Regulation of Ca$^{2+}$ and electrical alternans in cardiac myocytes: role of CAMKII and repolarizing currents

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Livshitz LM, Rudy Y. Regulation of Ca$^{2+}$ and electrical alternans in cardiac myocytes: role of CAMKII and repolarizing currents. Am J Physiol Heart Circ Physiol 292: H2854–H2866, 2007. First published February 2, 2007; doi:10.1152/ajpheart.01347.2006.—Alternans of cardiac repolarization is associated with arrhythmias and sudden death. At the cellular level, alternans involves beat-to-beat oscillation of the action potential (AP) and possibly Ca$^{2+}$ transient (CaT). Because of experimental difficulty in independently controlling the Ca$^{2+}$ and electrical subsystems, mathematical modeling provides additional insights into mechanisms and causality. Pacing protocols were conducted in a canine ventricular myocyte model with the following results: 1) CaT alternans results from refractoriness of the sarcoplasmic reticulum Ca$^{2+}$ release system; alternation of the L-type calcium current has a negligible effect; 2) CaT-AP coupling during late AP occurs through the sodium-calcium exchanger and underlies AP duration (APD) alternans; 3) increased Ca$^{2+}$/calmodulin-dependent protein kinase II (CaMKII) activity extends the range of CaT and APD alternans to slower frequencies and increases alternans magnitude; its decrease suppresses CaT and APD alternans, exerting an antiarrhythmic effect; and 4) increase of the rapid delayed rectifier current (I$_{Ks}$) also suppresses APD alternans but without suppressing CaT alternans. Thus CaMKII inhibition eliminates APD alternans by eliminating its cause (CaT alternans) while I$_{Ks}$ enhancement does so by weakening CaT-APD coupling. The simulations identify combined CaMKII inhibition and I$_{Ks}$ enhancement as a possible antiarrhythmic intervention.

T-WAVE ALTERNANS IS CLOSELY ASSOCIATED with dispersion of repolarization, ventricular arrhythmias, and sudden death (47, 53). One hypothesis states that T-wave alternans originates from alternation of cardiac repolarization at the cellular level, particularly beat-to-beat variation of the action potential (AP) duration (APD) (36, 49). APD alternans can be electrical in nature, caused by ionic current restitution (36). Alternatively, alternation of the intracellular Ca$^{2+}$ transient (CaT alternans) can modulate electrical activation and induce APD alternans (38, 48). The mechanism of Ca$^{2+}$ alternans and its coupling to electrical activation is not completely understood (55). Several mechanisms of Ca$^{2+}$ alternans have been proposed: 1) restitution of L-type Ca$^{2+}$ current [I$_{Ca,L}$] (20); 2) refractoriness of sarcoplasmic reticulum (SR) Ca$^{2+}$ release channels [ryanodine receptor (RyR)] (50); 3) dependence of Ca$^{2+}$ uptake by the SR Ca$^{2+}$-ATPase/phospholamban complex (SERCA2a/PLB) on Ca$^{2+}$ concentration in myoplasm ([Ca$^{2+}]_{i}$) (31); 4) SR Ca$^{2+}$ overloading and Ca$^{2+}$ wave propagation (13); and 5) step dependence of SR Ca$^{2+}$ release on SR Ca$^{2+}$ concentration (2, 14).

Interactions among these processes and with metabolic and/or Ca$^{2+}$-dependent regulatory pathways can promote alternans (6, 48).

Due to tight coupling between the Ca$^{2+}$ and electrical cellular subsystems, it is difficult to determine cause and effect experimentally, because the ability to independently control each subsystem is limited (23). Even more challenging is the study of interactions between specific SR processes and sarcoplemmal currents (50). Several studies have shown that CaT alternans persists when the cell is voltage clamped, with either constant voltage (13, 48, 50) or constant duration APs (9), suggesting that Ca$^{2+}$ oscillation plays the primary role in alternans generation at a moderately fast rate of pacing. Theoretical modeling can realize precise independent control of individual components and is, therefore, very useful for studying the highly interactive mechanism of alternans. While important insights have been obtained from simplified models (1, 2, 20, 55, 59), processes relevant to alternans formation, such as dynamic ion accumulation and regulation by Ca$^{2+}$-dependent regulatory pathways, have not been considered.

Moreover, properties of CaT-AP coupling are species-dependent (4, 15, 23, 50), each with remarkably different CaT and AP morphologies and durations. In addition, there is the well-documented transmural heterogeneities in AP and CaT cycling properties in the same species, which have been documented to affect the onset and amplitude of alternans (51, 68). It has been observed that the large CaT during beat-to-beat (large-small) CaT alternans is accompanied by a short APD in some species (or certain experimental conditions) (33, 46), while in other species by a prolonged APD (48, 50). It was suggested that the Na$^{+}$/Ca$^{2+}$ exchanger (I$_{NaCa}$) is responsible for prolongation of APD during large CaT, while Ca$^{2+}$-dependent inactivation of I$_{Ca,L}$ is the mechanism of APD shortening (48, 59). However, the specific mechanism of CaT-APD coupling during alternans and its modulation by the whole cell environment require further exploration.

A delicate balance between repolarizing and depolarizing currents provides for precise control of the AP time course (54). Because this balance is modulated by [Ca$^{2+}$], it is important to use physiologically detailed models of the cardiac myocyte for studying the interaction between the Ca$^{2+}$ and electrical subsystems in the study of alternans. Here, we investigate the cellular mechanism of alternans that involves both CaT and APD alternation. Specifically, we examine the following hypotheses. 1) Calcium alternans drives APD alternans via coupling of the Ca$^{2+}$ and electrical subsystems through I$_{NaCa}$. 2) Calcium alternans is caused by refractory...
properties of the SR Ca\(^{2+}\) release process and steep dependence of Ca\(^{2+}\) release on SR Ca\(^{2+}\) load. 3) Repolarizing currents have a modulatory effect on alternans by influencing APD in a Ca\(^{2+}\)-independent manner. 4) By modulating SR Ca\(^{2+}\) cycling, Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) is a major determinant of alternans and its rate dependence.

