Divergent action potential morphologies reveal nonequilibrium properties of human cardiac Na channels

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Abstract

Objective: Fast inward Na current (\(I_{Na}\)) carried by the voltage-gated Na channel (NaV1.5) is critical for action potential (AP) propagation and the rapid upstroke of the cardiac AP. In addition, a small fraction of NaV1.5 channels remains open throughout the plateau of the AP, and this current is termed as late \(I_{Na}\). In patients with mutant NaV1.5-based congenital long Q–T (LQT) syndrome, mutant channels pass more late \(I_{Na}\) compared to wild-type channels in unaffected patients. Although LQT mutant NaV1.5 channels are well studied, there is no careful evaluation of the effects of cardiac APs on early and late current. This is important with the recent documentation of nonequilibrium \(I_{Na}\).

Methods: We measured AP-stimulated \(I_{Na}\) through NaV1.5 wild-type and two LQT mutant channels (\(\Delta KPQ\) and \(N1325S\)). Three distinct AP morphologies were used: human embryonic stem cell-derived cardiac myocyte (hES-CM) APs with a relatively slow upstroke and canine endocardial and epicardial ventricular myocytes with rapid upstrokes.

Results: All three APs elicited both early and late \(I_{Na}\). For wild-type NaV1.5, the hES-CM AP elicits more early and late \(I_{Na}\) than either the endocardial or epicardial AP. The mechanism for this difference is that the hES-CM has a relative slow \(dV/dt_{max}\) that causes a maximal open channel probability. Slower upstroke stimulation also allows greater Na flux through wild-type and \(N1325S\) channels, but not the \(\Delta KPQ\) mutant.

Conclusions: The inherent gating properties of NaV1.5 provide natural tuning of optimal \(I_{Na}\) density. Slower upstroke velocities can yield more \(I_{Na}\) and Na flux in some NaV1.5 variants.

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1. Introduction

Voltage-gated Na channel activity provides a pathway for inward current to promote a rapid upstroke of the action potential (AP) in excitable cells. In the heart, the rapid inward Na current (\(I_{Na}\)) is carried by the NaV1.5 Na channel, which opens quickly in response to a depolarization. In addition to carrying the classical fast and transient inward
the late $I_{Na}$ to contribute to AP duration (APD) in animal models [4,5]. Decrease of $I_{Na}$ by TTX causes APD shortening [6,7], while increased late current significantly lengthens the AP and may cause severe arrhythmia [8,9].

The most compelling reason to study late NaV1.5 current arose from the identification of long Q–T (LQT) syndrome linkage to NaV1.5 mutations. There are now >20 known mutations of NaV1.5 expressed by LQT patients [10]. Most of these mutants exhibit more late $I_{Na}$ compared to consensus wild-type Na channel late $I_{Na}$. Late $I_{Na}$ can prolong APD nonuniformly from cell to cell by virtue of the stochastic nature of rare late openings and can directly contribute to AP dispersion [7]. Dispersion of APD across the ventricular wall allows the development of severe arrhythmias by triggered automaticity and reentry [11], increasing the lethality of LQT. Thus, it is imperative to gain a complete understanding of the factors that contribute to late NaV1.5 current. Although many thorough studies have been published about the biophysical properties and genetic changes of NaV1.5 [10,12,13], few have considered the effects of AP morphology on NaV1.5 current. The vast majority of the biophysical characterization of LQT mutant NaV1.5 channels uses square voltage-clamp steps to describe channel function. However, the complex gating mechanism of NaV1.5 causes these channels to gate differently, stimulated by voltage protocols that mimic APs than those from simple step clamps [8].

The goal of this study was to use distinct APs instead of square wave protocols to determine the response of heterologously expressed wild-type and LQT mutant NaV1.5 channels. Human embryonic stem cell-derived cardiac myocyte (hES-CM) APs, canine endocardial APs, and canine epicardial ventricular APs were recorded and used to stimulate heterologously expressed NaV1.5 channels. We chose the AP of hES-CM because the hES-CMs are an important system for use in cell-based regenerative cardiac therapy, and the very incomplete characterization of hES-CM function is one of the several obstacles to realize the therapeutic utility of these cells. Canine epicardial and endocardial APs reflect, in part, ventricular wall AP heterogeneity. These APs have different shapes, durations, and slopes of phase 0; therefore, they provide distinct nonequilibrium conditions for sodium channels.

