A multi-modal composition of the late Na⁺ current in human ventricular cardiomyocytes

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Abstract

Objective: We reported an ultraslow late Na⁺ current (I_{NaL}) in ventricular cardiomyocytes of human hearts. I_{NaL} has been implicated in regulation of action potential duration in normal hearts and repolarization abnormalities in failing hearts. We have also identified sodium channel (NaCh) gating modes including bursts (BM) and late scattered openings (LSM) that together comprise I_{NaL}; however, the contribution of these gating modes to Na⁺ current (I_{Na}) remains unknown. In the present study, the late NaCh activity was recorded, analyzed, and modeled for heterologously expressed NaCh, Nav1.5, and for the native NaCh of ventricular mid-myocardial cardiomyocytes from normal and failing hearts.

Methods and Results: We found that LSM gating was significantly slower in failing compared to normal myocytes and Na,1.5 (τ = 474 ± 10 vs. 299 ± 9, and 229 ± 12 ms, m ± SEM; P < 0.05, n = 5–6). Total burst length of BM decreased with depolarization and was larger in failing compared to normal myocytes and Na,1.5. A complete I_{Na} decay was then numerically approximated as composed of NaCh populations operating in three gating modes described by separate Markov kinetic schemes: transient mode (TM), LSM, and BM. The populations of NaCh operating in each gating mode were estimated as 79.8% for TM, 20% for LSM, and 0.2% for BM, yielding an apparent four-exponential I_{Na} decay at −30 mV (maximum I_{Na})(τ ≈ 0.4, 4, 50, and 500 ms). Whole-cell recordings confirmed the existence of all four predicted components. The model also predicted voltage and temperature dependence of I_{Na} as well as I_{Na} increase and slower decay in failing hearts and acceleration by amiodarone.

Conclusions: The early phase of Na⁺ current decay (<40 ms) involves all three NaCh gating modes, the intermediate phase (from 40 to 300 ms) is produced by BM + LSM, although the contribution of BM decreases with depolarization, and ultra-late decay (>300 ms) is determined solely by LSM. The concept of multi-mode composition for I_{Na} provides a new rationale for I_{Na} modulation by factors such as voltage, temperature, pharmacological agents, and pathological conditions.

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1. Introduction

We reported an ultraslow inactivating and reactivating late Na⁺ current (I_{NaL}) in human ventricular myocytes (VM) from both normal and failing hearts that has been implicated in the action potential (AP) plateau [1]. In VM of failing human and dog hearts, whole-cell I_{NaL} was augmented and I_{NaL} decay was slower as reported by our group and others [2–4]. Partially blocking this current with toxins or lidocaine returned AP to its normal duration and halted early afterdepolarizations [1,3]. Recently I_{NaL} has been recognized as one of the major factors contributing to abnormal repolarization in heart failure [5].

In spite of its importance, to our knowledge the complete I_{Na} decay time course, including I_{NaL}, has never been

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examined in humans or approximated numerically. Until recently such an examination has been hampered by technical difficulties related to simultaneous recording and analysis of relatively large fast/transient currents ($I_{Na,T} \sim 10$ nA and 1 ms, respectively) and small slow/late currents ($I_{Na,L} \sim 10$ pA and 1 s). Also, because the difficulty in working with human cardiac tissues in general, and recording the late Na$^+$ channel (NaCh) [6] in particular, a statistically significant difference in late NaCh gating has not been demonstrated.