CaMKII is a regulatory pathway that modulates its activity in response to frequency, amplitude, and duration of Ca\(^{2+}\) pulses (10, 26). It plays an essential role in frequency-dependent augmentation of cardiac contractility (69) and acceleration of relaxation (12), particularly during stress or exercise. CaMKII hyperactivity can lead to structural heart disease and arrhythmias (3, 32, 75).

For the purpose of this study, an updated mathematical formulation of SR Ca\(^{2+}\) release (I_{Rel}) was developed. It includes activation of RyR by I_{Ca(L)} and its regulation by junctional SR (JSR) Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]_{JSR}) and CaMKII. This formulation was incorporated into theoretical models of ventricular epicardial myocytes of two species, guinea pig (16, 43) and canine (28). The article outline is as follows. First, reformulated I_{Rel} is validated by reproducing experimental protocols that reveal properties of the Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) process. Second, the dependence of CaMKII on CaT and APD and its inotropic effect are simulated and compared with experiment. Third, the roles of I_{Ca(L)}, SR Ca\(^{2+}\) fluxes, and CaMKII in alternans onset and termination at moderately fast rate are studied. Fourth, the nature of bidirectional CaT-APD coupling during alternans is investigated, particularly the role of I_{Ca(L)}, I_{NaCa}, and rapid delayed rectifier K\(^{+}\) current (I_{K1}). Aspects of this work have been presented in abstract form (41).

METHODS

Myocyte Models

Table 1 in the APPENDIX contains parameter definitions. The theoretical Luo-Rudy dynamic (LRd) (16, 43) and Hund-Rudy dynamic (HRd) (28) models of mammalian ventricular AP provide the basis for the simulations. The LRd model is based on guinea pig data; it includes membrane ion-channel currents, pumps, and exchangers, and accounts for dynamic concentration changes of Na\(^{+}\), K\(^{+}\), and Ca\(^{2+}\). The HRd model (Fig. 1A) is based on epicardial canine data (28) and adds to LRd processes of chloride (Cl\(^{-}\)) homeostasis and the CaMKII regulatory pathway. The model includes the following phosphorylation targets of CaMKII: Ca\(^{2+}\) uptake flux by SERCA pump (I_{SERCA}), I_{Ca(L)}, and I_{Rel}. I_{Ca(L)} includes effects of CaMKII on both the SERCA pump maximal turnover rate and its affinity to Ca\(^{2+}\); I_{Ca(L)} and I_{Rel} interact in a subsarcolemmal restricted subspace for Ca\(^{2+}\) distribution. Models equations and computer codes can be found in the research section of http://tudylab.wustl.edu.

CICR process (I_{Rel}). We formulated a two-state (closed-open) model of SR Ca\(^{2+}\) release kinetics (Fig. 1B) and incorporated it in LRd and HRd. We assumed that, in nonfailing myocytes, CICR is a spatially uniform process (50). Based on this assumption and the assumptions that RyRs are independent and identical, the two-state model can describe the kinetics of the SR Ca\(^{2+}\) release process (61, 62, 64). Transition kinetics between release (open) and no-release (closed) states depend on I_{Ca(L)} (42, 59) [Ca\(^{2+}\)]_{JSR} (19, 67), and CaMKII activity. This is consistent with experiments (7, 18, 34) and early modeling work (16, 43, 58), where efficiency of release was linked to the rate of Ca\(^{2+}\) elevation in myoplasm and not to the level of myoplasmic Ca\(^{2+}\) per se. The differential equations for the model are presented in the APPENDIX.

Simulation protocols. The 0.5 ms or 1 ms of −80 μA/μF current stimuli were used to pace LRd or HRd, respectively. The stimulus current was assigned Cl\(^{-}\} and/or K\(^{+}\) ions as charge carrier to ensure charge conservation and model stability (29). Numerical integration was performed using Matlab (Mathworks, Natick, MA) (56), with error tolerance of 10\(^{-6}\). Steady state was defined when all state variables showed <0.1% variability over 100 beats (1 min). The models were tested for convergence and long-term stability over the entire frequency range and parameter values considered. Steady-state APD (90% repolarization) and peak CaT (or ΔCa\(^{2+}\)=max(CaT)−min(CaT)) were computed and used to create rate-adaptation curves. Results are shown for HRd simulations, except for Fig. 4, where alternans are also shown for LRd to demonstrate model (species) independence of the alternans phenomenon.

RESULTS

Model Properties Validation

Because we hypothesize that SR Ca\(^{2+}\) cycling plays a key role in CaT alternans, it is essential to verify that the models of SR Ca\(^{2+}\) release and CaMKII activity reproduce the experimentally observed behaviors that are relevant to alternans generation. The following sections and Figs. 1–3 provide such validation. Tables 4 and 5 in the APPENDIX contain documentation of the electrophysiological data used for the canine model validation and CaMKII data, respectively.

