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CaMKII-dependent Activation of Late I_{Na} Contributes to Cellular Arrhythmia in a Model of the Cardiac Myocyte

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Abstract—**Cardiac voltage-gated Na^+ channels underlie membrane depolarization during the upstroke of the action potential (AP). These channels also exhibit a late, slowly-inactivating component of current (late I_{Na}) that may be enhanced under pathological conditions such as heart failure, and may therefore promote AP prolongation and increase the likelihood of arrhythmia. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) functionally modifies Na^+ channels, however it remains unclear if the CaMKII-dependent changes in late I_{Na} are a major contributor to cellular arrhythmias such as early after depolarizations (EADs). In this study we develop a model of I_{Na}, including CaMKII-dependent effects, based on experimental measurements. The Na^+ channel model is incorporated into a computational model of the whole myocyte which describes excitation-contraction coupling via stochastic simulation of individual Ca^{2+} release sites. Simulations suggest that relatively small augmentation of late I_{Na} is sufficient to significantly prolong APs and lead to the appearance of EADs.**

I. Introduction

CARDIAC voltage-gated Na^+ channels allow for the rapid influx of Na^+ into cardiac myocytes which drives membrane depolarization. These channels exhibit fast activation kinetics, and the inward Na^+ current (I_{Na}) is responsible for the rapid upstroke at the start of the cardiac action potential (AP). These Na^+ channels may also play an important role in setting the AP duration (APD). Mutations in the gene encoding the α-subunit of the Na^+ channel have been associated with long-QT syndrome as well as Brugada syndrome [1, 2]. Mutant channels exhibit slower recovery from inactivation as well as a negative shift in voltage-dependent availability. In addition, these channels exhibit "late I_{Na}", a relatively small persistent inward current that fails to fully inactivate during the AP and likely contributes to APD prolongation. Furthermore, studies have demonstrated that enhancement of late I_{Na} under conditions of oxidative stress, as may occur in heart failure, leads to AP prolongation and cellular arrhythmias such as early after depolarizations (EADs) [4].

Ca^{2+}/calmodulin –dependent protein kinase II (CaMKII) is a Ca^{2+}-dependent protein that regulates the function of a number of transporters, pumps, and ion channels via protein phosphorylation. CaMKII activity has been linked to alterations in Na^+ channel behavior and its expression has been shown to be increased in heart failure [5]. Recently, a number of notable studies have elucidated functional effects of CaMKII-mediated Na^+ channel phosphorylation. Deschenes et al [6] cloned Na^+ channels in HEK293 and C2C12 cells and found that the CaMKII inhibitor KN-93 (but not the CaMKII inhibitor AIP) slowed current decay, shifted steady-state inactivation in the depolarizing direction, and slowed the kinetics of entry into inactivated channel states. Maltsev et al [7] found that in canine ventricular myocytes, late I_{Na} decay was accelerated by KN-93 in the presence of elevated cytosolic Ca^{2+} levels. Wagner et al [8] studied the effects of adenovirus mediated and transgenic CaMKII over-expression and observed that CaMKII over-expression caused a negative shift in the voltage dependence of Na^+ channel availability, enhanced intermediate inactivation, slowed recovery from inactivation, and increased the amplitude of late I_{Na}. The latter results form the basis for a CaMKII-dependent Na^+ channel computational model by Grandi et al [9].

While these previous experimental results revealed the functional importance of CaMKII-mediated phosphorylation of Na^+ channels, the acute effects of CaMKII on channel function remained unclear. Recently, Aiba et al [3] studied the direct effects of CaMKII on Na^+ channels in guinea pig ventricular myocytes. Using whole cell patch clamp techniques, they found that CaMKII-dependent phosphorylation caused a positive shift in the voltage dependence of Na^+ channel availability, hastened recovery from inactivation, and decreased intermediate inactivation. In addition, Aiba et al [3] also found that CaMKII-dependent phosphorylation increased late I_{Na}. These recent results are the basis for the Na^+ channel state model presented in this study, which describes the behavior of unphosphorylated and CaMKII-phosphorylated Na^+ channels based on a pre-existing model [9]. It is further constrained by voltage- and temperature-specific I_{Na} kinetics, and it accounts for the acute effects of CaMKII phosphorylation on Na^+ channels. Furthermore, it has been integrated into our computational model of the canine ventricular myocyte [10] that describes excitation-contraction coupling via stochastic simulation of individual Ca^{2+} release sites (dyadic clefts) [11] with local CaMKII-mediated phosphorylation of L-type Ca^{2+} channels (LCCs) [12] and ryanodine receptors (RyRs) [10]. This allows us to use the model to isolate the role each CaMKII target plays in determining AP shape and duration.

