Novel, Ultraslow Inactivating Sodium Current in Human Ventricular Cardiomyocytes

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Background—Alterations in K⁺ channel expression and gating are thought to be the major cause of action potential remodeling in heart failure (HF). We previously reported the existence of a late Na⁺ current (I_{NaL}) in cardiomyocytes of dogs with chronic HF, which suggested the importance of the Na⁺ channel in this remodeling process. The present study examined whether this I_{NaL} exists in cardiomyocytes isolated from normal and failing human hearts.

Methods and Results—A whole-cell patch-clamp technique was used to measure ion currents in cardiomyocytes isolated from the left ventricle of explanted hearts from 10 patients with end-stage HF and from 3 normal hearts. We found I_{NaL} was activated at a membrane potential of −60 mV with maximum density (0.74±0.05 pA/pF) at −30 mV in cardiomyocytes of both normal and failing hearts. The steady-state availability was sigmoidal, with an averaged midpoint potential of −94±2 mV and a slope factor of 6.9±0.1 mV. The current was reversibly blocked by the Na⁺ channel blockers tetrodotoxin (IC_{50}=1.5 μmol/L) and saxitoxin (IC_{50}=98 nmol/L) in a dose-dependent manner. Both inactivation and reactivation of I_{NaL} had an ultraslow time course (τ≈0.6 seconds) and were independent of voltage. The amplitude of I_{NaL} was independent of the peak transient Na⁺ current.

Conclusions—Cardiomyocytes isolated from normal and explanted failing human hearts express I_{NaL} characterized by an ultraslow voltage-independent inactivation and reactivation. (Circulation. 1998;98:2545-2552.)

Key Words: heart failure • myocytes • action potentials • saxitoxin • tetrodotoxin

Mechanisms of ventricular arrhythmias in heart failure (HF) remain poorly understood despite intensive investigation. Studies in isolated ventricular myocardial fibers and cardiomyocytes obtained from failing human hearts demonstrated a prolongation of action potential (AP) duration.1-4 The prolongation was less prominent at rates >150 beats/min and was different in HF of different origins.3 AP prolongation was also described in animal HF models.5-8 Patch-clamp studies highlighted the importance of alterations in K⁺ channel expression and gating in the prolongation of AP in HF.3,4 Given that a delicate balance exists between the inward and outward currents in modulating AP duration, a role for inward currents in the AP prolongation in HF cannot be discounted. A late inward Na⁺ current, which exceeded the current in ventricular cardiomyocytes of normal and failing human hearts.

Key Words: heart failure • myocytes • action potentials • saxitoxin • tetrodotoxin

Methods
Cell Isolation
Cardiomyocytes were isolated from 10 failing explanted human hearts and from 3 normal donor hearts that for technical reasons were not suitable for transplantation. On harvesting, hearts were immersed in ice-cold cardioplegic solution and delivered to the laboratory within 10 minutes. The composition of the cardioplegic solution was (in mmol/L) NaCl 110, CaCl₂ 1.2, MgCl₂ 16, and KCl 16 (pH 7.8 adjusted with NaHCO₃). A transmural tissue block was obtained from the left ventricle apex. Five to 8 midmyocardial longitudinal slices, ~10×20 mm and 0.5 to 1 mm thick, were obtained with a blade and rinsed in oxygenated trituration solution (TTS) at room temperature. The composition of TTS was (in mmol/L) NaCl 140, KCl 5.4, MgCl₂ 2, glucose 5, and HEPES 10 (pH 7.4). All subsequent procedures were performed in O₂-saturated and constantly triturated TTS at 37°C. To remove interstitial Ca²⁺, specimens were immersed in 100 mL of TTS for 20 minutes, and the procedure was repeated twice. Slices were transferred into TTS containing 25 μmol/L Ca²⁺ and protease type XXIV (Sigma Chemical Co), 4 U/mL for 3 to 10 minutes, and subsequently treated with a mixture of collagenase (Worthington, type II, 291 U/mg) and hyaluronidase (Sigma, type IV-S) 0.5 mg/mL for 15 to 20 minutes. Finally, slices were incubated for 20 minutes with collagenase only.