Relationship between CaT, I_{Ca(L)}, and SR Ca\(^{2+}\) loading. The SR Ca\(^{2+}\) release model is validated by reproducing a number of key properties of excitation-contraction coupling (ECC) that determine CaT. 1) Variable gain: Ca\(^{2+}\) influx via I_{Ca(L)} is an order of magnitude smaller than the Ca\(^{2+}\) flux via RyR. The ratio I_{Rel}/I_{Ca(L)} [or CaT/I_{Ca(L)}] (63) is ECC gain; it depends on membrane voltage variable gain. 2) Graded release: SR Ca\(^{2+}\) release and, consequently, CaT are under tight control of I_{Ca(L)}, i.e., the magnitude of I_{Rel} is graded with the amplitude of I_{Ca(L)}. 3) Fractional SR Ca\(^{2+}\) release: Percentage of total available SR Ca\(^{2+}\) ([Ca\(^{2+}\)]_{JSR}) released via RyR. Experiments show that fractional release is a steep nonlinear function of [Ca\(^{2+}\)]_{JSR} (57). The above properties of ECC are often evaluated experimentally when exploring the mechanisms underlying alternans and arrhythmias (13, 50). Figure 1C shows ECC gain ratio of CaT to peak I_{Ca(L)} at different membrane voltages. CaT is the ratio of max(CaT) to min(CaT), a definition consistent with experimental measurements of CaT as peak to minimum fluorescence ratio (63). The cell was clamped to −40 mV for 50 ms, followed by 50-ms pulse to varying voltages. Both experimental (63) and simulated data show EC gain to be monotonically decreasing function of voltage. Figure 1D shows total Ca\(^{2+}\) released during one cycle [cycle length (CL) = 1,000 ms] as a function of peak [Ca\(^{2+}\)]_{JSR}. [Ca\(^{2+}\)]_{JSR} was the same (7.8 mmol/l) at the beginning of the pacing cycle for each value of I_{Ca(L)}. The simulation shows an almost linear dependence of Ca\(^{2+}\) released on trigger Ca\(^{2+}\) entry. Figure 1E shows fractional SR Ca\(^{2+}\) release. Note the steep dependence on [Ca\(^{2+}\)]_{JSR} at high SR loading. This strong, nonlinear dependence has two consequences. At low SR Ca\(^{2+}\) load, it helps terminate SR Ca\(^{2+}\) release, preventing further depletion of SR Ca\(^{2+}\). However, at high SR Ca\(^{2+}\) load, it can cause Ca\(^{2+}\) cycling instability (13, 50, 57).

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Sensitivity of CaMKII to CaT and APD. In the model-experiment comparison, we use in vitro experimental results from neuronal CaMKII isoform due to lack of direct data regarding the rate of CaMKII phosphorylation in cardiac myocytes. However, as shown by Gaertner et al. (22), all CaMKII isoforms have very similar catalytic and regulatory properties (5). It should be noted that CaMKII isoforms with very similar biochemical characteristics can demonstrate remarkably different targeting properties (e.g., anchoring to target proteins) (25, 72). Simulated CaMKII activity and steepness of frequency dependence increase at a fast rate. The CaMKII model reproduces qualitatively the experimentally (in vitro) observed dependence of CaMKII activity [fraction of active CaMKII binding sites (CaMK_{active})] on CaT frequency, amplitude, and duration (CaTd) (10). Figure 2 shows simulated (A) and measured (10) (B) time course of CaMK_{active} (% of maximum) at rates of 1, 2.5, and 4 Hz (CaTd and amplitude held constant at 200 ms and 20 μmol/l). Both experiment (10) and simulation show increased CaMK_{active} and steepness at fast rate (slope at 4 Hz is twice steeper than at 2.5 Hz). This rate dependence is due to the autocatalytic nature of the autophosphorylation reaction (27). The different time course in model and experiment is due to faster kinetic parameters used in the simulations (28), reflecting higher activity of cardiac isoform-δ compared with isoforms-α and -β in the experiments (22). Figure 2 also shows simulated (C) and measured (10) (D) frequency dependence of CaMKII activity for different CaTd: 500, 200, 80, and 40 ms (40-ms data are not available in the experiment, but are included in simulation, because it is comparable to CaTd in the restricted subspace). As CaTd increases, the curve shifts to lower frequencies. Note that the same CaMKII activity can be reached at slower rates for longer CaTd. For example, CaMKII activity of 20% (horizontal line) is achieved at 0.4, 1, 3, and 6 Hz for CaTd of 500, 200, 80, and 40 ms, respectively (arrows). Both experiment (10) and model show a threshold phenomenon, i.e., no CaMKII activity occurs for specific CaTd until minimal frequency is reached. Ability of CaMII to respond to CaT frequency and morphology is important, because CaMKII senses different CaT transients when tethered (26) to different targets; for example, RyR and L-type calcium channel in the restricted tubular subspace experience a different CaT and consequently CaMKII activity than SERCA/PLB in the bulk...
myoplasm (Fig. 1A). Both experiment (10) and model show a threshold phenomenon, i.e., no CaMKII activity occurs for specific CaTd until minimal frequency is reached. In addition, model simulations show time-dependent saturation of CaMKactive (Fig. 2A).

Figure 2, E and F, shows modulation of CaMKII activity by APD. Figure 2E shows (clockwise) time course of AP, CaT, [Ca^{2+}]_{JSR}, and CaMKactive at 1-Hz stimulation rate. Control AP waveform (solid lines) is prolonged to double APD at CL = 1,000 ms from 215 to 430 ms (shaded lines). The prolonged AP is used as command waveform to pace the cell to steady state (shaded lines). APD prolongation leads to dramatic increase of CaT and [Ca^{2+}]_{JSR}, >200% of initial values; consequently, CaMKactive is increased by 75%. These simulations show that APD can modulate CaMKII activity by increasing intracellular Ca^{2+} loading and CaT. Figure 2F shows CaMKactive at rates from 0.5 to 3 Hz for control APD and APD increased by 50 or 100%. The same level of CaMKII activity is achieved at lower frequency for longer APD. For example, CaMKII activity of 20% (horizontal line) is achieved at 1.8, 2.4, and 2.8 Hz for 2.0, 1.5, and 1.0 control APD, respectively (arrows). The sensitivity of CaMKII activity level to APD is important when considering that CaMKII serves as a frequency sensor in different species (mouse, guinea pig, dog, human), which have remarkably different AP morphologies and durations (4, 21, 40).