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II. METHODS

The Na^+ channel model of Grandi et al [9] was used as a framework to build a model that could reproduce the recent experimental results of Aiba et al [3], who measured the acute effects of CaMKII on cardiac Na^+ currents in guinea pig ventricular myocytes in the presence of CaMKII or AIP. In order to constrain the model, it was assumed that in the presence of AIP all Na^+ channels were unphosphorylated, whereas upon the addition of CaMKII (without AIP), all channels were assumed to be phosphorylated. The experiments were performed at room temperature.

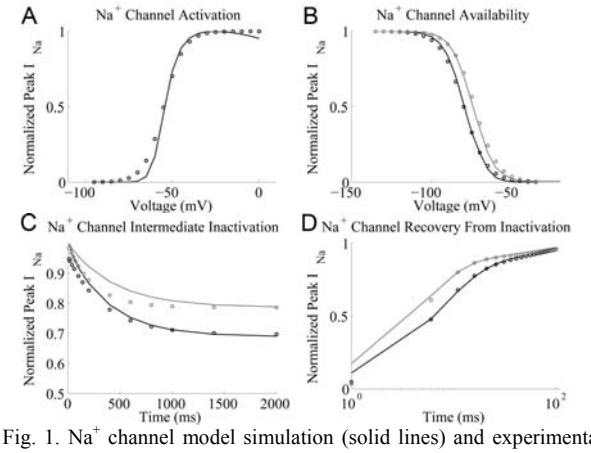

Fig. 1. Na^+ channel model simulation (solid lines) and experimental data (circles) [3] under phosphorylated (grey) and unphosphorylated (black) conditions. (A) Activation: I_{Na} is recorded during a 50 ms test potential from a holding potential of -120 mV. (B) Availability: a 500ms prepulse to a variable voltage is followed by a voltage clamp to -20mV, during which I_{Na} is recorded. (C) Recovery from intermediate inactivation: holding potential of -140 mV is followed by a variable duration prepulse to -20mV, during which I_{Na} is recorded. The membrane is then clamped to -140 mV for 20 ms and then to -20 mV for 50 ms, during which I_{Na} is recorded again. The ratio of peak I_{Na} (-20 mV vs. prepulse) is shown as a function of prepulse duration. (D) Recovery from inactivation: a holding potential of -140 mV is followed by a prepulse to -20mV for 300 ms, during which I_{Na} is recorded. The membrane is then clamped to -140 mV for a variable duration and then clamped to -20 mV for 50 ms, during which I_{Na} is recorded again. The ratio of peak I_{Na} (2^{nd} vs. 1^{st} pulse) is shown as a function of the interpulse interval.

As in the model of Grandi et al [9], the Na^+ channel model presented here consists of two 13-state deterministic models; the first represents an unphosphorylated Na^+ channel and the second represents a CaMKII-phosphorylated channel. The transition rates within each of the models were re-fit to match the results of a variety of experimental protocols [3] including activation, steady-state availability, recovery from inactivation, intermediate inactivation and late I_{Na} amplitude, as described in [9]. Fig. 1 shows a comparison of model simulations and experimental data for I_{Na}.

Aiba et al [3] found that CaMKII phosphorylation of Na^+ channels increases late I_{Na} significantly. This discovery corroborates the findings of Wagner et al [8], who also found increased late I_{Na} in cardiac myocytes from transgenic mice over-expressing CaMKII and from myocytes in which CaMKII had been adenovirally over-expressed. When measured under voltage-clamp, late I_{Na} is 0.23 ± 0.1% of peak I_{Na} in both control and CaMKII inhibited myocytes, whereas it is 0.95 ± 0.47% of peak I_{Na} in cells that have been acutely exposed to CaMKII [3]. After fitting model parameters, the model exhibits late I_{Na} with a magnitude of 0.16% of peak I_{Na} in unphosphorylated channels, and 0.79% of peak I_{Na} in phosphorylated channels. Experiments [3] showed that late I_{Na} is not significantly different between control and CaMKII-inhibited myocytes. Thus for the model, it was assumed that under control conditions Na^+ channels are unphosphorylated.