The cell suspension was centrifuged for 1 minute at 100g, and the
cardiomyocyte pellet was resuspended in MEM (Sigma) with 200 μmol/L CaCl₂. The yield of viable, CaCl₂-tolerant, rod-shaped myocytes varied from 5% to 50%. The mean capacitance of myocytes was 245±17 pF (n=57). The study was approved by the Henry Ford Health System Human Rights Committee (Institutional Review Board).

**Voltage-Clamp and Recording Technique**

Ion currents were recorded by whole-cell patch-clamp technique (Axopatch 200A patch-clamp amplifier, Axon Instruments Inc). The resistance of the glass patch pipettes (K150F, WPI Inc) was 600 to 800 kΩ (to solve, see Table 1). The requirement for stable measurement of the small (in the picoampere range) ion currents was a large total patch-pipette cell resistance (5 to 10 GΩ). The leak current was not subtracted during experiments. However, when characteristics of the late Na⁺ current (I_{NaL}) was assessed, the leak current was obtained after tetrodotoxin (TTX, 25 μmol/L) application and was subtracted from the current traces. Currents were filtered at 2 or 5 kHz (6 dB, 4-pole low-pass Bessel filter) and digitized at a sampling rate of 10 kHz (Digida 1200, Axon Instruments). Currents were recorded at room temperature (22°C to 24°C). The quality of the voltage clamp was controlled in each cell as previously described.17

KCl 143 5.4
NaCl 10 10 140 140
LiCl 140
KCl 143 143
CsCl 133 133 5.4 5.4 145.4
CaCl₂ 1.8 1.8 1.8 1.8
MgCl₂ 2 2 2 2
MgATP 2 2 2
TEA 0.002 0.002 0.002
EGTA 10 10 10
HEPES 5 5 5 5 5 5
pH 7.3 7.3 7.3 7.3 7.3 7.3
(KOH) (CsOH) (CsOH) (NaOH) (CsOH) (CsOH)

**AP Recording Technique**

APs were recorded in amphotericin-B–perforated patch-clamp configuration at 37°C in solution B (Table 1). Amphotericin-B (0.32 mmol/L) was added to the pipette solution B₃. Cardiomyocytes were stimulated by use of current pulses of 0.1 ms duration with an amplitude of 2.5 times the excitation threshold. AP duration was measured at room temperature (22°C). The toxin dose-response curve describing the percentage of the I_{NaL} block (B%) was determined by a 1-binding-site model:

\[ B\% = \frac{100\%}{1+\frac{[\text{toxin}]}{IC_{50}}} \]

Steady-state activation was evaluated from current-voltage relationships. Maximum Na⁺ conductance (g_{max}) and reversal potential (V_r) were estimated from a linear fit of the current-voltage relationship in the range from 0 to 60 mV. Na⁺ conductance (g) at a test potential (V_t) was calculated as

\[ g = \frac{I_{NaL} (V_t - V_r)}{I_{NaL} (V_t - V_r)} \]

Data points of normalized conductance (G=g/g_{max}) were fitted to a Boltzmann function:

\[ G = \frac{1+exp(V_t - V_{1/2G_{max}})/k_G)}{1+exp(V_t - V_{1/2G_{max}})/k_G)} \]

The time course of I_{NaL} decay was evaluated by a single exponential model:

\[ I_{NaL}(t) = I_0 \cdot e^{-\tau t} + I_i \]

where \( \tau \) is the time constant and \( I_0 \) and \( I_i \) are the amplitude and the steady-state component, respectively. I_{NaL} was fitted within the time interval from 0.2 to 2 seconds after the onset of membrane depolarization.