CaMKII underlies frequency-dependent acceleration of relaxation and positive force-frequency relation. When the heart rate increases, greater force (implying greater CaT) (6) is generated. The increase of force (or CaT) with frequency is termed positive force-frequency relation (PFFR). At a fast rate, less time is available for cardiac relaxation or pumping Ca^{2+} back into the SR. In this subsection, we study the effects of CaMKII on the frequency dependence of CaT amplitude and decay. These properties affect CaT in a rate-dependent manner and are therefore relevant to formation of alternans. Experimental data on CaMKII regulation in ventricular myocytes are limited. For experiment-model comparison, we use the measured time derivative of left ventricular pressure (69) or twitch relaxation (12) as surrogates of CaT when data for myocyte CaT are not available. Figure 3A shows experimental (bottom) (60) and simulated (top) CaT at different rates. Both experiment and simulation show that CaT amplitude and rate of decay increase at a fast rate. Note that simulated and measured (60) peak CaT increase monotonically with pacing frequency (PFFR). The descending limb of CaT is fit by a single expo-
ential, with time constant of relaxation $\tau$. Both experiments
and simulations show that, with increasing frequency, $\tau$ de-
creases monotonically. Tenfold increase in frequency from
0.25 to 2 Hz results in about twofold increase of relaxation rate,
with $\tau$ decreasing from 450 to 200 ms. This phenomenon is
called frequency-dependent acceleration of relaxation (FDAR)
and is essential for normal diastolic function. Complete sup-
pression of CaMKII effect on all targets in the model (Fig. 3
B, shaded line) slows FDAR and blunts its frequency dependence
compared with control (panel B, solid line). Similar behavior is
seen experimentally (panel C) (12).

Figure 3, D and E, shows the effect of CaMKII inhibition on
force-frequency relation. For control with CaMKII active
(solid curves), experiment (E) (69) and simulation (D) show
similar 40% increase of contractility or CaT, respectively, as
rate increases over the range shown. Total CaMKII inhibition
greatly suppresses this rate dependence.

Figures 3 was generated by setting CaMKII activity to zero
for all of its targets [i.e., $I_{Ca(L)}, I_{up}$, and $I_{Rel}$], which mimics
the acute effect of KN-93 application to the whole cell. The
agreement between model and experiment is qualitative. The
onset of increased contractility (or CaT) is shifted to lower
frequencies in simulations relative to experiments, reflecting
the slower heart rate of canine (simulation) compared with
rabbit (experiment) (69).

$CaT$ and $APD$ Alternans

Frequency dependence of alternans. Figure 4, A and B,
shows steady-state APD and $\Delta Ca^{2+}$ rate dependence (adaptation curves) generated by the guinea pig (LRd) model. Figure
4, C and D, shows similar curves for canine (HRd). As pacing
rate is increased, APD shortens, until it reaches a point of
bifurcation at which for the same pacing rate APD oscillates
between long and short values. Figure 4, B and D, shows
Corresponding CaT adaptation curves; CaT amplitude increases
at a fast rate until, exactly at the same frequency as APD,
bifurcation occurs. The bifurcation portions of APD and CaT
curves are shown in insets on the expanded scales. The guinea
pig model alternates at CL from 150 to 250 ms, consistent with
experimental data (49). Maximal APD and CaT differences
between two consecutive beats occur at CL = 200 ms, with
magnitudes of 12 ms and 0.75 $\mu$mol/l, respectively. The canine
model alternates at CL = 155–275 ms, also consistent with
experimental data (23). Maximal APD and CaT differences
between two consecutive beats occur at CL = 250 ms, with
magnitudes of 35 ms and 0.5 $\mu$mol/l, respectively. APD and
CaT curves bifurcate also when plotted against the preceding
diastolic interval (DI) (not shown), as frequently presented (23,
35). Note that the bifurcation points of APD adaptation
curves are smooth functions of CL; as CL decreases, alternans
amplitude increases to a maximum and then decreases (23) (Fig. 4C, inset). Both canine and guinea pig model simulations are shown in Fig. 4, demonstrating model (species) independence of the alternans phenomenon. The simulated frequency ranges and amplitudes of alternans are consistent with corresponding experimental data (24, 68).

CaT-AP coupling during alternans and its mechanism. To elucidate the link between APD (electrical) and CaT (mechanical) alternans, we pace the cell under conditions of AP clamp or CaT clamp (Fig. 5). Figure 5A shows AP (top) and CaT (bottom) during alternans at 4 Hz; note that large CaT is accompanied by long APD. In Fig. 5B, steady-state behavior is...
shown during pacing at $CL = 250\text{ ms}$ with $\text{AP (top)}$ clamped to either its short $\text{AP} = 133\text{ ms}$ (shaded line) or long $\text{AP} = 165\text{ ms}$ (solid line). Despite elimination of $\text{AP}$ alternans by the clamp protocol, $\text{CaT}$ alternans persists (Fig. 5B, bottom). In Fig. 5C, $\text{CaT (bottom)}$ is clamped to either its small (shaded line) or large (solid line) morphology. In either case, $\text{AP}$ alternans is eliminated (Fig. 5C, top). The SR $\text{Ca}^{2+}$ subsystem continues to oscillate during clamping with large $\text{CaT}$ morphology, and the SR $\text{Ca}^{2+}$ release rate is higher during large depletion than during small depletion (not shown). The results reveal that, at this pacing rate, $\text{CaT}$ alternans is causing $\text{AP}$ alternans; in other words, oscillation of the $\text{Ca}^{2+}$ subsystem is driving the $\text{APD}$ oscillations. Simulations over the entire bifurcation range ($170 < CL < 270$) show the same $\text{Ca}^{2+}$-driven mechanism of $\text{AP}$ alternans.

To explore the role of $I_{\text{Ca(L)}}$ in $\text{CaT}$ alternans, we conducted the simulations in Fig. 5D; the $\text{bottom}$ shows that clamping $I_{\text{Ca(L)}}$ to either its small (shaded line) or large (solid line) morphology does not eliminate either $\text{AP}$ or $\text{CaT}$ alternans (top), indicating that alternation of SR $\text{Ca}^{2+}$ release is not due to alternation of its $I_{\text{Ca(L)}}$ trigger. However, clamping $I_{\text{Ca(L)}}$ to either its small (shaded line) or large (solid line) morphology reduces the $\text{APD}$ alternans amplitude by $32\text{ ms}$ to $21$ or $14\text{ ms}$, respectively, indicating a role of $I_{\text{Ca(L)}}$ in $\text{CaT}$-$\text{AP}$ coupling. $\text{APD}$ alternans amplitude is defined as the difference between long and short $\text{AP}$.