Based on a detailed patch-clamp examination of late NaCh activity, we believe the present study shows for the first time that both bursts (BM) and late scattered openings NaCh activity, we considered any period of inactivity as a gap within the depolarization step. Distributions for overlapping events were considered, omitting the last half the amplitude of the single-channel current as evaluated channel analysis. The channel opening threshold was set to 25 \%. Changing the gap criterion from $I_{Na,L}$ was measured using a physiological concentration of Na$^+$ (in mM): 140 NaCl, 5 CsCl, 1.8 CaCl$_2$, 2 MgCl$_2$, 5 glucose, 0.002 nifedipine, and 5 HEPES–CsOH buffer (pH 7.3). $I_{Na,L}$ was also measured without averaging in a bath solution containing a physiological concentration of Na$^+$ (in mM): 140 NaCl, 5 CsCl, 1.8 CaCl$_2$, 2 MgCl$_2$, 5 glucose, 0.002 nifedipine, and 5 HEPES–CsOH buffer (pH 7.3). $I_{Na,L}$ was inactivated by a short voltage pre-pulse of 5 ms to $+50$ mV Multi-exponential function was fit to $I_{Na}$ decays by Clampfit 9 program (Axon Instruments) with the Levenberg–Marquardt method, utilizing the sum of squared errors.

Single-channel cell-attached recordings were obtained with low-resistance pipettes (1.8–2.4 MΩ) to increase the number of low-probability late NaCh in the patch (up to 25) and thereby improve the success rate. Depolarizations of 816 ms were applied at a stimulation rate of 0.2 Hz, so that late channel activity would recover completely. The composition of the pipette solution was (in mM): 280 NaCl, 1.8 CaCl$_2$, 2.0 MgCl$_2$, 10 tetraethylammonium, 0.002 nifedipine, and 10 HEPES–NaOH buffer (pH 7.3). The depolarizing bath solution contained (in mM): 150 KCl aspartate, 2 MgCl$_2$, 10 TEA-Cl, 5 glucose, and 10 HEPES–KOH buffer (pH 7.2).

Custom-made software was used for primary single-channel analysis. The channel opening threshold was set to half the amplitude of the single-channel current as evaluated from Gaussian fit to amplitude histograms. Only non-overlapping events were considered, omitting the last closure within the depolarization step. Distributions for single-channel events were analyzed using custom-made software and logarithmic histograms. When analyzing burst activity, we considered any period of inactivity as a gap when its length ($\tau_{gap}$) was greater than $4\tau_{close1}$ (gap criterion). Changing the gap criterion from $\tau_{gap} > 3\tau_{close1}$ to $\tau_{gap} > 6\tau_{close1}$ resulted in only minor changes in envelope distribution (13.4 vs. 12.6 ms), supporting our contention.

2.3. Computer simulations

Single-channel openings were simulated employing a previously described algorithm [7]. The numerical model of $I_{Na}$ decay was developed using Delphi-7 software (Borland).

2.4. Statistical analysis

Data are expressed as mean±SEM. NaCh latency was adjusted to provide optimum voltage control. The entire time course of $I_{Na}$ ($I_{Na}=I_{Na,T}+I_{Na,L}$) was measured with, $[Na^+]_{bath}=5$ mM and experimental traces were averaged ($\sim$50). Average “zero” current ($I_{0}$) was obtained after applying tetrodotoxin (25 μM) and subtracted from total average current ($I$), so that $I_{Na}=I-I_{0}$. The bath contained (in mM): 5 NaCl, 133 CsCl, 1.8 CaCl$_2$, 2 MgCl$_2$, 5 glucose, 0.002 nifedipine, and 5 HEPES–CsOH buffer (pH 7.3). The pipette solution contained (in mM): 5 NaCl, 133 CsCl, 2 MgATP, 20 TEA-Cl, 10 EGTA, and 5 HEPES–CsOH buffer (pH 7.3). $I_{Na,L}$ was also measured without averaging in a bath solution containing a physiological concentration of Na$^+$ (in mM): 140 NaCl, 5 CsCl, 1.8 CaCl$_2$, 2 MgCl$_2$, 5 glucose, 0.002 nifedipine, and 5 HEPES–CsOH buffer (pH 7.3). $I_{Na,T}$ was inactivated by a short voltage pre-pulse of 5 ms to $+50$ mV Multi-exponential function was fit to $I_{Na}$ decays by Clampfit 9 program (Axon Instruments) with the Levenberg–Marquardt method, utilizing the sum of squared errors.

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the groups, and therefore standard analysis of variance (ANOVA) was employed. Tukey’s Studentized Range was used to adjust for multiple comparisons. Differences were considered significant at a value of $P < 0.05$.

