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\(_{Na}\)) 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\(_{Na}\) 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 (≥−100-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\(_{Na}\) 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 \(\mu\)mol/L) accelerated the rate of time-dependent loss of availability and markedly delayed recovery from availability, inducing significant use-dependent reduction of I\(_{Na}\). However, at drug concentrations ≥60 \(\mu\)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,\(^14,7\) suggesting that Na\(^+\) current (I\(_{Na}\)) 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.\(^14\)

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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.₆ Iₙa 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 mutations¹⁵-₁⁷ and subsidiary (β₁) subunit coexpression¹₈-₁₉ 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.₉,₁₀,₁₁ We examined whether Na⁺ channels of IZs of the 5-day infarcted heart exhibit altered functional sensitivity to the antiarrhythmic agent lidocaine. Unexpectedly, both IZs and myocytes from the noninfarcted heart (NZs) exhibited the same use-dependent current reduction with lidocaine exposure, despite marked differences in fast-inactivation gating under drug-free conditions.

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.²² 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 cardiectomy 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 scalpel blade was used to quickly remove a thin slice 1 cm² by 3 cm² by 2 mm of epicardial muscle (EBZ) from this area on the anterior left ventricle adjacent to the left anterior descending coronary artery. Single Ca²⁺-tolerant ventricular myocytes were enzymatically dispersed from the slice of EBZ tissue (IZs) and from slices of epicardial tissue obtained in a similar fashion from the same anatomic region of control noninfarcted hearts (NZs), as previously described by our laboratory.⁴,₅ Briefly, the tissue was rinsed twice in a Ca²⁺-free solution containing (mmol/L) NaCl 115, KCl 5, sucrose 35, dextrose 10, HEPES 10, and taurine 4, pH 6.95, to remove blood. Then it was triturated in 20 mL of enzyme-containing solution (collagenase B, 0.38 mg/mL, Worthington Biochemical Corp; protease, 0.25 mg/mL, Sigma; 36°C to 37°C) for 30 minutes, after which the solution was decanted and discarded. For a second trituration, the solution contained only collagenase and was discarded after 30 minutes. The next 6 to 7 triturations were each performed for 15 minutes. Each time, the solution was centrifuged at 500 rpm for 3 minutes to collect the supernatant and the dispersed myocytes. Resuspension solution was changed every 30 minutes for solutions containing increasing concentrations of Ca²⁺ (50 to 500 μmol/L). With this procedure, the living NZ yield was ~30% to 40%. For NZs, only rod-shaped cells with staircase ends, clear cross striations, and surface membranes free from blebs were used for study. IZs chosen for study, however, had a ruffled appearance, were less rodlike appearing, and had somewhat irregular cross striations and small dark droplets on the membrane. Our recent studies show that these morphological features indicate the IZs that exhibit abnormal transmembrane action potentials similar to those of the multicellular preparation of the EBZ.³-₄,₂₃

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 proper 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 pinned with a mica stage (type M-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 gigahm 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 11, 4-aminopyridine 3, and MnCl₂ 2 (pH 7.3 with CsOH), designed for proper Iₙa measurements. Mn²⁺ is known to affect Iₙa,₂₄ and preliminary experiments showed that the Mn²⁺ effect on Iₙa in IZs was similar to its effect in NZs.⁶ With this combination of external and internal solutions, Iₙa would be of manageable size and isolated from other possible contaminating currents.

Voltage-Clamp and Recording Techniques

Whole-cell Iₙa 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, 8; 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 capacitive transient induced by the local current 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 0.01 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 Iₙa 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 (\(I_{\text{Na}}\max\) curve) in the hyperpolarizing direction have been observed.\(^5\) Typically, these changes are known to occur within minutes after membrane rupture. We have previously established the degree of shift of \(I_{\text{Na}}\max\) inactivation curves with time after membrane rupture under our recording conditions.\(^6\) From 20 to 50 minutes after membrane rupture, half-maximal activation voltage (\(V_{\text{Na}}\max\) values showed a similar rate of shifting, 0.14 mV/min on average 0.029 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 (\(V_{\text{H}}\)) 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 (\(I_{\text{Na}}\max\)) 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.\(^6,26,29\)

The time course of recovery of \(I_{\text{Na}}\max\) 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 \(I_{\text{Na}}\max\) by Lidocaine