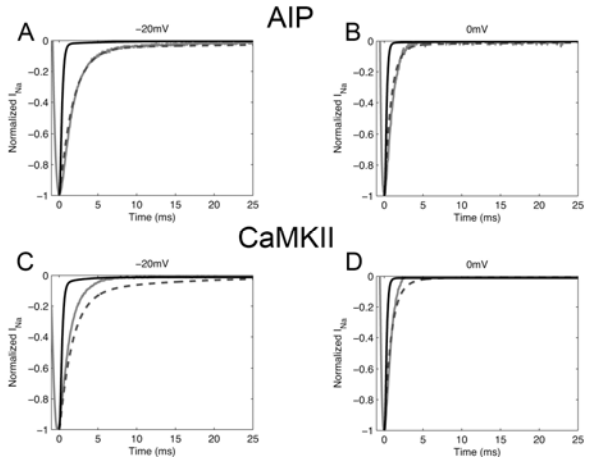

Fig. 2. Simulated vs Experimental I_{Na} under voltage clamp. A 50 ms test clamp was applied from a holding potential of -120 mV. Experimental results [3] at 23°C in guinea pig myocytes (solid grey line) are compared with simulated I_{Na} at 23°C (dashed line) and 37°C (solid black line). I_{Na} is shown in the presence of CaMKII inhibitor AIP at -20 mV (A) and 0 mV (B) and in the presence of CaMKII at -20 mV (C) and 0 mV (D).

The transition rates within each model were scaled for body temperature. Studies have shown that Na^+ channel kinetics are considerably faster at 37°C (body temperature) than at 23°C (room temperature) [13]. In addition, Na^+ channel conductance has been shown to increase with temperature [14]. Nagatomo et al [13] found that the rate of I_{Na} decay increased ~3-fold from 23°C to 33°C. Rates were adjusted to match this temperature-dependent increase in kinetics at voltage clamp test potentials of -30 mV, -20 mV, -10 mV and 0 mV. All rates were increased by a factor of 2.5. Certain rates were further adjusted to match depolarizing shifts in activation curves at higher temperatures, as observed in experiments [14] and to maintain the sigmoidal shape of the activation and availability curves. Fig. 2 shows simulated I_{Na} at both room and body temperature for voltage clamps to -20 mV and 0 mV compared to I_{Na} data obtained at room temperature [3] for unphosphorylated and phosphorylated channels. I_{Na} decay rates increase by a factor of ~3-5 at body temperature. Transition rate definitions are identical to those described in [9], with the exception of a_6, which is now defined as $P_{1a4} \times e^{V/(P2a4 \times P2a4\_2)} / P_{1a6}$. Kinetic parameters of the Na^+ channel model are given in Table I.

It was necessary to fit the Na^+ channel model for channel conductance. Using the late I_{Na} magnitude reported by Valdivia et al [15], and accounting for reported temperature-induced increases in channel conductance [14], Na^+ channel conductance was fit and a value of 8 mS/μF was obtained.

Finally, minor adjustments to the whole cell model were made to fine tune AP properties in the presence of a new Na^+

channel model which now included significant late I_{Na}. The number of channels that carry the Ca^{2+}-dependent Cl^- current (I_{to2}) was set to the level originally reported by Greenstein and Winslow [11]. In addition, I_{K1} conductance was increased by 10%, in agreement with recent experimental measurements in canine myocytes [16].

TABLE I
NA^+ CHANNEL PARAMETERS

	Unphosphorylated		*Phosphorylated*	
Parameter	*23℃*	*37℃*	*23℃*	*37℃*
P_{1a1}	7.9852	3.9926	7.9852	3.9926
P_{2a1}	0.0204	0.0204	0.0204	0.0204
P_{1a4}	1.1516	5.758	1.1516	5.758
P_{2a4}	26.9168	107.6672	26.9168	107.6672
P_{1a5}	$6.5x10^{-9}$	$1.625x10^{-8}$	$1.0x10^{-8}$	$2.5x10^{-8}$
P_{2a5}	6.2134	6.2134	6.2134	6.2134
P_{1b1}	0.0012	0.003	0.0012	0.003
P_{2b1}	9.3532	9.3532	9.3532	9.3532
P_{1b2}	0.0147	0.0367	0.0147	0.0367
P_{2b2}	6.6636	6.6636	6.6636	6.6636
P_{1b3}	$6.7x10^{-4}$	$1.675x10^{-3}$	$6.7x10^{-4}$	$1.675x10^{-3}$
P_{2b3}	17.3338	17.3338	17.3338	17.3338
P_{1b5}	0.0053	0.0132	0.0037	0.0132
P_{2b5}	$-2.88x10^{-6}$	$-7.2x10^{-6}$	$-1.82x10^{-6}$	$-4.55x10^{-6}$
P_{1a6}	27.2731	27.2731	1.9948	1.9948
P_{1b6}	$9.8x10^{-7}$	$2.45x10^{-6}$	$1.51x10^{-6}$	$3.775x10^{-6}$
P_{2b6}	11.7989	11.7989	11.7989	11.7989
P_{1a7}	0.0019	0.0047	0.0007	0.0018
P_{2a7}	25.9073	25.9073	151.5985	151.5985
P_{1b7}	0.0012	0.003	0.0012	0.003
P_{2b7}	53.4430	53.4430	53.4430	53.4430
P_{1a8}	$6.46x10^{-7}$	$1.615x10^{-6}$	$5.17x10^{-6}$	$7.238x10^{-6}$
P_{1b8}	$6.16x10^{-4}$	$1.54x10^{-3}$	$6.16x10^{-4}$	$1.54x10^{-3}$
P_{1a4_2}	1.2109	1.2109	0.4089	0.1022