In addition to the wild-type sodium channel (hH1a) [14], we expressed thoroughly studied mutant channels AKPQ and N1325S in HEK 293 cells. The AKPQ mutant is an in-frame deletion of three amino acids in the intracellular linker between domains III and IV, while N1325S is a point mutation located in the S4–S5 linker. Both regions are involved in the inactivation of the sodium channel. Consistent with the role of these regions, AKPQ and N1325S mutations cause the defect of inactivation and allow repeated channel reopening during depolarization, evoking late $I_{Na}$ [10]. In this study, we show unique responses of NaV1.5 that are not apparent in traditional voltage-clamp experiments using square steps or voltage ramps.

2. Materials and methods

2.1. Cell transfection and culture

HEK 293 cells were transfected with 0.006 mg of cDNA encoding NaV1.5 channels. The wild-type channel was hH1a; DKPQ and N1325S mutants were on the hH1a backbone.

2.2. Measurement of APs

Single ventricular myocytes were isolated from canine hearts using collagenase enzyme as described earlier [15]. Human undifferentiated ES cells of the clone H9.2 [16] were grown on mouse mitotically inactivated (mitomycin C) embryonic fibroblast feeder layer as previously described [16,17]. To induce differentiation, hES cells were cultured in suspension for 7–10 days, where they aggregated to form EBs. At 22–35 days, beating areas were dispersed into isolated cells for AP measurement.

APs of single canine cells were recorded using large-resistance (30–60 MΩ), 3-mol/l KCl-filled glass microelectrodes coupled to the input of an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA). The canine cells were continuously paced at 1 Hz frequency using 1-ms-wide rectangular current pulse with 120% threshold amplitude. On hES-CM, the spontaneous APs were recorded with a patch electrode also at 120% threshold amplitude. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US NIH.

2.3. Measurement of $I_{Na}$

$I_{Na}$ of transfected HEK 293 cells was recorded using the whole cell mode of patch-clamp technique. The cells were perfused with Tyrode solution (containing, in mmol/l: NaCl 140, KCl 5.4, CaCl2 1.8, MgCl2 1.0, HEPES 10, and glucose 10, at pH 7.4) at room temperature (20–24 °C). Electrodes were prepared from borosilicate glass and filled with solution containing (in mmol/l): CsCl 140, NaCl 5, EGTA 10, Mg,ATP 3, and HEPES 10, pH 7.36, with CsOH. The signals were amplified and filtered at 10 kHz by Axopatch 200 B amplifier. The pulse generation and data collection were controlled by pClamp 8.1. and Digidata 1200 A/D card (Axon Instruments) digitized the data. The sampling rate was 50 kHz. To maximize voltage control for $I_{Na}$, we used low-resistance pipettes (1.0–1.5 MΩ), series resistance compensation, and small spherical cells without visible processes. The series resistance was 2–6 MΩ before compensation (usually 70%). The cells without graded shape of activation were excluded from analysis.

Single channel sodium currents ($I_{Na}$) were measured in the cell-attached patch of transfected HEK 293 cells. The membrane potential was nullified by a high K+ external solution containing (in mmol/l): 140 KCl, 2.0 MgCl2, 10...
the amplitude of $I_{\text{Na}}$ was augmented using high Na$^+$ pipette solution (containing, in mmol/l: 300 NaCl, 0.5 MgCl$_2$, 1.8 CaCl$_2$, 10.0 HEPES, pH 7.30, with NaOH). The signals were sampled at 200 kHz and filtered at 5 kHz. The leak and capacitive transients were removed by subtracting the average of null traces of the same patch. Unitary channel amplitude was obtained by fitting the Gaussian distribution of amplitude histogram. The data analysis was performed using Clampfit 9.0 software.

3. Results

3.1. Measurement of late current

To approximate the late $I_{\text{Na}}$ during the repolarization phase of cardiac APs, we used different AP waveforms as a voltage-clamp command. The applied APs were previously recorded from human embryonic cardiac stem cells (hESC-CM), or canine endocardial (ENDO) or canine epicardial (EPI) cells. HEK 293 cells have outward rectifying endogenous current [18]; however, in our experiments, $<300$ pA endogenous current was activated by different AP waveforms. Most importantly, the amplitude of his endogenous current was independent of the holding potential ($V_{\text{hold}}$). To eliminate the endogenous current of HEK 293 cells, the AP waveforms were applied from $V_{\text{hold}}$ of $-120$ and $-60$ mV every 5 s. At $V_{\text{hold}}$ of $-60$ mV, $I_{\text{Na}}$ was fully inactivated (Fig. 1). This current trace was digitally subtracted from a current recorded from a $V_{\text{hold}}$ of $-120$ mV. The obtained difference currents are shown below the different AP waveforms in Fig. 2.

