecules. Lidocaine could be shifting the inactivation gating equilibrium in a manner that favors occupancy of slow-inactivated states, which may not differ for NZs and IZs. Support for this model derives from accumulating evidence that site-directed mutations or α-βi subunit interactions directed toward shifting the inactivation gating equilibrium away from slow inactivation are effective in attenuating use-dependent lidocaine action.

At this time, we cannot exclude the possibility that use-dependent effects of lidocaine are somehow related to a drug interaction with fast inactivation in both cell types. First, it is possible that differences between IZs and NZs are blunted in lidocaine because of a marked drug-induced change in fast inactivation gating kinetics that somehow masks the distinctive gating properties seen in rapid, drug-free gating conditions. The nonlinear behavior of multistate Na+ channel gating models with a number of additional drug-associated kinetic states may predict such behavior. Alternatively, it is possible that Na+ channels in IZs are less “sensitive” to lidocaine and that the drug may therefore accelerate tNa inactivation and slow its recovery from inactivation more in NZs than in IZs. Finally, given the effects of the βi subunit in heterologous expression systems on lidocaine-induced use dependence, fast- and slow-inactivation gating, and channel density, the influence of myocardial infarction on Na+ channel density, gating, and lidocaine action in myocytes surviving in the border zone may be partly related to primary effects of the disease on the status of the Na+ channel βi subunit. Our results motivate future studies to examine the role of structural elements, such as subsidiary subunits, in Na+ channel gating and antiarrhythmic drug action in cells that survive in the infarcted heart.

Acknowledgments
This study was supported by NIH grant HL-30557 (Dr. Boyden). Salary support for Dr. Balser was from NIH grant ROI GM-56307 and the Clinician Scientist Award of the American Heart Association.

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Circ Res. 1998;83:431-440
doi: 10.1161/01.RES.83.4.431
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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