Figure 6A shows (clockwise) superimposed $\text{AP}$, $I_{\text{Kr}}$, $\text{CaT}$, and $I_{\text{NaCa}}$ during alternans for two consecutive beats, with long (solid line) and short (shaded line) $\text{APD}$. The higher early plateau of the short $\text{AP}$ (70 ms, arrow) is mainly due to enhanced $I_{\text{Ca(L)}}$ caused by less $\text{Ca}^{2+}$-dependent inactivation (Fig. 6A, bottom) during the small $\text{CaT}$ (shaded line). Early plateau $\text{Ca}^{2+}$-dependent transient outward $\text{Cl}^{-}$ current ($I_{\text{NaCl}}$) is also $\text{Ca}^{2+}$ dependent, but is a small current, and its effect on $\text{AP}$ morphology changes during alternans is small. During the large $\text{CaT}$ (solid line), $I_{\text{NaCl}}$ is more inward than during the small $\text{CaT}$ (shaded line), slowing $\text{AP}$ repolarization to cause crossovers of the $\text{APs}$ and prolongation of $\text{APD}$. The higher plateau of the short $\text{AP}$ and the $\text{APs}$ crossover in agreement with experimental data (24) (Fig. 6B) from canine ventricular myocytes. The simulations identify $I_{\text{NaCl}}$ as the major coupling link between $\text{CaT}$ alternans and $\text{AP}$ alternans, due to its major role late in the $\text{AP}$, when repolarization and $\text{APD}$ depend on a delicate balance of currents and are easily modulated.

**SR $\text{Ca}^{2+}$ content and $\text{CaT}$ alternans.** To explore the role of SR $\text{Ca}^{2+}$ fluxes in onset and offset of $\text{CaT}$ alternans, we conducted the simulations in Fig. 7. Figure 7A shows SR-releasable $\text{Ca}^{2+}$ content changes during alternans at 5 Hz. $\Delta \text{Ca}^{2+}$ is shown for two different levels of SR $\text{Ca}^{2+}$ loading during alternans. In the simulation (top), increase of $[\text{Ca}^{2+}]_{\text{ISR}}$ by 40% leads to a fourfold increase in $\Delta \text{Ca}^{2+}$, demonstrating that small changes in $[\text{Ca}^{2+}]_{\text{ISR}}$ lead to large changes in $\Delta \text{Ca}^{2+}$. Such steep dependence is consistent with experimental findings (bottom) (13). Total SR $\text{Ca}^{2+}$ content ($\text{ISR}$ + network $\text{SR}$ (NSR)) increases as a function of pacing rate (Fig. 7B) (45). In addition, during alternans, change of total SR content is very small, in accordance with the experiment (13, 50). However, $[\text{Ca}^{2+}]_{\text{ISR}}$ and consequently the releasable pool of $\text{Ca}^{2+}$, is slightly decreased with rate after reaching a maximum at 1.5 Hz (15% decrease at 4 Hz, Fig. 7C). This property of the model is consistent with experimental observations that refractoriness of the global CICR process has a time constant in the range of 0.3 to 1 s (8, 50, 65). At slow rates in the absence of alternans, $\text{SR}$ fluxes are in balance, i.e., the amount of $\text{Ca}^{2+}$ transported from NSR to JSR ($I_{\text{leak}}$, solid thin line) during one beat at steady state equals the amount of $\text{Ca}^{2+}$ released ($I_{\text{Ca(L)}}$) and the net flux into the SR $[\text{Ca}^{2+}]_{\text{ISR}}$ (bottom) from SR; shaded line, Fig. 7D). In addition, free diastolic $\text{Ca}^{2+}$ is in equilibrium over the entire $\text{SR}$ (not shown). However, at a moderately fast rate during alternans following a large $\text{CaT}$ (defined as even beat), $\text{NSR}$ reloading and $\text{Ca}^{2+}$ transfer to $\text{JSR}$ are less than the amount released (Fig. 7D, even beat). Consequently, less $\text{Ca}^{2+}$ is available for release during the next beat (Fig. 7C, odd beat), and, due to the steep dependence of fractional $\text{Ca}^{2+}$ release on $[\text{Ca}^{2+}]_{\text{ISR}}$ (Fig. 7E), less $\text{Ca}^{2+}$ is released during this beat (Fig. 7D, odd beat). Following a small $\text{CaT}$ (odd beat), there is accumulation of releasable $\text{Ca}^{2+}$ (Fig. 7C, even beat) because of imbalance between $\text{SR}$ reloading and release, resulting in a large $\text{CaT}$. This alternating behavior repeats to cause sustained alternans. When pacing rate is further increased (>6 Hz), time for $\text{Ca}^{2+}$ accumulation after a small release is decreased, and the alternans gradually disappear.

**Effect of CaMKII.** Figure 8A shows $\text{APD (top)}$ and $\Delta \text{Ca}^{2+}$ (bottom) adaptation curves for three different levels of CaMKII activity modulated by changing the fraction of low-affinity calmodulin binding sites (CaMKII<sub>0</sub>) (27), which mimics the effect of KN-93 (12). Setting CaMKII<sub>0</sub> to zero completely inhibits CaMKII activity. Increase of CaMKII activity by 25% shifts onset of $\Delta \text{Ca}^{2+}$ and $\text{AP}$ alternans to slower frequencies, from 3.3 to 2 Hz, while the frequency of maximal alternans is unchanged (3.6 Hz). However, the amplitudes of $\Delta \text{Ca}^{2+}$ and $\text{APD}$ oscillations increase by 10 ms and 0.4 $\mu\text{mol/l}$, respectively. Increase of CaMKII activity has no effect on offset of $\text{CaT}$ and $\text{APD}$ alternans, while CaMKII inhibition...
suppresses alternans (dashed curves), thereby exerting an antiarrhythmic effect. Unfortunately, decrease of CaMKII activity blunts the PFFR (Fig. 3, E and F) and FDAR (Fig. 3, C and D), thereby compromising cardiac mechanical function.