During the upstroke of the AP, a large inward $I_{\text{Na}}$ flows through open Na$^+$1.5 channels. For a square voltage step to a potential corresponding to the mean AP plateau, this $I_{\text{Na}}$ inactivates nearly completely within 20 ms. For longer sustained depolarization duration, however, the channels continue to open and close, and the current through the channels can be seen as a small late current. In hH1a and N1325S transfected cells but not for $\Delta KPQ$ mutants, the hES-CM AP elicited a larger peak $I_{\text{Na}}$ than ENDO or EPI APs. The peak $I_{\text{Na}}$ amplitudes were as follows (hES-CM, ENDO, and EPI): hH1a, 5.9±0.6, 2.1±0.3, and 2.4±0.4 nA; N1325S, 5.4±0.7, 3.5±0.2, and 3.0±0.5 nA; $\Delta KPQ$, 0.5±1, 1.7±0.2, and 1.8±0.3 nA.

The different AP waveforms elicited late current for all three mutants. To better demonstrate the late current, the amplitude axis is expanded in the lower panels of Fig. 2. Late current was present during the length of the applied APs, until the end of phase 3 repolarization. The amplitude of late current was not constant but it increased during the process of AP repolarization. These general characteristics are elicited by all three AP types and by all channel types, although they are most apparent for the $\Delta KPQ$ channels (Fig. 2, bottom row).

To quantify the late current, the amplitudes were measured at a time when the AP command waveform repolarization phase reached 0, $-10$, $-20$, $-30$, and $-40$ mV. In case of the EPI AP, the membrane potential reaches 0 mV several times; therefore, on Fig. 2, an arrow indicates the time when the late current amplitude was measured. The current traces of Fig. 2 and the data of Fig. 3A show that the same AP elicited a larger late current on $\Delta KPQ$ channels than on hH1a and N1325S mutants. It is also obvious that ENDO and EPI APs could evoke smaller late current than the hES-CM AP.

The late current measured at 0 mV (repolarization phase) was expressed as a percentage of corresponding peak current and plotted on Fig. 3B. In hH1a and N1325S mutants, the late current densities were only 0.35–0.72% of peak $I_{\text{Na}}$. There was no significant difference in the late/peak current ratio for the different AP waveforms in these mutants. This indicates that the larger peak $I_{\text{Na}}$ predicts larger late current in hH1a and N1325S mutants. In $\Delta KPQ$ mutants, the late current amplitudes elicited by hES-CM, ENDO, and EPI AP waveforms were $12.0±2.6\%$, $2.5±2\%$, and $2.7±5\%$ of the peak current, respectively. These relative late/early values are much higher than that of the other mutants because in $\Delta KPQ$ mutant, the peak $I_{\text{Na}}$ was smaller while the late current amplitude was larger than in other studied mutants.
3.2. Sodium current amplitude depends on ramp duration

Although the maximal upstroke velocity and the overshoot of hES-CM AP are smaller than that of the ENDO and EPI AP, the hES-CM AP elicited larger peak and late $I_{Na}$ in hH1a and N1325S mutants. To systematically evaluate this discrepancy, we applied a series of ramp pulses. The ramps were applied from $V_{\text{hold}}$ –120 to –60 mV in 5-s intervals. The current trace recorded from a $V_{\text{hold}}$ of –60 mV was digitally subtracted from a current recorded from a –120 mV holding potential. The difference currents are shown below the AP waveforms. The time scale of APs and current traces presented in this figure are identical. For comparison of the late current the current of hH1a, N1325S, and ΔKPQ mutants are shown on an extended scale. Dashed lines indicate 0 and –40 mV. For the epicardial AP, an arrow indicates 0 mV repolarization analyzed below.
subtract the endogenous current of HEK cells, the current traces recorded from −60 mV holding potential were digitally subtracted from data obtained from −120 mV holding potential. These difference currents are shown in Fig. 4A. After the digital subtraction for a square pulse, we cannot detect late current neither for hH1a nor for N1325S and ΔKPQ mutants. To evaluate the effect of voltage rate of rise on $I_{Na}$ amplitude, we normalized the responses elicited by a ramp to that elicited by a square pulse (control). Such normalized data were plotted as the function of applied ramp duration (Fig. 4B). An increase of ramp duration increases the $I_{Na}$ amplitude for all three mutants (Fig. 4). Maximal $I_{Na}$ was elicited by durations of 4.5 ms for hH1a channels and 6 ms for the N1325S. The maximal $I_{Na}$ of hH1a and N1325S channels was 195±4% and 227±27% of the square pulse elicited current, respectively. Longer ramps caused a progressive reduction in the $I_{Na}$ amplitude, but peak $I_{Na}$ remained larger than the control in the studied duration range. By contrast, ΔKPQ channels’ maximal $I_{Na}$ was obtained for 2 ms ramp duration, and the current amplitude was only 14±5% larger than the control. When the ramp duration was longer than 2 ms, the current amplitude decreased monotonically, and at 28 ms ramp duration, the current amplitude of ΔKPQ channel was reduced to only 10±2% of the control.