<table>
<thead>
<tr>
<th>(V_{\text{H}})</th>
<th>20 (\mu)mol/L</th>
<th>Control</th>
<th>Drug</th>
<th>60 (\mu)mol/L</th>
<th>Control</th>
<th>Drug</th>
<th>120 (\mu)mol/L</th>
<th>Control</th>
<th>Drug</th>
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<tr>
<td>−90 mV</td>
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<tr>
<td>NZs</td>
<td>−1419±196</td>
<td>−1086±168†</td>
<td></td>
<td>−1471±423</td>
<td>−850±280†</td>
<td></td>
<td>−1907±228</td>
<td>−973±178†</td>
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<td>n</td>
<td>13</td>
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<td>10</td>
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<tr>
<td>% Decrease</td>
<td>25±2.8</td>
<td>46.5±4.7*</td>
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<td>50.7±2.8*</td>
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<tr>
<td>IZs</td>
<td>−717±64</td>
<td>−395±59†</td>
<td></td>
<td>−526±121</td>
<td>−323±90†</td>
<td></td>
<td>−1253±190</td>
<td>−356±50†</td>
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<td>n</td>
<td>5</td>
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<tr>
<td>% Decrease</td>
<td>45.2±5.9</td>
<td>41.8±6.9*</td>
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<td>70±3.3*</td>
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<td>(P)</td>
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<td>−100 mV</td>
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<tr>
<td>NZs</td>
<td>−1594±209</td>
<td>−1411±197†</td>
<td></td>
<td>−1513±327</td>
<td>−1188±294†</td>
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<td>−2181±261</td>
<td>−1569±216†</td>
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<td>n</td>
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<tr>
<td>% Decrease</td>
<td>12.4±2</td>
<td>24.6±2.9*</td>
<td></td>
<td>28.7±3.7*</td>
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<tr>
<td>IZs</td>
<td>−1008±113</td>
<td>−821±99†</td>
<td></td>
<td>−634±133</td>
<td>−460±90†</td>
<td></td>
<td>−1262±286</td>
<td>−754±150†</td>
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<td>36±4.2*</td>
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<tr>
<td>(P)</td>
<td>NS</td>
<td>NS</td>
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<td>&lt;0.05</td>
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<td>−110 mV</td>
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<tr>
<td>NZs</td>
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<td>−1435±209†</td>
<td></td>
<td>−1892±531</td>
<td>−1666±480†</td>
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<td>−2120±252</td>
<td>−1741±245†</td>
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<td>n</td>
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<tr>
<td>% Decrease</td>
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<td>13.1±3.4</td>
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<td>19.3±3.9</td>
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<tr>
<td>IZs</td>
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<td>−918±101†</td>
<td></td>
<td>−825±203</td>
<td>−635±144‡</td>
<td></td>
<td>−1525±232</td>
<td>−1113±199†</td>
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<td>n</td>
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<tr>
<td>% Decrease</td>
<td>11.6±4.4</td>
<td>21.3±3</td>
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<td>28.2±2.8*</td>
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<td>(P)</td>
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Tonic block was determined by comparing \(I_{\text{Na}}\max\) from the first beat of the pace protocol with and without lidocaine. \(I_{\text{Na}}\max\) was induced from different \(V_{\text{H}}\) levels (−90, −100, and −110 mV) to −25 mV for 40 ms. The tonic block showed a dose and \(V_{\text{H}}\) dependence in both cell types. Block was greater in IZs than in NZs at all \(V_{\text{H}}\) levels and reached significance with 120 \(\mu\)mol/L lidocaine at \(V_{\text{H}}\) of −90 and −100 mV. The greatest amount of tonic block occurred at \(V_{\text{H}}\) of −90 mV in both cell types, with 120 \(\mu\)mol/L lidocaine causing a 70% decrease in \(I_{\text{Na}}\max\) 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 (\(P<0.05\)).

\(P\) 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.
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braner rupture (at 20 μmol/L, 26.9±1.1 and 49±1.1 minutes for NZs and 25.7±0.9 and 497±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).

where τ1 and τ2 are the fast and slow time constants, respectively, and A1 and A2 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 Vh at −90, −100, and −110 mV with at least a 10-s interval between each train. The reduction of INa at the first pulse after lidocaine was defined as tonic block. The use-dependent block was determined by reduction of INa at the end of the 20th pulse, where the reduction of INa 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.

Statistics
All values are represented as mean±SEM. A value of P<0.05 was considered statistically significant. For 2-sample comparison, an unpaired t test was used to compare a single mean value between the 2 independent cell groups. A paired t test was used to compare the mean values obtained from the same cell group before and after drug intervention. For multiple comparisons, an ANOVA was used, followed by an F test to determine that the sample mean values between groups were significantly different from each other. If so, a modified t test with the Bonferroni correction was used (Sigmastat, Jandel Scientific).