**Effect of IKr.** In general, peak $I_{Kr}$ amplitude is smaller at faster rates (28). However, our simulations and experiments (23) show that, during APD alternans, $I_{Kr}$ is larger during the shorter than longer AP. The simulations indicate that this behavior (large $I_{Kr}$ at short AP) is due to the combined effect of residual activation due to shorter DI after the long APD and greater activation due to high early plateau potential of the short AP. Figure 8B shows APD (top) and CaT (bottom) adaptation curves for three different levels of $I_{Kr}$ conductance. Fifty percent decrease of conductance increases APD by 15 ms over the entire stimulation range (dashed line). In addition, the magnitude of APD alternans increases by 15 ms. However, CaT and magnitude of CaT alternans are not affected. Note that this modest increase of APD prevents one-to-one capture at 5 Hz because the DI following the long APD approaches zero. Increase of $I_{Kr}$ conductance by 200% (note shown) decreases APD alternans magnitude by 50% (15 ms), with no effect on onset frequency and magnitude of CaT alternans. A large threefold increase of $I_{Kr}$ conductance is necessary to completely eliminate APD alternans, consistent with the experiment (23). Even with such large increase, the onset frequency and magnitude of CaT alternans (not measured in the experiment) are not affected. The 300% increase of $I_{Kr}$ decreases APD by 50% and CaT amplitude by 50% (shaded line) at slow rate; it extends the frequency range of CaT alternans by shifting its termination to 10 Hz (not shown) from 5.5 Hz. The inset shows overlapped consecutive APs at 5 Hz for 300% $I_{Kr}$ conductance. While APDs are almost identical, there are significant differences in AP morphologies: the AP plateau during small CaT (dashed line) is more convex than AP during large CaT.

**DISCUSSION**

This study shows that, at a moderately fast rate (between 3.5 and 5.5 Hz), the SR Ca$^{2+}$ subsystem, strongly modulated by CaMKII, can initiate CaT alternation that induces APD alternans.

Fig. 7. Steady-state SR Ca$^{2+}$ flux balance during alternans. A: dependence of $\Delta$Ca$^{2+}$ = max(CaT) − min(CaT) on JSR Ca$^{2+}$ content at 5 Hz. Simulation (top) is compared with experiment (bottom) (13, reproduced with permission). Only 40% change in [Ca$^{2+}]_{SR}$ leads to fourfold change in $\Delta$Ca$^{2+}$. [Ca$^{2+}]_{JSR} = $ free and buffered [Ca$^{2+}]_{JSR}$ before release; solid and shaded bars correspond to larger and smaller [Ca$^{2+}]_{JSR}$, respectively. Data are normalized to small [Ca$^{2+}]_{JSR}$. B: total end-diastolic SR Ca$^{2+}$ content ([Ca$^{2+}]_{JSR}$ and [Ca$^{2+}]_{NSR}$) as function of rate. C: free Ca$^{2+}$ in JSR ([Ca$^{2+}]_{JSR}$) as function of frequency. D: total Ca$^{2+}$ released by RyR ($I_{rel} \, dt$, solid thick line), reloaded into NSR ($I_{upl} \, dt$, shaded lines) and translocated from NSR to JSR ($I_{trans} \, dt$, solid thin line) over one cycle, as function of frequency.

Fig. 8. A: APD (top) and $\Delta$Ca$^{2+}$ (bottom) adaptation curves for three different levels of CaMKII activity: control (100%), 25% elevation (125%), or complete block (0%). B: effect of $I_{Kr}$ on APD and CaT alternans. APD (top) and CaT (bottom) adaptation curves for three different levels of $I_{Kr}$ conductance are shown: control (100%), 300% elevation, and 50% reduction. Inset: superimposed consecutive APs at 5 Hz for $I_{Kr}$ increase of 300%.
CaT Alternans

At a moderately fast rate, the guinea pig (4–6.5 Hz) and canine (3.5–5.5 Hz) models produce sustained alternans of both APD and CaT. Simulated AP and CaT clamp protocols confirm (38) that oscillation of the Ca\(^{2+}\) subsystem is driving the APD alternans in both species. The mechanism underlying CaT alternans is explored by evaluating the roles of the trigger for SR Ca\(^{2+}\) release \(I_{\text{Ca(L)}}\), SR load, SR Ca\(^{2+}\) fluxes, and CaMKII activity during alternans. Model simulations show that refractoriness of the SR Ca\(^{2+}\) release process is the main mechanism of CaT alternans. Specifically, two rate-limiting processes, \(I_{\text{AP}}\) and \(I_r\) (Fig. 7D), in conjunction with steep dependence of SR Ca\(^{2+}\) release on SR Ca\(^{2+}\) load (Fig. 1E), determine the onset and offset of sustained alternans at moderately fast rates.

\(I_r\) in the model represents both (local) RyRs intrinsic recovery from refractoriness and (global) Ca\(^{2+}\) diffusion (8) through the SR. While the steep dependence of release and rate of uptake are sufficient to induce alternans in the model (see also Ref. 70), \(I_r\) also contributes to alternans formation (Fig. 7D). In addition, the model predicts that, during Ca\(^{2+}\) overload, the SR Ca\(^{2+}\) cycling subsystem can oscillate even without corresponding beat-to-beat oscillations of CaT (not shown). In contrast to previous modeling reports (20, 55), we find that alternation of \(I_{\text{Ca(L)}}\) is not necessary to evoke steady-state CaT alternans; such alternans are not eliminated under \(I_{\text{Ca(L)}}\) clamp, only reduced in amplitude (Fig. 5D). This observation is consistent with experimental data (13, 48, 50) showing that contraction or CaT alternans can occur without \(I_{\text{Ca(L)}}\) fluctuations.