III. RESULTS

A. Rate Dependent AP Shape and Duration

Fig. 3A shows average simulated APs over 50 s (1Hz pacing) and 25 s (0.5 Hz and 2 Hz pacing). APD increases with cycle length, as in experimental data [16]. Average APD at 90% repolarization (APD_{90}) is 314.0 ± 7.2 ms during 2 Hz pacing, 383.3 ms ± 7.0 ms during 1 Hz pacing and 463.3 ± 23.8 ms during 0.5 Hz pacing (data are presented as mean ± S.E.M.). Experimental APD measurements are highly variable. The APD_{90} data presented by Li et al [16] in canine myocytes is close to that shown here (305 ms, 375 ms, and 422 ms at 2 Hz, 1 Hz, and 0.5 Hz pacing, respectively).

B. AP Duration and Late I_{Na}

Fig. 3B shows the relationship between fractional Na^+ channel phosphorylation and APD_{90}. As the fraction of Na^+ channels that are phosphorylated increases, so does APD. This result makes intuitive sense since late I_{Na} contributes significant inward current during the plateau of the AP. Profiles of average late I_{Na} at 0% and 10% Na^+ channel phosphorylation during 1 Hz APs are shown in Fig. 3C. Mean I_{Na} during the 100 ms to 300 ms time interval of the AP is -0.177 pA/pF and -0.265 pA/pF at 0% and 10% Na^+ channel phosphorylation, respectively. Due to late I_{Na} and significant increases in APD, occasional EADs are observed in the simulated APs (see Fig. 3D). EAD frequency increases with fractional Na^+ channel phosphorylation.

C. Differential LCC and Na^+ Channel Phosphorylation

APD is very sensitive to both late I_{Na} and the late phase of the L-type Ca^{2+} current. In order to better understand APD variation with differential channel phosphorylation, simulations were performed in which the maximum CaMKII-mediated LCC phosphorylation rate and fractional Na^+ channel phosphorylation were varied (Fig. 4). The LCC phosphorylation model has been described previously [12].

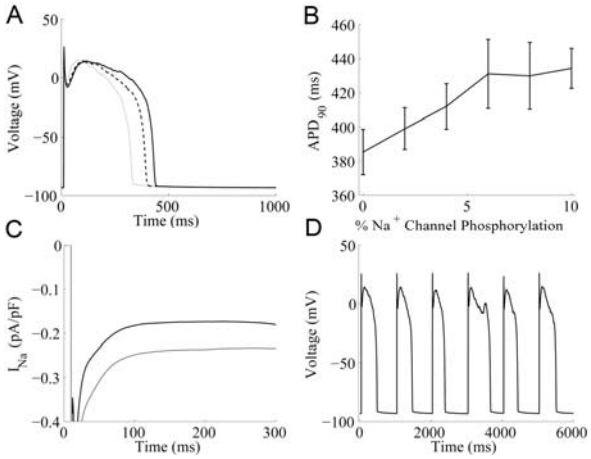

Fig. 3. Simulated late I_{Na} and APD. (A) Average simulated APs at 0.5 Hz (solid black line), 1 Hz (dotted line), and 2 Hz pacing (solid grey line). (B) APD_{90} as a function of Na^+ channel phosphorylation (mean ± S.E.M.) . (C) Average late I_{Na} underlying steady-state 1-Hz APs with 0% (black) and 10% (grey) Na^+ channel phosphorylation. (D) Simulated EAD at 1-Hz with 10% Na^+ channels phosphorylated.