The area of the Na current is an index of the amount of sodium ion fluxing across the channel. To evaluate the amount of Na flux for a given ramp, the integral of the individual measured current traces was calculated (Fig. 4C). In hH1a and N1325S channels, the integral of current traces increased monotonically. As a result, slower upstroke $dV/dt$ elicits more Na flux. For example, for a 6-ms ramp duration, the integral of $I_{Na}$ of hH1a and N1325S mutants are approximately three times larger than the integral of the current elicited by a square pulse, respectively. In contrast, if the ramp duration was less than 7 ms, there was no change in the integral of sodium current of ΔKPQ mutants, but longer ramps caused the reduction of ΔKPQ peak $I_{Na}$ amplitude and the integral of the current.
A 6.0-ms ramp corresponds to the duration of the rising phase of the hES-CM AP, while the 0.5-ms ramp refers to the time for the upstroke of ENDO and EPI APs. The results detailed in Fig. 4 explain our previous finding that for hH1a and N1325S mutants, the hES-CM AP elicits larger \( I_{Na} \); while for the \( \Delta KPQ \) mutant, the hES-CM AP elicits smaller \( I_{Na} \) than ENDO or EPI APs.

3.3. Slower dV/dt induces larger \( I_{Na} \) via increased \( P_{o} \) and increased driving force

The behavior of \( I_{Na} \) in response to ramp pulses was unexpected; therefore, we examined the mechanism employing a well-established Na channel kinetic model. There are two mechanistic explanations for this \( I_{Na} \)
amplitude dependence on upstroke \( \frac{dV}{dt} \). The first is that the peak current is reached at a potential corresponding to increased driving force compared to that following a square step. The second mutually exclusive explanation is that channel open probability \( P_o \) has a maximum for an intermediate upstroke \( \frac{dV}{dt} \). To evaluate whether the alteration of driving force or the alteration of channel gating is responsible for these observations, we used the kinetic model of \( hH1a \) sodium channel developed by Clancy et al. [8] and Luo and Rudy [19]. Using this published model, a series of simulated sodium currents was obtained in response to ramp pulses ranging from \(-120\) to \(+20\) mV. The applied ramp durations were 0, 2, 5, 10, 15, 20, and 30 ms.

Fig. 5A shows that the simulated \( I_{Na} \) (Fig. 5A) mimics recorded data. The model predicts a 63% increase in the