3. Results

3.1. Three modes of NaCh activity, experimental data

Three major gating modes of NaCh activity were found in all cell-attached patches of heterologously expressed Nav1.5 ($n = 9$ patches), normal VM ($n = 8$) and failing VM ($n = 9$) (Fig. 1A–C): a transient mode (TM), a late scattered mode (LSM), and “bursts” (BM). Statistical analysis of the LSM openings revealed significantly slower latency in failing VM compared to normal VM and clone (Fig. 1E).

We previously found that bursts had two close states characterized by $\tau_{\text{close}1}$ and $\tau_{\text{close}2}$ [6]. Burst activity was manifested by periods of rapid switching between the open state and the first close state, separated by long gaps without channel activity (second close state). Fig. 1C shows one such burst, indicating periods of rapid channel switching activity (“envelopes”) and total burst length determined from single-channel measurements. Total burst length was potential-dependent (decreased with depolarization) and was largest in VM of failing hearts (Fig. 1F).

3.2. Simulations of single-NaCh currents

3.2.1. Transient mode (TM)

We employed a simplified non-unique model for early NaCh gating of heterologously expressed Na1.5 [8]. Based on our patch-clamp data (Fig. 1 and [6]), we made the following modifications (Fig. 2A):

1) The transition $I_6 \rightarrow I_5$ was prohibited, thereby eliminating the steady-state current produced by channel re-openings.
2) c and e were slightly changed to c = 1 / 0.4 = 2.5 ms\(^{-1}\) and e = 1 / 4.21 = 0.2375 ms\(^{-1}\), where 0.4 and 4.21 ms are the time constants for open times and latency distributions of early openings at −30 mV [6].

3) Transitions \( C_3 \rightarrow I_5 \) and \( I_5 \rightarrow C_3 \) were omitted, as they were originally introduced only for scaling purposes.

4) We made \( d = 0.1 \) ms\(^{-1}\) to bring the contribution of \( s_2 \) in line with our estimate of \( \sim 9\% \) for ensemble current (Fig. 1D) and other reports [9].

### 3.2.2. Late scattered mode (LSM)

While the TM model describes early openings and re-openings in cardiomyocytes very well it does not reproduce the slowly inactivating late currents generated by late scattered openings and bursts [6]. Accordingly, we added two more channel populations operating in LSM and BM (Fig. 2). For LSM, \( e = 1 / 474 \) ms = 0.00211 ms\(^{-1}\), where 474 ms is the time constant for latency of scattered openings at −30 mV (Fig. 1E). Note that we used the value measured for myocytes in heart failure for further comparison of model predictions with single-channel data obtained at −30 mV (see text for details). Traces in (A), (B), and (C) were generated from 3, 5, and 1 channel(s), respectively. Total simulation times are indicated above the traces. Simulated traces had a noise amplitude and bandwidth similar to actual single-channel recordings.

3.2.3. Burst mode (BM)

The burst mode was described by a kinetic scheme with one close state (\( C_1 \)), one open state (\( O_2 \)), and two inactivated states (\( I_3, I_4 \)) (Fig. 2C), yielding two close states observed in NaCh bursts in human VM [6]. Rapid switching between \( C_1 \) and \( O_2 \) forms the burst itself, whereas transitions to the inactivated state \( I_3 \) result in gaps (Fig. 1C) corresponding to the second close time found in bursts [6]. Return from the “deep” inactivated state \( I_4 \) back to \( I_3 \) was not included, as we observed no burst reappearance once initial burst activity ceased. Rate constants were estimated based on single-channel data (Table 1) and confirmed backward and forward compatibility of simulated currents with experimental single-channel distributions (not shown) and whole-cell currents (see below), respectively.
3.3. Model predicts whole-cell Na\(^+\) current