The recovery time constant (\( \tau_r \)) was assessed by a double-pulse protocol. The membrane was depolarized for 2 seconds to −30 mV by a conditioning pulse followed by a recovery period (T). The test pulse to −30 mV was then applied. The amplitude (Iₜ) of I_{NaL} elicited by the test pulse was normalized to a maximum I_{NaL} (I_{max}) and fitted to a single exponential model:

\[ I_t/I_{max} = 1 - e^{-\tau r} \]

**Statistical Analysis**

All measurements are reported as mean±SEM. Comparison between mean values was performed with unpaired Student’s t test. A value of \( P<0.05 \) was considered significant.

**Results**

**Steady-State Current Balance**

An inward current limb was found in the steady-state current (Iₕ)-membrane potential (Vₐ) relationship (Figure 1). TTX reversibly shifted the steady-state ion current balance toward outward currents (Figure 1). The difference in Iₕ-Vₐ relationships before and after TTX application (inset in Figure 1) revealed an activation threshold of approximately −60 mV, a maximum of approximately −20 mV, and a reversal potential close to 60 mV. This indicated the presence of an inward current, possibly of Na⁺ origin, that may contribute to the steady-state current balance.

**TABLE 1. Extracellular (Bath) and Intracellular (Pipette) Solutions Used in the Study**

<table>
<thead>
<tr>
<th>Pipette Solutions, mmol/L</th>
<th>Bath Solutions, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁</td>
<td>P₂</td>
</tr>
<tr>
<td>NaCl</td>
<td>10</td>
</tr>
<tr>
<td>LiCl</td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>143</td>
</tr>
<tr>
<td>CsCl</td>
<td>133</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1.8</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>2</td>
</tr>
<tr>
<td>MgATP</td>
<td>2</td>
</tr>
<tr>
<td>TEA</td>
<td>20</td>
</tr>
<tr>
<td>Nifedipine</td>
<td></td>
</tr>
<tr>
<td>EGTA</td>
<td>10</td>
</tr>
<tr>
<td>HEPES</td>
<td>5</td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
</tr>
<tr>
<td>(KOH) (CsOH) (CsOH) (NaOH) (CsOH) (CsOH) (CsOH)</td>
<td></td>
</tr>
</tbody>
</table>

TEA indicates tetraethylammonium.
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current density (I_{NaL})–membrane potential (V_m) relationship (Equation 1; Figure 2). The Na⁺ channel is known to be highly permeable for Li⁺ (ionic permeability ratio P_{Li⁺}/P_{Na⁺}=0.93^{19}). Indeed, a current with similar density (92±2%, n=4, measured at −30 mV), current-voltage relationship, and an ultrasonic decay (τ=0.54±0.01 seconds at −30 mV, n=4) was detected when Na⁺ was replaced on an equimolar basis by Li⁺ (Figure 2, A and B). The Na⁺ channel is impermeable for Cs⁺ (P_{Cs⁺}/P_{Na⁺}<0.016^{19}). When Na⁺ was replaced by Cs⁺, the current was almost completely abolished (Figure 2, C and D). The Na⁺-selective current, obtained as the difference in current before and after Na⁺ replacement by Cs⁺, was activated near −60 mV, reached its maximum at −30 mV, and reversed at 64 mV (Figure 2C). These data confirmed the Na⁺ origin of I_{NaL} and indicated that I_{NaL} was not related to the electrogenic Na⁺/Ca²⁺ exchange because Li⁺ is not transferred by the exchanger.

**Current-Voltage Relationship and Na⁺ Selectivity of the Late Current**

In the experimental configuration in which K⁺ and Ca²⁺ were blocked, we found a late inward current, I_{NaL}, that persisted long after I_{NaT} was completely inactivated. I_{NaL} was present in cardiomyocytes in 9 of the 10 failing hearts and in 2 of 3 normal donor hearts (Table 2). To rule out the possibility that I_{NaL} is related to Ca²⁺ current, we also changed [Ca²⁺]o reduction from 1.8 to 67 mV; indeed, a current with similar density (92±2%, n=4, measured at −30 mV), current-voltage relationship, and an ultrasonic decay (τ=0.54±0.01 seconds at −30 mV, n=4) was detected when Na⁺ was replaced on an equimolar basis by Li⁺ (Figure 2, A and B). The Na⁺ channel is impermeable for Cs⁺ (P_{Cs⁺}/P_{Na⁺}<0.016^{19}). When Na⁺ was replaced by Cs⁺, the current was almost completely abolished (Figure 2, C and D). The Na⁺-selective current, obtained as the difference in current before and after Na⁺ replacement by Cs⁺, was activated near −60 mV, reached its maximum at −30 mV, and reversed at 64 mV (Figure 2C). These data confirmed the Na⁺ origin of I_{NaL} and indicated that I_{NaL} was not related to the electrogenic Na⁺/Ca²⁺ exchange because Li⁺ is not transferred by the exchanger.