CaT-AP Coupling

While the magnitude of CaT alternans is comparable in guinea pig and canine (100% relative to minimum CaT, Fig. 4, B and D), that of APD alternans is twice as large in canine (20% canine, 10% guinea pig, of maximum APD, Fig. 4, A and C), indicating stronger CaT-AP coupling in this species. These values are comparable with experimental data (23, 36, 50) that reflect a modest level of CaT-APD coupling during alternans. This is in contrast to recently published simulations (55) where 50% alternation of CaT caused greatly exaggerated (>100%) alternation in APD. Such strong dependence of APD on CaT during alternans has never been observed experimentally (24, 30, 36, 49, 50). While the roles of \(I_{\text{NaCa}}\) and \(I_{\text{Ca(L)}}\) in CaT-AP coupling during alternans were discussed previously (70), the precise nature of these interactions in detailed myocyte models was not addressed. The stronger CaT-AP coupling in the canine compared with guinea pig is due to differences in ion channel expression levels and kinetic properties. On the background of smaller \(I_{\text{Kr}}\) and slow delayed rectifier K\(^+\) current \(I_{\text{Ks}}\) in the canine (27), in conjunction with a much smaller \(I_{\text{Ca(L)}}\) during the late AP plateau (4), CaT-induced changes in \(I_{\text{NaCa}}\) have a much greater modulatory effect on AP repolarization and APD. This makes the canine myocyte more susceptible to Ca\(^{2+}\)-induced AP alternans and suggests that similar sensitivity to arrhythmia is characteristic of the human heart, the cell electrophysiology and AP morphology of which resemble those of the canine (21). The results show that prolongation of APD secondary to a large CaT is mainly due to large inward \(I_{\text{NaCa}}\) at the late AP plateau and repolarization phase, identifying \(I_{\text{NaCa}}\) as the major CaT-APD coupler during alternans. The other Ca\(^{2+}\)-dependent currents, \(I_{\text{Ca(L)}}\) and \(I_{\text{KCa}}\), play a role in shaping the AP during its initial plateau phase, causing crossover between consecutive APs during alternans (Fig. 8A), but have a minimal effect on APD. Transient outward K\(^+\) current \(I_{\text{K1}}\) that contributes to APD rate adaptation (28) has little effect on AP morphology during alternans. The situation can be different, with \(I_{\text{Ca(L)}}\) playing a role in APD alternans, in species, where \(I_{\text{Ca(L)}}\) persists into the late phase of the AP (e.g., the guinea pig) and Ca\(^{2+}\)-dependent \(I_{\text{Ks}}\) has a large conductance (17, 40). Under such conditions, a large CaT can lead to APD shortening during alternans, due to increased Ca\(^{2+}\)-dependent inactivation of \(I_{\text{Ca(L)}}\). Heart failure shifts the onset of APD alternans to slower frequencies and causes a remarkable increase in its amplitude (71). Upregulation of \(I_{\text{NaCa}}\) has been reported in human and animal models of heart failure (6). This observation supports the role of \(I_{\text{NaCa}}\) as the major CaT-APD coupler during alternans. It should be commented that exploration of such mechanistic details requires detailed species-specific and ionic-based cell models. It cannot be accomplished with simplified models (55, 58), where the levels of Ca\(^{2+}\)-dependent and voltage-dependent inactivation of \(I_{\text{Ca(L)}}\) are treated as model parameters, not based on experimental data.

Modulation of CaT and APD Alternans by CaMKII and Repolarizing Currents

Elevated CaMKII activity, as occurs in hypertrophy and heart failure (3), extends the range of CaT alternans and consequently APD alternans to slower frequencies and increases alternans magnitude, suggesting its role in arrhythmia and sudden death in these pathologies. Decrease of CaMKII activity suppresses both CaT and APD alternans, thereby exerting an antiarrhythmic effect. Unfortunately, the decrease blunts the PFFR and FDAR (Fig. 3, E and D), thereby compromising cardiac mechanical function. Modification of \(I_{\text{Ks}}\) has been suggested as a possible intervention for reducing APD alternans (20, 23). Here we describe the first study of the role of Ca\(^{2+}\)-independent currents during Ca\(^{2+}\)-driven APD alternans. The simulations show (Fig. 8, A and B) that only a large threefold increase of \(I_{\text{Ks}}\) can completely suppress APD alternans, which limits its potential use as antiarrhythmic intervention. Moreover, increase of \(I_{\text{Ks}}\) has no effect on the onset and magnitude of CaT alternans (Fig. 8D). Thus, unlike CaMKII inhibition that suppresses APD alternans by eliminating its cause, CaT alternans, increased \(I_{\text{Ks}}\) weakens CaT-AP coupling, thereby suppressing APD alternans by disrupting its link to persistent alternans of CaT. Similar results were obtained by modulating other repolarizing currents, namely the \(I_{\text{KCa}}\), the inward rectifier K\(^+\) current \(I_{\text{K1}}\), and the ATP-dependent K\(^+\) current \(I_{\text{KATP}}\) (not shown). However, results are shown only for \(I_{\text{Ks}}\), because the conductance of \(I_{\text{Ks}}\) is Ca\(^{2+}\)-dependent, increase of \(I_{\text{K1}}\) markedly decreases excitability and conduction velocity (44), and \(I_{\text{KATP}}\) is activated only during pathological conditions of ischemia (acidosis) (54). These results suggest two possible antiarrhythmic strategies for alternans suppression: 1) prevention of CaT alternans by partial CaMKII inhibition; or 2) modification of the coupling between the Ca\(^{2+}\) and electrical subsystems by modulating repolarizing currents such as \(I_{\text{Kr}}\) or \(I_{\text{KATP}}\). A combined approach of 1 and 2 above seems
reasonable, providing more flexibility for alternans suppression with minimization of deleterious effects on contractility and mechanical performance. At a very fast rate (>7 Hz), APD alternans is primarily an electrical phenomenon. This electrical alternans (not shown) has been attributed to slow recovery from inactivation of either \(I_{\text{Na}}\) (44, 52, 66) or \(I_{\text{Ca(L)}}\) (70). Several studies have shown (73) that cells in the heart can be exposed to such fast and even faster rates (e.g., 11 Hz) (73) during fast ventricular tachycardia and fibrillation. APD alternans at these rates can lead to propagation failure and transition from ventricular tachycardia to fibrillation via wavebreak mechanisms (73).