On average, we observed 4 late scattered openings in 10 traces in an average 5-channel patch; this opening rate could be simulated with one LSM channel, suggesting LSM % population \(\approx 20\%\). The number of bursts per trace per channel in each patch was \((1.93 \pm 0.38) \times 10^{-3}\) as defined from 1,951 traces recorded in 6 patches; this estimates BM % population \(\approx 0.193\%\). Simulations of total \(I_{Na}\) (Fig. 3) were performed with a total of 100,000 NaCh satisfying the previously reported density of 0.35 pA/pF for \(I_{NaL}\) measured at 200 ms depolarization in human VM [1]. Thus an average human VM with a membrane capacitance of 150 pF yields \(I_{NaL} \approx 50\) pA (Fig. 3B). The predicted peak of \(I_{NaT}\) was \(\approx 50\) nA (Fig. 3A) with a ratio \(I_{NaL}/I_{NaT} \approx 10^{-3}\), close to the experimentally measured ratio of \(~0.7 \times 10^{-3}\) for Nav1.5 [10]. We then calculated the percent contribution of each gating mode to \(I_{Na}\) during its time course (Fig. 3D). The experimental and simulated currents appear almost identical in two time scales, 20 and 2000 ms (see insets in Fig. 3A and C).

The multi-mode model of complete \(I_{Na}\) decay (Fig. 2), by definition, manifests an apparent decay composed of...
four exponentials with the following time constants: \( \tau_1 = 1/c_1 = 1/2.5 = 0.4 \) ms (NaCh inactivation in TM and LSM), \( \tau_2 = 1/e_1 = 1/0.2375 = 4.2 \) ms (inactivation of re-openings in TM), \( \tau_3 = 54.6 \) ms (apparent time constant for burst inactivation), and \( \tau_4 = 1/e_2 = 1/0.00211 = 474 \) ms (inactivation of re-openings in LSM). To validate this prediction (and thus the model itself), we performed a four-exponential fit of the entire average \( I_{Na} \) decay measured in a low [Na+] bath (5 mM). Indeed, all four predicted \( I_{Na} \) decay components were found in the whole-cell recordings (Fig. 4A and B). The slight difference in percent contributions and \( \tau \) values for the exponentials predicted by the model and those measured experimentally (Table 2) could be related to the difference in experimental conditions, particularly extracellular [Na⁺], 5 compared with 280 mM (whole-cell compared with cell-attached configuration from which the model was derived).

To minimize differences related to the experimental conditions, we also performed whole-cell experiments with bath [Na⁺] = 140 mM. Since the first and second decay components (\( \tau_1 \) and \( \tau_2 \); Table 2) representing the transient Na⁺ current (Fig. 1F) are well known [11], we

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**Fig. 4.** Multi-exponential decay function closely fits whole-cell \( I_{Na} \) recordings in human ventricular myocytes. (A) and (B) Four-exponential fit for a total \( I_{Na} \) (in gray) recorded at a low bath Na⁺ (5 mM) and shown as transient (\( I_{NaT} \)) and late (\( I_{NaL} \)) currents, respectively, at two different time scales. (C) Simplified two-exponential fit for \( I_{NaL} \) decay recorded with 140 mM Na⁺ in the bath. The respective fitting functions are shown as solid black lines, with a boxed numerical representation indicated by the arrows.
focused on the two new slow exponents with time constants \( \tau_3 \) and \( \tau_4 \), mainly comprising \( I_{NaL} \). \( I_{NaT} \) surge was eliminated with a short voltage pre-pulse of 5 ms to +50 mV \([1]\) and \( I_{NaL} \) decay (Fig. 4C) was fit to a two-exponential function beginning at 40 ms membrane depolarization, when any remaining TM activity is ceased (Fig. 3D). The average \% contributions and time constants for the two slow exponents determined from the whole-cell recordings in 24 cells from 3 hearts were: 48.0 \( \pm \) 4.7 ms and 42.5 \( \pm \) 1.6\%, 563 \( \pm \) 32 ms and 57.5 \( \pm \) 1.2\%, in line with the predicted time constants of 54.6 and 474 ms, respectively, for BM and LSM (see above). The nearly equal percent contribution by the two identified exponentials also fits very well with the predicted BM and LSM contributions at 40 ms membrane depolarization (48% and 52\%, respectively; Fig. 3D).