**Blockade of I_{NaL} by Specific Toxins**

To distinguish between nerve, skeletal, and cardiac Na⁺ channel isoforms, 2 toxins, TTX and STX, were used. 20 The cardiac isoform is 10 times less sensitive to TTX (50% of maximum blockade, IC_{50}=1 to 5 μmol/L) and almost 10² times less sensitive to STX (IC_{50}=100 μmol/L) than nerve and skeletal muscle isoforms. 21 Both toxins reversibly blocked I_{NaL} (Figure 3). The I_{NaL} values were 1.53 μmol/L and 98 mmol/L for TTX and STX, respectively, as anticipated for the heart Na⁺ channel.

**Density, Activation, and Inactivation of I_{NaL}**

The density of I_{NaL} and the midpoint of the availability curve varied widely among patients, whereas the slope (k_A) re-

![Figure 1. Effect of TTX (25 μmol/L) on the net steady-state current density (I_{NaL})–membrane potential (V_m) relationship (C), before TTX; (D), after TTX application. Data points are mean±SEM (4 cells each from patients 1, 2, and 3 in Table 2).](image)

**TABLE 2. Characteristics of I_{NaL} in Human Ventricular Cardiomyocytes**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Diagnosis</th>
<th>Medications</th>
<th>I_{NaL} Density, pA/pF</th>
<th>V_{1/2}A, mV</th>
<th>k_A, mV</th>
<th>τ, seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16/M</td>
<td>IDC</td>
<td>B, D, Db, L</td>
<td>0.54±0.08 (5)</td>
<td>−85.3±1.5 (4)</td>
<td>7.2±0.1</td>
<td>0.57±0.04</td>
</tr>
<tr>
<td>2</td>
<td>58/M</td>
<td>ICM</td>
<td>ACE, B, D, L</td>
<td>0.24±0.04 (6)</td>
<td>−84.5±3.6 (4)</td>
<td>7.4±0.2</td>
<td>0.61±0.06</td>
</tr>
<tr>
<td>3</td>
<td>59/M</td>
<td>ICM</td>
<td>ACE, D, L, M</td>
<td>0.11±0.03 (5)</td>
<td>−90.6±3.2 (5)</td>
<td>7.3±0.9</td>
<td>0.64±0.04</td>
</tr>
<tr>
<td>4</td>
<td>54/M</td>
<td>IDC</td>
<td>A, ACE, D, L</td>
<td>UD (2)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>51/M</td>
<td>ICM</td>
<td>A, ACE, B, D</td>
<td>0.34±0.07 (5)</td>
<td>−99.1±4.8 (5)</td>
<td>7.3±1.0</td>
<td>0.53±0.02</td>
</tr>
<tr>
<td>6</td>
<td>56/F</td>
<td>ICM</td>
<td>B, L, D</td>
<td>0.15±0.02 (7)</td>
<td>−97.3±1.5 (7)</td>
<td>7.6±0.4</td>
<td>0.62±0.04</td>
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<tr>
<td>7</td>
<td>42/M</td>
<td>IDC</td>
<td>ACE, D, Db, L</td>
<td>0.57±0.14 (4)</td>
<td>−104.5±2.1 (4)</td>
<td>6.6±0.1</td>
<td>0.61±0.09</td>
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<tr>
<td>8</td>
<td>13/F</td>
<td>IDC</td>
<td>ACE, D, L</td>
<td>0.49±0.06 (6)</td>
<td>−98.3±4.5 (6)</td>
<td>7.0±0.8</td>
<td>0.60±0.04</td>
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<tr>
<td>9</td>
<td>49/M</td>
<td>IDC</td>
<td>D, Db, I, L</td>
<td>0.37±0.11 (5)</td>
<td>−99.5±5.4 (5)</td>
<td>7.1±0.3</td>
<td>0.58±0.03</td>
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<tr>
<td>10</td>
<td>60/M</td>
<td>ICM</td>
<td>ACE, B, D, I, L</td>
<td>0.25±0.02 (12)</td>
<td>−86.6±1.2 (12)</td>
<td>6.2±0.2</td>
<td>0.63±0.01</td>
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<tr>
<td>11</td>
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<td>NDH</td>
<td>UD (4)</td>
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<tr>
<td>12</td>
<td>N/A</td>
<td>NDH</td>
<td>0.35±0.02 (7)</td>
<td>−96.9±1.7 (7)</td>
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<tr>
<td>13</td>
<td>N/A</td>
<td>NDH</td>
<td>0.35±0.03 (9)</td>
<td>−91.7±1.2 (9)</td>
<td>6.3±0.3</td>
<td>0.53±0.02</td>
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</tr>
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</table>