When LCC phosphorylation rates remain at their control values (light grey curve) and there is no Na^+ channel phosphorylation, APD_{90} is 383 ms. APD_{90} increases steadily with increased fractional Na^+ channel phosphorylation, reaching 481 ms with 10% phosphorylated Na^+ channels. Under these conditions, EADs are observed at 4% Na^+ channel phosphorylation.

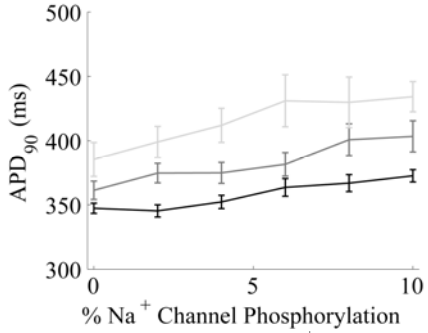

Fig. 4. APD as a function of LCC and Na^+ channel phosphorylation (1-Hz pacing). Mean APD_{90} ± S.E.M. is shown (n = 30). Black: reduction of LCC phosphorylation rate to 60% of control (~1.4% LCCs phosphorylated); dark grey curve: reduction of LCC phosphorylation rate to 80% of control (~2.5% LCCs phosphorylated); light grey curve: control LCC phosphorylation rate (~3.9% LCCs phosphorylated).

Decreasing LCC phosphorylation rates to 80% of normal (dark grey curve) lowers APD_{90} to 361 ms and 446 ms at 0% and 10% Na^+ channel phosphorylation, respectively. At this rate of LCC phosphorylation, EADs appear at 10% Na^+ channel phosphorylation. Lowering LCC phosphorylation

rates to 60% of normal (black curve) diminishes APD_{90} to 350 ms and 416 ms at 0% and 10% Na^+ channel phosphorylation, respectively, and no EADs were observed.

IV. Discussion

The cardiac myocyte model presented here builds upon a Na^+ channel representation that accounts for more recently observed Na^+ channel gating phenomena, such as burst mode states. Simulations show that this model is able to reproduce features of I_{Na} at room temperature, and rate parameters have been scaled to match experimentally observed temperature-dependent shifts in gating properties.

With the addition of the new Na^+ channel model, the whole cell model contains two channels that conduct inward currents, carried by Na^+ and Ca^{2+}, which have augmented function as a result of CaMKII phosphorylation. Therefore, it is not surprising that APD is greatly augmented in comparison with results from our original model [11].

Our results indicate that APD and shape are highly sensitive to Na^+ channel phosphorylation. Even a small fraction of Na^+ channel phosphorylation has the potential to result in occasional EADs (see Fig. 3D). It should be noted that the experimental observations of late I_{Na} from Aiba et al [3] had a large amount of variability (0.95 ± 0.47% of peak I_{Na} in phosphorylated channels). In simulations, the late I_{Na} produced by phosphorylated channels is 0.8% of peak. If the rate into the Na^+ channel burst mode states (see [9]), and thus late I_{Na}, were reduced, sensitivity to Na^+ channel phosphorylation would also decrease. Fig. 4 shows that the model is also very sensitive to LCC phosphorylation. Even a small reduction in CaMKII-dependent LCC phosphorylation rate, which leads to reduced occupancy of the LCC high activity gating mode (mode 2, see [12]), results in a significant decrease in APD.

This model is limited by the fact that it does not include dynamic CaMKII-mediated phosphorylation of Na^+ channels. We took the simple approach of assuming steady-state fractional phosphorylation of Na^+ channels because data on the dynamics of CaMKII-dependent Na^+ channel phosphorylation are not available, and therefore the associated rates cannot be reasonably constrained. Currently, the location of the Na^+ channel phosphorylation site, as well as its phosphorylation and dephosphorylation rates, remains unknown. However, the model includes a representation of cytosolic CaMKII, so that when the phosphorylation rates become available, it will be relatively straightforward to describe dynamic transitions between unphosphorylated and phosphorylated Na^+ channels. Despite this model limitation, this study has helped further illustrate the sensitivity of the cardiac AP to Na^+ channel phosphorylation.

The model presented here has demonstrated a mechanistic link between augmentation of late I_{Na}, AP prolongation and the appearance of EADs in agreement with previous experimental observations [4]. Recently, the generation of reactive oxygen species under conditions of increased oxidative stress, such as during heart failure, has been linked to CaMKII-mediated enhancement of late I_{Na} and consequent arrhythmias [17]. Therefore, therapies that target late I_{Na}, such as block by ranolazine [18], may have promise in treating patients that are at risk for arrhythmia.

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