### 3.4. Voltage dependence of \( I_{Na} \)

We previously identified three voltage-dependent parameters in single-NaCh inactivation in human VM \([6]\). One was the second ("slow") voltage-dependent inactivation component of early openings, represented by the constant \( e \) in the kinetic scheme for the transient mode (Fig. 2A). This component was not examined further in the present study, as it relates to \( I_{NaT} \) and has already been characterized extensively in a variety of species including human cardiomyocytes \([9]\). The other two voltage-dependent parameters are related to the burst mode: the open time (\( s_o \)) and the slow component of distribution of close times (\( s_{C2} \)) within bursts \([6]\). We used a linear approximation of \( s_o \) and \( s_{C2} \) voltage dependency based on their respective linear regression lines from our previous data \([6]\), as follows (in ms and mV): \( s_o(V) = 0.062 \times V + 3.78 \) and \( s_{C2}(V) = 0.21 \times V + 12.5 \). These voltage dependencies were incorporated into the model (Fig. 2C) with \( b = 1/\tau_o(V) \) and \( d = \tau_{C2}(V) \), respectively. This modified model reproduces experimentally observed voltage dependence of NaCh bursts (Figs. 1F and 5A) and predicts that both \( P_{open} \) and average current decay accelerate with membrane depolarization (Fig. 5B and C), so that increasing gaps between envelopes (reflected by \( \tau_{C2} \)) have a greater effect on \( P_{open} \).
than longer openings within the envelope. The faster burst inactivation at higher voltages can readily be explained: by staying longer in a gap, the channel can more easily fall into the stable inactivated state $I_A$.

3.5. Temperature dependence of $I_{NaL}$

Increasing temperature from 23 to 33 °C reportedly accelerated $I_{NaL}$ decay from $\sim 143$ to $\sim 65$ ms ($Q_{10} \sim 2.2$), but did not change the ratio of $I_{NaL}$ to peak $I_{NaT}$ [12], indicating that human NaCh clone conductance had the same $Q_{10}$ for both currents. Assuming $Q_{10}=2.2$ for late NaCh gating and $Q_{10}=1.5$ [13,14] for NaCh conductance, $I_{NaL}$ simulations at 37 °C (Fig. 6) closely reproduced the $I_{NaL}$ decay acceleration observed in NaCh clone [12] and in our studies of canine ventricular myocytes (“gating” $Q_{10} \sim 2$; unpublished data).

3.6. $I_{NaL}$ of Na$_{A,1.5}$ and normal and failing hearts

Based on our single-channel data (Fig. 1E and F), we evaluated LSM and BM for Na$_{A,1.5}$ expressed in HEK293 cells, normal VM, and failing VM. Simulated current decays for both modes: BM and LSM were significantly slower in failing VM compared to either normal VM or Na$_{A,1.5}$ (Fig. 7) at $\sim 30$ mV. An important new prediction of the model was that the slower LSM gating in failing VM resulted in a larger $I_{NaL}$ amplitude (by $\sim 30\%$; 65 pA compared to 50 pA in normal VM) measured 200 ms after depolarization. Also, $I_{NaL}$ transferred significantly more Na$^+$ to failing cells (see inset in Fig. 7A). The total charge transferred by $I_{NaL}$ from 10 to 2000 ms was predicted as 28.5 and 45 pC for normal and failing VM, respectively, or a $\sim 58\%$ increase.

3.7. Model predicts amiodarone effect on $I_{NaL}$

As we reported previously [15], amiodarone effectively and selectively reduced amplitude ($I_{NaL}$ not $I_{NaT}$) and significantly accelerated decay of $I_{NaL}$ (see example in Fig. 8A). Our $I_{NaL}$ model (Fig. 2B) predicts that the only way $I_{NaL}$ decay could accelerate so dramatically is by speeding up the transition $I_5 \rightarrow I_6$ (controlled by constant $e$). However, when we adjusted constant $e$ to yield the experimentally observed acceleration, it resulted in only a relatively small drop in $I_{NaL}$ amplitude of $\sim 23\%$ (not shown) compared to 50% observed experimentally, indicating that amiodarone has a more complex effect on NaCh gating. One possibility that would explain remaining 27% to