Averaged parameters

| Overall (11 hearts) | 0.34±0.05 | −94.2±2.0 | 6.9±0.1 | 0.59±0.01 |
| Donor (2 hearts)    | 0.35±0.0  | −94.3±2.6 | 6.4±0.1 | 0.55±0.02 |
| ICM (5 hearts)      | 0.22±0.04 | −91.6±2.9 | 7.2±0.2 | 0.61±0.02 |
| IDC (4 hearts)      | 0.50±0.04 | −96.9±4.1 | 6.9±0.1 | 0.59±0.01 |

ICD indicates idiopathic dilated cardiomyopathy; B, β-blocker; D, digoxin; Db, dobutamine; L, lasix; ICM, ischemic cardiomyopathy; ACE, ACE inhibitor; M, metolazine; A, amiodarone; I, isorbid; NDH, normal donor heart; UD, undetectable current density of <0.02 pA/pF; and ND, not determined.

Data are mean±SEM (no. of cells). V_{1/2A} and k_A indicate the availability curve midpoint potential and slope, respectively (Equation 3); τ indicates time constant of exponential fit to I_{NaL} decay at −30 mV (Equation 6).

Bath/pipette solutions B2/P2 (Table 1).
mained nearly the same (Table 2; Figure 4). Steady-state activation for $I_{NaL}$ (Figure 4B) was characterized by the midpoint potential ($V_{1/2} = -34.3 \pm 5.6$ mV, $n = 4$, patient 5) and the slope ($k = 7.1 \pm 0.7$ mV).

$I_{NaL}$ was almost completely inactivated after 2 seconds of membrane depolarization ($I_{s0}$, $0.05 \pm I_{o}$; Equation 6; Figure 5A). The decay time constant of $I_{NaL}$ was voltage independent within a voltage range from $-50$ to $40$ mV (Figure 5B). The time constant was similar in patients and normal donor hearts (Table 2).

The time course of $I_{NaL}$ recovery from inactivation was assessed at several holding potentials ($V_h$) by the conventional double-pulse method (Figure 6). The data were well fitted by a single exponential function (Figure 6C). Recovery was slow ($\tau_r = 0.56 \pm 0.04$ seconds, $V_h = -120$ mV, $n = 7$) and, unlike $I_{NaT}$, was not voltage dependent (Figure 6C).

**I$_{NaL}$ Is Independent of Peak I$_{NaT}$**

We investigated the relationship between $I_{NaL}$ and $I_{NaT}$ using a protocol shown in the inset of Figure 7A. A portion of $I_{NaT}$ was inactivated by a short ($\Delta t = 2$ to 5 ms) depolarization prepulse to 50 mV preceding a test pulse to $-30$ mV. The amplitude of the $I_{NaT}$ elicited by the test pulse was dependent on the prepulse duration. Increase in the prepulse duration gradually reduced the $I_{NaT}$ peak, but $I_{NaL}$ remained unchanged (Figure 7), indicating the independence of $I_{NaL}$ and $I_{NaT}$.