Results
Tonic Block of INa in NZ and IZ Cells
To examine whether IZs and NZs exhibit differences in tonic block, peak whole-cell INa 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 INa in IZs (5.5±0.53 pA/pF, n=38, Vh=−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.6 Furthermore, the reduction of INa due to lidocaine (120 μmol/L) was significantly greater in IZs than in NZs (Vh=−90 mV) (Table 1) (P<0.05). Even at lower lidocaine concentrations, there was a consistent trend suggesting greater sensitivity of INa in IZs. Nevertheless, in the absence as well as presence of lidocaine, INa in IZs was significantly reduced compared with that of NZs.

When tonic block was assessed at more hyperpolarizing pre pulses (−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 INa in IZs.6 To determine whether differences in steady-state inactivation might explain these voltage-dependent differences in INa block in IZs and NZs, we examined the availability of INa over a wide range of inactivating membrane potentials (Figure 1). Under drug-free conditions, the fitted V0.5 of INa 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 V0.5 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 allows drug-free conditions. Recent studies of lidocaine action in drug-free condition, minimal, tonic block of 

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 NZs 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 = NS) (Figure 2B). Lidocaine accelerated the rate at which channels became unavailable from preopen states in both cell types. Hence, IZs held at −90 mV exhibit greater tonic block than do NZs, whereas at more hyperpolarized potentials where the degree of inactivation for both IZs and NZs is minimized, tonic block of t in IZs is not significantly different.

TABLE 2. Decrease in Time Constants of Development of Na+ Channel Unavailability Induced by Lidocaine

<table>
<thead>
<tr>
<th></th>
<th>20 μmol/L Control</th>
<th>20 μmol/L Drug</th>
<th>60 μmol/L Control</th>
<th>60 μmol/L Drug</th>
<th>120 μmol/L Control</th>
<th>120 μmol/L Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>t1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZs</td>
<td>97 ± 9.8</td>
<td>64 ± 5.5†</td>
<td>78 ± 9.6</td>
<td>45 ± 5.7†</td>
<td>86 ± 5.5</td>
<td>45 ± 4.8†</td>
</tr>
<tr>
<td>A1</td>
<td>59 ± 3.3</td>
<td>54 ± 3.8</td>
<td>63 ± 2.4</td>
<td>48 ± 4.7†</td>
<td>54 ± 3.5</td>
<td>37 ± 2.7†</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>13</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>% Decrease</td>
<td>33 ± 3</td>
<td>42 ± 4</td>
<td>47 ± 3.6</td>
<td>29 ± 2.4†</td>
<td>47 ± 2.7</td>
<td>28 ± 2.7†</td>
</tr>
<tr>
<td>IZs</td>
<td>43 ± 4.5</td>
<td>24 ± 2.4†</td>
<td>47 ± 3.6</td>
<td>29 ± 2.4†</td>
<td>47 ± 2.7</td>
<td>28 ± 2.7†</td>
</tr>
<tr>
<td>A2</td>
<td>58 ± 4.1</td>
<td>56 ± 3.6</td>
<td>60 ± 4.1</td>
<td>44 ± 4.8†</td>
<td>54 ± 6.8</td>
<td>46 ± 5.4</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>12</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>% Decrease</td>
<td>43 ± 3</td>
<td>37 ± 2</td>
<td>40 ± 2.7</td>
<td>37 ± 2</td>
<td>40 ± 2.7</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Lidocaine accelerated the development of Na+ channel unavailability (inactivation and block) by decreasing t1 and t2 in both cell types. The effect was accompanied by a decrease in the relative amplitude of the t1 component (A1) and an increase in the t2 component (A2). However, this effect was dose dependent in NZs but not in IZs. In IZs, 20 μmol/L lidocaine produced a maximal effect. % Decrease refers to change in t values. P value indicates the comparison of percent decrease in t1 or t2 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.
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 $I_{Na}$ ($I_{Na}$) 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).

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. 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 $V_{Na}$ 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 $I_{Na}$-NZ differences in use-dependent reduction of $I_{Na}$ at 3 Hz were exaggerated at more depolarized recovery potentials ($V_{Na}$, Figure 3B).

Lidocaine increased the use-dependent reduction of $I_{Na}$ in a dose-dependent manner in both NZs and IZs (3 Hz). As in the drug-free condition, IZs exhibited significantly greater use-dependent reduction of $I_{Na}$ compared with NZs with the lowest lidocaine concentration (20 μmol/L) (circles, Figure 4). However, at the higher concentrations (60 and 120 μmol/L), differences between NZs and IZs were eliminated. Under drug-free conditions, $I_{Na}$ is reduced (pulse 20/pulse 1) by $10.7\pm1.5\%$ in IZs but only by $6.2\pm0.9\%$ in NZs (3 Hz, $V_{Na}$=−100 mV, $P<0.01$ versus NZs). Conversely, in 60 μmol/L lidocaine (squares, Figure 4), there was no difference between NZs and IZs ($50.9\pm4.5\%$ in IZs and $47.3\pm3.2\%$ in NZs, $P=NS$). Similar results were obtained in 120 μmol/L lidocaine ($62.3\pm2.5\%$ in IZs and $59.9\pm2.3\%$ 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 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 IpI 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 IpI 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 drug-free conditions (Figure 5B). However, 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 this phenomenon influenced our results. First, we found 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 can 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 I/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.