![Graphs showing temperature dependence and amiodarone effect on $I_{NaL}$](https://example.com/graph.png)

Fig. 6. Model predicts acceleration of $I_{NaL}$ decay and increase in $I_{NaL}$ amplitude as temperature increases from 24 °C (A, B) to 37 °C (C, D). The figure shows simulated cumulative activity of 20,000 late scattered mode channels (LSM, Panels A and C) and 193 burst mode channels (BM, panels B and D) at different voltages indicated at the traces. All channels were available and activated upon depolarization. Channel numbers were chosen to correspond to a human ventricular myocyte (same as in Fig. 3). Gating schemes for LSM and BM are shown in Fig. 2B and C, respectively. Temperature dependence was explored using Q10 factors as discussed in the text. Single-channel currents for 24 °C were calculated with a conductance of 11 pS. Equilibrium Na$^+$ potential was calculated as $E_{Na}=(RT/F)\ln([Na]_o/([Na])$. The LSM currents were low-pass (100 Hz) filtered.
the total blocking effect is to assume that amiodarone stabilizes the inactivated state \( I_5 \), Fig. 8B) controlled by the constant \( d \). We have shown that amiodarone effectively interacts with the inactivated state of the late NaCh with the dissociation constant of 0.15 \( \mu \text{M} \) [15]. Thus, a combined change of \( e \) and \( d \) closely predicts the experimentally observed effect on \( \tau \) and \( I_{\text{NaL}} \) amplitude (Fig. 8). Future single-channel experiments will test this prediction.

**4. Discussion**

We believe the present study represents the first report of significantly slower gating of late NaCh in human failing VM. Also, based on our single-channel data, we developed the first stochastic numerical model of complete \( I_{\text{Na}} \) decay during long-lasting membrane depolarizations (up to 2 s) that included \( I_{\text{NaL}} \) in adult human VM. The model permitted important predictions about instant contributions of various gating modes to \( I_{\text{NaL}} \) and their modulation by voltage, temperature, pharmacological factors (such as amiodarone) and heart failure.

**4.1. Theoretical importance: comparison with previous models**

Böhle et al. [16] used Hodgkin–Huxley formalism [17] to describe ensemble-averaged currents produced by five distinct NaCh gating modes that comprise the fast peak component of \( I_{\text{Na}} \) in human VM (late NaCh was not examined). Another approach describing channel gating as a Markov process provides a much better approximation,
as it reproduces single-channel behavior. However, existing Markov models for the cardiac NaCh are incomplete as they only describe transient \( I_{\text{Na}} \) [18–21] and miss late NaCh activity lasting hundreds of milliseconds. The most recent Markov model for the human cardiac NaCh clone Na,1.5 [8] included a late component that faithfully described a persistent current produced by long-QT-related NaCh mutants. With respect to wild-type NaCh, that model also generated a substantial non-inactivated current (~50 pA for 100,000 channels, not shown) that is absent in whole-cell recordings of both human VM [1] and heterologously expressed Na,1.5 (our unpublished data) and also conflicts with single-channel latency data for scattered openings that become inactivated with time [6].

Our new Markov model for human cardiac NaCh prohibits transition \( I_5 \rightarrow I_3 \) (Fig. 2), ruling out persistent \( I_{\text{Na,L}} \) produced by LQT3 NaCh mutants [8]. The major advantage of the new model is that it handily predicts both single-channel kinetics of late NaCh (Fig. 2) and the complex composition of \( I_{\text{Na}} \) decay (Fig. 3), which is governed by a four-exponential decay process. All four exponentials predicted by the model were confirmed in the whole-cell recordings (Fig. 4), thereby validating the model and providing mechanistic insight into gating mode origin.