**TTX Decreases AP Duration**

To test the possible physiological importance of $I_{NaL}$, we assayed the effect of TTX on AP duration (Figure 8). TTX decreased AP duration at all 4 stimulation frequencies (0.2, 0.5, 1, and 2 Hz) in cells isolated from a normal donor heart (Table 3). In cardiomyocytes from failing hearts, TTX reduced AP duration and abolished early afterdepolarizations (Figure 8B).

**Discussion**

**Sodium Channel Underlying $I_{NaL}$**

A late Na$^+$ current, but not similar to the $I_{NaL}$ reported in the present study, was previously described in the mammalian heart. In contrast to our finding, $I_{NaL}$ has not previously been detected in human cardiomyocytes. The lack of data on $I_{NaL}$ in human cardiomyocytes may be explained by differences in experimental conditions. In previous studies, to improve voltage control, 5 mmol/L of Na$^+$ on both membrane sides was used. In such a voltage-clamp configuration, it is
impossible to distinguish $I_{\text{NaL}}$ from the experimental noise (our unpublished observation).

Voltage-dependent Na$^+$ channels can be distinguished by their toxin sensitivity. Compared with neuronal and skeletal muscle Na$^+$ channels, $I_{\text{NaL}}$ has low sensitivity for both TTX and STX, a property of the cardiac Na$^+$ channel clone hH1$^{23}$ underlying $I_{\text{NaT}}$. The $I_{\text{NaL}}$ IC$_{50}$ for TTX was comparable to that measured for $I_{\text{NaT}}$ in human atrial cardiomyocytes (1.1 $\mu$mol/L$^{21}$). The position and shape of the steady-state activation and availability curves for $I_{\text{NaL}}$ are also similar to those documented for the human $I_{\text{NaT}},^{21,22}$ which suggests that $I_{\text{NaL}}$ is produced by an Na$^+$ channel isoform that is similar to hH1. Because the most striking difference

Figure 3. Blockade of $I_{\text{NaL}}$ by specific toxins. A and B, Non-leak-subtracted whole-cell current recordings showing block of $I_{\text{NaL}}$ by different concentrations of TTX (patient 5, Table 2) and STX (patient 3), respectively. Voltage-clamp protocols are shown in insets. C and D, Dose-response block of $I_{\text{NaL}}$ by TTX and STX, respectively. Solid lines represent fit to a single-site binding model (Equation 2). Data points are mean±SEM, $n=7$ for TTX and $n=9$ for STX. Current recordings were low-pass filtered (25 Hz) and truncated. Bath/pipette solutions: $B_2/P_2$ (Table 1).

Figure 4. Voltage dependency of steady-state availability and activation of $I_{\text{NaL}}$. Example of $I_{\text{NaL}}$ traces (A) and steady-state availability and activation curves (B) obtained in patient 3. B, Voltage-dependent availability ($\Phi$) $A(V_p)$ (Equation 3) for $I_{\text{NaL}}$. Voltage-clamp protocol for $A(V_p)$ shown in inset to Figure 4A. Voltage-dependent activation ($\tau$), $A(V_m)$ (Equation 5) was determined from $I_{\text{NaL}}$-voltage relationship (see Methods for details). Bath/pipette solutions: $B_2/P_2$ (Table 1).

Figure 5. Voltage-independent inactivation time course of $I_{\text{NaL}}$. A, Representative current traces recorded at $-40, -20$, and 0 mV are shown along with single exponential fits (Equation 6; patient 5, Table 2). Dashed lines indicate zero current. B, Mean $\tau$ values measured at different membrane potentials (data from 22 cells obtained from 5 patients). Solid line represents linear regression. Slope and correlation coefficient ($r^2$) are indicated. Current recordings were low-pass filtered (25 Hz) and truncated. Bath/pipette solutions: $B_2/P_2$ (see Table 1).
between $I_{NaL}$ and $I_{NaT}$ was found in their inactivation, the difference, if any, between isoforms would probably be within the intracellular III-IV linker but not within the channel vestibule.