**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 I/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
I inactivation in the cardiac isoform. Similarly, development
in the negative shift in the voltage dependence of steady-state
Na block effects of lidocaine in cardiac versus skeletal muscle
proportion of Na channels have proposed an identical mechanism based
on the modulated receptor model, which suggests that lido-
caine affinity for the Na channel is highest in the inactivated
conformational state. However, recent studies using mutant
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 $I_{Na}$.

Although $I_{Na}$ density in IZs was significantly lower than in
NZs, we found no statistical relationship between $I_{Na}$ 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 hyper-
polarized. These effects are readily explained by differences
in the voltage dependence of drug-free steady-state inactiva-
tion relations for IZs and NZs (Figure 1). Since the voltage
dependence of drug-free steady-state inactivation is shifted negative to that of NZs, a larger
portion of Na channels in IZs is inactivated at relatively
depolarized $V_{th}$. 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 isof orm. Similarly, development-
tal changes in Na channel blocking properties of lidocaine in
the postnatal rat heart have been attributed to changes in
inactivation gating.

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 (Fig-
ure 5), causing a significant enhancement of drug-free use-
dependent reduction of $I_{Na}$ in IZs relative to NZs. In both cell
types, lidocaine accelerated the rate at which channels be-
came unavailable to open (Figure 2), delayed the recovery of
availability (Figure 5), and enhanced the use-dependent
reduction of $I_{Na}$ 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.

### Table 3. Increase in Time Constants of Recovery From Na Channel Unavailability by Lidocaine

<table>
<thead>
<tr>
<th></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>$\tau_1$ NZs</td>
<td>$40\pm3.4$</td>
<td>$59\pm4.2$</td>
<td>$46\pm3.9$</td>
</tr>
<tr>
<td>$A_l$ NZs</td>
<td>$78\pm2.6$</td>
<td>$54\pm1.4$</td>
<td>$72\pm4.4$</td>
</tr>
<tr>
<td>$n$</td>
<td>15</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>% Increase</td>
<td>$53\pm7$</td>
<td>$111\pm19^*$</td>
<td>$498\pm80^*$</td>
</tr>
<tr>
<td>$\tau_1$ IZs</td>
<td>$67\pm8.6$</td>
<td>$110\pm21.3$</td>
<td>$66\pm5.8$</td>
</tr>
<tr>
<td>$A_l$ IZs</td>
<td>$81\pm1.9$</td>
<td>$54\pm2.6$</td>
<td>$68\pm5.1$</td>
</tr>
<tr>
<td>$n$</td>
<td>9</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>% Increase</td>
<td>$59\pm11$</td>
<td>$90\pm23^*$</td>
<td>$241\pm59^*$</td>
</tr>
<tr>
<td>$P$</td>
<td>NS</td>
<td>$&lt;0.05$</td>
<td>$&lt;0.01$</td>
</tr>
</tbody>
</table>

Lidocaine produced an increase in the fast recovery time constant $\tau_1$, which was accompanied by decreasing the relative amplitude of the $A_l$ component ($A_1$) and increasing the $A_1$ component ($A_2$) dose-dependently in both cell types. This effect was greater in NZs than in IZs at 120 μmol/L. Lidocaine also increased $\tau_1$ in NZs but not in IZs; this difference reached significance at 60 and 120 μmol/L.

Data used to construct these relationships were obtained at similar times after membrane rupture (at 20 μmol/L, 28 ± 1.1 and 51 ± 1.6 minutes for NZs and 30 ± 1.3 and 55 ± 2.9 minutes for IZs; at 60 μmol/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 μmol/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 $\tau$ 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.
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.\(^6\) 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\(^{44}\) or \(\alpha\)-\(\beta\) subunit interactions\(^{41}\) 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 and that the drug may therefore accelerate \(I_{\text{Na}}\) inactivation and slow its recovery from inactivation more in NZs than in IZs. Finally, given the effects of the \(\beta\) subunit in heterologous expression systems on lidocaine-induced use dependence,\(^5\)\(^6\) fast- and slow-inactivation gating, and channel density,\(^7\)\(^8\)\(^9\) 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 \(\beta\) 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

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Lidocaine Action on Na⁺ Currents in Ventricular Myocytes From the Epicardial Border Zone of the Infarcted Heart
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