4.2. Interplay of gating mode contributions: paradoxes and their mechanisms

We discovered an interesting interaction among the contributions of the various gating modes to \( I_{\text{Na}} \) (Fig. 3D). Early after the onset of membrane depolarization, TM provides most \( I_{\text{Na}} \); however, its dominance is short-lived, as TM becomes completely inactivated after ~40 ms. All three modes have an almost equal contribution at ~16 ms when BM briefly takes the lead. After ~33 ms BM progressively decreases so that \( I_{\text{Na}} \) is formed entirely by LSM after ~300 ms. This is a paradoxical outcome, as it means most \( I_{\text{Na,L}} \) is produced by relatively rare scattered openings (Fig. 1B) rather than bursts characterized by abundant channel activity (Fig. 1C). We believe this is because bursts occur in a much smaller fraction of the NaCh than LSM (~0.193% compared to 20%, respectively) and they are inactivated much faster (with an apparent decay time constant of ~50 vs. 500 ms). Furthermore, the contribution from the bursting channels is expected to decrease with depolarization (Figs. 5B, C, and 6B, D).

Another important and unexpected finding is that despite a much smaller channel population operating in LSM compared to TM (20% vs. 79.8%) and a much smaller scale of \( I_{\text{Na,L}} \) compared to \( I_{\text{Na,T}} \), LSM and TM channels transfer almost the same amount of Na⁺ through the plasma membrane during 2 s depolarization. We integrated the respective simulated currents for a cell with \( 10^5 \) NaCh (similar to Fig. 3) and found that TM, LSM, and BM transferred total electrical charges of 42, 45 and 7 pC, respectively. In other words, the \( I_{\text{Na,T}} \) peak is about 3 orders of magnitude larger than \( I_{\text{Na,L}} \) (50 nA compared to 50 pA), yet its span is about 3 orders of magnitude shorter (2 ms compared to 2 s), resulting in both modes transferring almost the same total charge.

4.3. Physiological importance

We have previously reported the effect of ion currents produced by NaCh operating in BM and LSM on the AP plateau in human VM, suggesting that these gating modes are important for both plateau support and prolonging AP [6]. The present study evaluated populations of NaCh operating in different gating modes and examined the time, voltage, and temperature dependence of Na⁺ currents originating from different gating modes. These results provide a new theoretical basis for future modeling of AP in human VM, with predicted contributions for the NaCh gating modes to various phases of AP.

Previous studies suggested that NaCh could reopen after activation and thus contribute to the AP plateau [22]. The present study shows that not all NaCh re-openings contribute to the AP plateau. Both TM and LSM are capable of generating re-openings (Fig. 2A and B), but only LSM re-openings are sustained during membrane depolarization (up to ~1 s; Fig. 3C), thus contributing to the entire AP plateau. The current produced by BM might be important for the very early phase of the AP plateau, as its contribution to total \( I_{\text{Na}} \) (evaluated by the model) would be greatest ~30 ms after membrane depolarization (Fig. 4) and fade away almost completely after 300 ms. In contrast to LSM, BM gating shows a strong voltage dependence (Fig. 1F). Since its \( P_{\text{open}} \) decreased with membrane depolarization (Fig. 5), the BM current could be expected to contribute to AP only at relatively low voltages. Thus, only those NaCh operating in LSM and BM, not TM, contribute to the AP plateau.

The model provides important predictions that \( I_{\text{Na,L}} \) composed of LSM and BM has greater amplitude at 37 than at 24 °C (Fig. 6), and its duration is comparable with AP plateau (~250 ms) [1] in human VM at the physiological temperatures. Accordingly, \( I_{\text{Na,L}} \) is expected to be significantly larger at 37 °C, and may have an even greater impact on the AP plateau than at 24 °C as we suggested previously [6]. Our earlier reports of the profound effects of partial blockade of \( I_{\text{Na}} \) by TTX, STX, and lidocaine on AP duration at 37 °C in human and canine VM indirectly support this concept [1,3].