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Fig. 5. Modified Luo–Rudy cell model was used to simulate \( hH1a \) sodium current (A) and the alteration of its open probability during ramp pulses (B). Similarly to our experiments, simulated ramps from \(-120\) to \(+20\) mV were applied to elicit \( I_{Na} \). The durations of the applied ramps were 0, 2, 5, 10, 15, 20, and 30 ms. (C) The driving force of peak \( I_{Na} \) is calculated as the difference between the actual ramp potential and the reversal potential of \( I_{Na} \). The reversal potential for \( I_{Na} \) is +69 mV. The insert shows a 30-ms-long ramp pulse and the corresponding simulated current.
current amplitude, which is smaller than what we obtained on hH1a-transfected cells. The simulated current is maximal for a 5-ms ramp, which is close to the measured 4.5 ms. In contrast, $P_o$ initially increases with ramp duration and is maximal for a 2-ms ramp, and $P_o$ then tends to decrease before the $I_{Na}$ is maximal (Fig. 5B). However, this decrease in $P_o$ is compensated by the increase in the driving force on sodium ion (Fig. 5C). The driving force on sodium ion was calculated as a difference of sodium reversal potential and the actual membrane potential. When a square pulse is applied (0 ms ramp duration), the peak current occurs at +20 mV. In the case of ramps, the peak current develops before the ramp potential reaches +20 mV. For more shallow ramps, the peak develops at more negative potentials. The insert of Fig. 5C shows how we determined the membrane potential corresponding with peak $I_{Na}$. The insert displays a 30-ms-long ramp pulse from $-120$ to +20 mV. $I_{Na}$ develops in response to the stimulus and it reaches its maximum 19.8 ms after the onset of ramp. At this time, the ramp potential is $-27.3$ mV. In the model, the Na$^+$ reversal potential was +69 mV; therefore, the actual driving force for the current is +96.3 mV. For longer ramp durations, the larger driving force does not compensate the decrease of $P_o$ of NaV1.5 and the current amplitude decreases. Thus, the applied model predicts that the rate of the upstroke $dV/dt$ regulates $I_{Na}$ amplitude by regulation of channel gating and as a consequence of variable driving force at the time to peak $I_{Na}$.

3.4. Slower $dV/dt$ increases $P_o$ but does not influence the inactivation of Na$^+$ channel

Computer modeling predicts that ramps, in comparison to square steps, elicit a higher $P_o$. To directly test this, we performed single channel recordings. In the same patch, we compared unitary openings in response to ramps versus traditional square command steps. To activate unitary Na$^+$ channel openings, square wave and 5-ms ramp pulses were applied from $-140$ to +20 mV. Openings mainly occurred during the onset of square wave pulse (Fig. 6A). Similarly to the macroscopic current, the 5-ms ramp pulse elicited maximal channel activity before the ramp reached +20 mV. The unitary current amplitude elicited by a square wave pulse ($0.91\pm0.05$ pA) was slightly smaller than the amplitude elicited by a 5-ms ramp ($1.21\pm0.14$ pA) because ramp-induced openings occurred at higher driving force. The open-dwell time histograms were fitted by monoexponentials to evaluate effects on fast inactivation. There is no significant difference of mean open times stimulated by square wave versus by 5-ms-long ramp pulse ($\tau=87\pm8$ and $94\pm2.6$ ms, respectively; Fig. 6B). The probability of sodium channel opening was lower for the square wave ($0.18\pm0.03$) than for the ramp ($0.26\pm0.04$; Fig. 6C). These data show that the $I_{Na}$ amplitude dependence on upstroke $dV/dt$ is determined by a combination of $P_o$ and driving force, rather than effects on open state inactivation.

3.5. Steady-state inactivation of late current

Steady-state availability (conversely inactivation) is an important determinant of $I_{Na}$ amplitude. Our subaim was to compare the voltage dependence of steady-state inactivation of peak and late current elicited by either AP waveform or square pulses. The cells were clamped to various potentials between $-140$ and $-60$ mV for 5 s. This prepulse was followed by the test pulse consisting of either a 100-ms square step to 0 mV, or the hES-CM AP. The amplitude of late $I_{Na}$ elicited by the hES-CM AP was measured at the different repolarization potentials of the AP waveform as in
Fig. 3. Peak and late currents were normalized to the maximum amplitude. The data were plotted against the respective prepulse potential and fitted to a Boltzmann function to obtain a midpoint ($E_{0.5}$) and slope factor. Neither the midpoint of availability nor the slope factor was significantly different among the studied mutants (Fig. 7). Although the midpoint of peak $I_{Na}$ elicited by rectangle pulses was more negative than the $E_{0.5}$ of peak $I_{Na}$ elicited by the AP waveform, the difference was not significant. In the $hH1a$ channel, the voltage dependence of steady-state inactivation of late current measured at 0 mV repolarization is significantly shifted toward negative membrane potentials ($p<0.05$, $n=7$), but in the other mutants, the shift was statistically not significant. These subtle effects of AP versus square wave stimulation on steady-state inactivation suggest that AP morphology regulation of steady-state inactivation is not a major distinguishing determinant of channel, or mutant channel function.

4. Discussion

The main finding of this study is that a commonly utilized paradigm of square pulses for evaluation of $I_{Na}$ does not elicit maximal early and late $I_{Na}$. Unexpectedly, there is an optimal $dV/dr_{max}$ of the upstroke of the AP that activates a maximal early and late $I_{Na}$. Thus, we conclude that AP upstrokes with intermediate—or even relatively slow—upstrokes maximize both early and late $I_{Na}$.