Possible Mechanisms of $I_{NaL}$

**Bursting Mode of Na Channel**

The mechanism of late currents was believed to be a bursting behavior of the transient Na channel that can function in different gating "modes," which might have an implication in $I_{NaL}$. However, in contrast to the voltage-dependent slow mode, the inactivation and reactivation of $I_{NaL}$ was found to be voltage independent (Figures 5 and 6).

**New Isoform**

Given that $I_{NaL}$ and $I_{NaT}$ were independent of each other (Figures 6 and 7), it is interesting to speculate that $I_{NaL}$ may not be simply the result of multiple reopenings of a small fraction of the transient Na channel but might rather reflect the activity of another channel subtype. A new Na channel isoform was suggested to produce a late Na current in rat ventricular myocytes. Recently, multiple Na channel subtypes with a slowly inactivating component were found in sensory neurons and in human coronary smooth muscle cells. Discovery of a second Na channel gene subfamily, hNa2.1, in the human heart provides an additional evidence for greater evolutionary divergence among voltage-dependent Na channels and suggests that other Na channel gene subfamilies may exist that may include the $I_{NaL}$ reported in the present study.

**Na Channel Modification**

Na channel inactivation can be modulated by channel protein phosphorylation. In contrast to the neuronal Na channel, no modulatory effect by the $\beta$-subunit on the kinetics of the cardiac Na channel was detected. Transient Na channel inactivation is dependent on the channel environment, which includes the sarcolemma and underlying cytoskeleton. Sarcolemmal partition of the ischemic phospholipid metabolite lyso phosphatidylcholine or modification of the F-actin-based cytoskeleton produced transition of some Na channels into a bursting mode. Although all of above-discussed mechanisms might play a contributory role in the origin of $I_{NaL}$, the exact mechanisms remain to be elucidated.
Role of $I_{\text{NaL}}$ in Determining AP Duration

The clinical importance of AP prolongation was recently shown in the SWORD trial. It was demonstrated that d-sotalol, a potassium channel blocker, increases the incidence of sudden death in patients with left ventricular dysfunction. The ventricular AP plateau is maintained by a delicate balance between inward and outward currents. In concert with the diminished $K^+$ currents in HF, $I_{\text{NaL}}$ would be expected to prolong AP duration by shifting this balance in favor of inward currents (Figure 1). We showed that $I_{\text{NaL}}$ can modulate the duration of AP over a broad range of pacing rates and that it may have a greater impact in HF (Table 3; Figure 8). Accordingly, $I_{\text{NaL}}$ can be implicated in repolarization impairments shown in human HF. Also, $I_{\text{NaL}}$ may play a role in acquired long-QT syndrome and in instances of severe bradycardia.

In conclusion, data from this study demonstrate for the first time the existence of a late Na$^+$ current in ventricular cardiomyocytes of normal donor and explanted failing human hearts. The current is characterized by an ultraslow, voltage-independent inactivation and reactivation.

**Table 3. Effect of TTX (1.5 μmol/L) on AP Duration (AP-Δt50ms)**

| Stimulation |
| Frequency, Hz | AP Duration Before TTX, ms | AP Duration After TTX, ms | % of Change |
| 0.2 | 333.0±2.0 | 264.9±1.2 | 20.5 |
| 0.5 | 331.2±0.9 | 262.1±1.6 | 20.8 |
| 1 | 321.4±1.8 | 259.4±1.3 | 19.3 |
| 2 | 314.0±1.0 | 266.5±1.0 | 15.1 |

Amphotericin-B–perforated patch-clamp configuration, 37°C, averaged data from 4 cells (normal donor heart 13, Table 2). At all stimulation frequencies, TTX significantly ($p<0.01$) decreased AP-Δt50ms.

**Acknowledgments**

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