4.4. Clinical and pharmacological importance

We found significantly different gating of late NaCh in VM of patients with heart failure as compared to normal individuals (Fig. 1E, F). Our numerical model of \( I_{\text{Na,L}} \), utilizing the experimentally determined changes in the gating modes, predicted the augmented and slower whole-
cell $I_{\text{NaL}}$ (Fig. 7) already reported for VM in human failing hearts and canine models of heart failure (both diffused infarction and pacing models) [2–4]. An important result of the numerical model is that it suggests a mechanism for the increased $I_{\text{NaL}}$ (despite reduced peak $I_{\text{NaT}}$ [23]) that could result from an increased fraction of NaCh operating in LSM with a slower transition (Figs. 1E and 7A) to the “deep” inactivated state $I_e$ (Fig. 2B), and/or an increased fraction of NaCh operating in BM with a slower transition (Fig. 1F) into state $I_s$ (Fig. 2C), increasing both the number of bursts per channel and burst length [6].

The suggested changes in BM and LSM can indeed modulate the AP plateau, as the two gating modes are operable in failing human VM at a membrane potential of −10 mV, comparable to take-off voltages for the early afterdepolarization (from −18 to −2 mV) [6]. Furthermore, an 58% increase in Na+ influx via a larger and slower $I_{\text{NaL}}$ in failing hearts (see $I_{\text{Na}}$ integral in Fig. 7A, inset) may alter [Na+], homeostasis, resulting in enhanced Ca2+ influx through the Na+/Ca2+ exchanger and support the positive role of the sarcoplasmic reticulum Ca2+ load in force development at slow heart rates. In contrary, at high heart rates, increased $I_{\text{NaL}}$ can lead to diastolic Ca2+ overload and poor VM contraction [24]. These data indicate that our models of NaCh gating for LSM and BM could prove important for future computer simulations of AP remodeling and Ca2+ handling in heart failure.

We previously showed that amiodarone accelerated $I_{\text{NaL}}$ and preferentially blocked $I_{\text{NaT}}$ compared to $I_{\text{NaL}}$ [15]. Our model predicts that this effect is due to a complex preferential interaction between amiodarone and NaCh in the LSM mode, both accelerating the transition into the second inactivation state ($I_s\rightarrow I_{\text{NaL}}$) and stabilizing the first inactivation state $I_e$ (Fig. 8). This effect of amiodarone suggests that different NaCh gating modes in human VM may represent separate new pharmacological targets. Our $I_{\text{NaL}}$ model may provide a valuable tool to advance pharmacological interactions with NaCh operating in different gating modes.

4.5. Study limitations

Data were obtained from a limited number of normal human hearts ($n=3$), as donor hearts rarely become available for study. Data obtained from HEK293 cells expressing Na1,1.5 may only partially simulate the native channel. The difference between cardiomyocytes and HEK293 cells in the membrane environment (such as the membrane underlying cytoskeleton) as well as auxiliary subunit composition, cell signaling, and other factors may affect channel gating. Multi-channel patches allow us to describe some, but not all characteristics of the NaCh gating modes comprising $I_{\text{NaL}}$. Our analysis was performed on mid-myocardial VM to avoid possible deviations due to $I_{\text{NaL}}$ dispersion through the wall of the left ventricle [25,26]. How and if the multi-mode composition of $I_{\text{Na}}$ relates to the $I_{\text{NaL}}$ transmural profile awaits further study.

Limitations of the model include the absence of NaCh reactivation and the failure to explain the transition mechanism underlying different gating modes. Böhle et al. [16] reported transitions between modes of high-probability NaCh underlying peak sodium current within one trace, but the time course of switching was not resolved. The problem with resolving mode switches is that there must be only one channel in the patch, making it almost impossible to assess the low-probability late NaCh.

5. Conclusions

Sodium current decay in human ventricular myocytes is governed by a four-exponential decay process resulting from gradual termination of activity of Na+ channels operating in three gating modes: transient, burst and late scattered. Heart failure significantly slows late NaCh gating, both bursts and scattered openings, resulting in much greater Na+ influx during prolonged membrane depolarization. The concept of a multi-mode composition for $I_{\text{NaL}}$ provides a new rationale for $I_{\text{NaL}}$ modulation by factors such as voltage, temperature, pharmacological agents, and pathological conditions.

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