A small late $I_{Na}$ is present in ventricular myocytes of different species [2,20,21], and a single population of sodium channel underlies both fast and late $I_{Na}$ [1,2]. Although the amplitude of late $I_{Na}$ is less than 1% of the peak $I_{Na}$, it significantly contributes to the development of the AP plateau [4–6]. Direct proof for this is APD shortening by a decrease of $I_{Na}$ by TTX. [6,7]. In contrast, increased late current significantly lengthens the AP and may cause severe arrhythmia [8,9]. Mutations in the SCN5A gene are associated with such increased late current [10]. It was originally reported that mutation-induced gain of late current is the consequence of either the transient inactivation failure of sodium channel [22], or steady-state channel reopening [23]. More recently, however, Clancy et al. [8] reported an alternative mechanism. If the sodium channel recovers faster from inactivation, then the nonequilibrium condition during repolarization enhances channel reopening. The key to this principle is that the dynamics of the time dependence and voltage dependence for the development and recovery from inactivation is highly dependent on the dynamics of the voltage. Thus, the existence of non-equilibrium gating necessitates the use of APs to evaluate wild-type or mutant Na channel function.
In agreement with the previous findings in our experiments, the hH1a variant had the smallest late current while the ΔKPQ mutant had the largest one. The major difference of our finding from previous reports was that ΔKPQ late current elicited by hES-CM AP was as high as 12% of peak current, while Nagatomo et al. [18] reported 0.76%, 2.6%, and 4.0%, respectively. This discrepancy can be related to the time-dependent and voltage-dependent property of late current.

Surprisingly, the major determinant regulating iNa amplitude was the upstroke velocity. For the three AP types tested, human ES-CM APs had the slowest maximum upstroke velocity (dV/dtmax) yet yielded the largest peak and late iNa compared to mature canine epicardial or endocardial ventricular APs. However, hH1a and N1325S mutants displayed similar ratios of late/peak current for all three APs indicating that larger early current predicts larger late current.

Our results show that an optimal upstroke velocity exists where the alteration of PNa and the driving force on the Na ion conspire to yield maximal iNa. The optimal ramp durations to +20 mV were 4.5 and 6 ms for hH1a and N1325S mutants, respectively. These ramps nearly doubled the iNa of these mutants compared to the square-wave-elicited current. This behavior of sodium channels can be observed in native cardiomyocytes, too. In isolated heart cells of 10-day-old embryonic mice, the maximal iNa was elicited by 4-ms-long ramp from −80 to +20 mV, and it was 259±22% of the current of square wave pulse (not shown). In contrast to these findings in ΔKPQ current, the optimal 2-ms-long ramp caused only a 14±5% increase and we observed a large reduction in peak iNa with slower ramps. This unexpected finding argues for atypical activation gating in concert with the well-described disruption of inactivation in ΔKPQ channels.

Our results may have interesting physiological implications. In tissues with relatively slow AP upstroke dV/dt, maximal iNa will be elicited. This increased iNa will in turn speed dV/dtmax, which in turn will stimulate a submaximal iNa amplitude. Thus, natural or inherent tuning of Na channel kinetics provides maximum PNa for optimal upstroke velocities.

Slower dV/dtmax increases not only the amplitude of iNa but also sodium influx. Under pathological conditions, this may contribute to the sodium overloading of cardiomyocytes. This hypothesis is supported by those findings which show that voltage-gated sodium channels significantly contribute to the sodium overload of cells in the initial phase of ischemia [24]. Sodium overloading is an early step in a cascade leading to a rise in intracellular calcium, which may cause arrhythmia in cardiac myocytes.

Finally, our results show that late iNa exists during the whole length of repolarization of different APs. The amplitude of late current correlates with the amplitude of early current; thus, early current predicts larger late current, except for the ΔKPQ mutant. Our finding is a reasonable extension of the recent description of nonequilibrium iNa from NaV1.5 wild-type and mutant channels. These earlier studies of nonequilibrium gating only considered the rate of repolarization using voltage ramps. In our experiments, measured APs were applied. We were able to ascribe a clear current amplitude dependence on upstroke velocity. Late iNa increases along with early current. Therefore, it follows that midrange upstroke velocities will elicit maximal late current. Thus, heterogeneity of upstroke velocity among heart cells will lead to differing amounts of late iNa which in turn will result in dispersion of APD.

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