**Limitations**

A limitation of the study is that ECC spatial heterogeneity is known to be arrhythmogenic (13). However, Ca\(^{2+}\) waves are rarely observed in nonfailing myocytes during fast pacing (30, 50), the subject of our investigation. For the same reasons (the models are based on data from nonfailing myocytes), only acute up/downregulation of CaMKII activity was considered. The model does not include the -adrenergic/PKA regulatory pathway that, while sharing some common targets [\(I_{\text{Ca(L)}}\), \(I_{\text{Ca(L)}}\), and \(I_{\text{Rel}}\)] with CaMKII, by itself plays an important role in ECC and cardiac repolarization. Incorporating in the model the -adrenergic/PKA regulatory pathway, together with effects of chronic upregulation of CaMKII activity leads to upregulation of repolarizing (39) and \(I_{\text{Ca(L)}}\) (75) currents and can compensate for mechanical function impaired due to calcineurin overexpression (32).

The time constant of Ca\(^{2+}\) translocation flux from NSR to JSR (\(I_{\text{NS}}\)) in the model represents both (local) RyRs intrinsic recovery from refractoriness and (global) Ca\(^{2+}\) diffusion (8) through the SR. Separation of these time-limiting processes requires additional experimental data that are not yet available and development of a detailed kinetic model of RyR gating. This can be important future work, considering that some studies (50) stress the importance of intrinsic RyR refractoriness in CaT alternans development. The simulations also indicate the need for detailed experimental studies of CaMKII properties in ventricular myocytes and its interactions with RyRs and SERCA/PLB during alternans.

**APPENDIX**

Equations and parameters of the guinea pig (Lrd) and canine (HRd) models used in this study are as in previously published papers (16, 28, 74) and in the research section of http://rudylab.wustl.edu. Definitions are provided in Table 1 of this APPENDIX. Changes made for the purpose of this study are summarized below.

**Formulation of \(I_{\text{Rel}}\)**

See Table 2. The differential equation that describes \(I_{\text{Rel}}\) is of the form

\[
\frac{dI_{\text{Rel}}}{dt} = - \frac{I_{\text{Rel} + L} + I_{\text{Rel}}}{\tau_{\text{Rel}}} \tag{1}
\]

where

\[
I_{\text{Rel}} = \frac{\alpha_{\text{Rel}}}{1 + (K_{\text{Rel}}(\text{Ca}^{2+})_{\text{JSR}})^{\beta_{\text{Rel}}}} \tag{2}
\]

and

\[
\tau_{\text{Rel}} = \frac{\beta_{\text{Rel}}}{1 + K_{\text{Rel}}(\text{Ca}^{2+})_{\text{JSR}}} \tag{3}
\]

\(\alpha_{\text{Rel}}\) is an amplitude coefficient, \(K_{\text{Rel}}\) is a half-saturation coefficient, and \(\beta_{\text{Rel}}\) is a maximal CAMKII-independent value of \(\tau_{\text{Rel}}\).
We make $\tau_{rel}$ in Eq. 3 dependent on CaMKII to incorporate CaMKII-dependent facilitation into the model with a maximal change that produces a 100% facilitation of peak $\Delta \beta_{Ca,CMK}$:

$$\beta_r = \beta_0 (1 + \Delta \beta_{Ca,CMK})$$

$$\Delta \beta_{Ca,CMK} = \Delta \beta_0 [1 + (K_0/CaMKactive)^{h_r}]$$

In addition, we make $\tau_{rel}$ in Eq. 3 a function of $[Ca^{2+}]_{JSR}$ to prevent an unphysiological draining of JSR. Sensitivity of the release flux $I_{rel}$ to luminal $[Ca^{2+}]_{JSR}$ is modeled by Hill equation with a coefficient $h_{rel}$ (14, 57) and half-saturation constant $K_{rel}$ (37).

**Gating Variables of Icalc** (See Table 3)

Fast Ca2+-dependent inactivation ($I_{Ca}$ gate) formulation:

$$f_{Ca} = 0.3/[1 - I_{Ca}(L)/0.05] + 0.55/[1 + [Ca^{2+}]_L/0.002] + 0.15$$

$$\tau_{Ca} = \Delta \tau_{Ca,CMK} / (1 + K_{CMK}/CaMKactive)$$

$$+ 1.5/[1 + ([Ca^{2+}]_L/0.002)] + 0.75$$

where $\Delta \tau_{Ca,CMK}$ is the maximal CaMKII-dependent change of $\tau_{Ca}$ (time constant of $f_{Ca}$ gate) set to 5 ms, $K_{CMK}$ is a half-saturation coefficient, $f_{Ca,ss}$ is the steady-state value of $f_{Ca}$. In addition, to reflect higher $[Ca^{2+}]_{L}$ in the subspace, activity coefficient $\gamma_{Ca}$ was replaced by $\gamma_{Ca} = 0.341$ in the constant field equation for $I_{Ca,L}$.

Steady state formulation of activation d gate was modified as follows

$$d_{Ca} = 1/[1 + e^{-[\gamma_{Ca} - 0.26]/6.74}]$$

where $V$ is membrane voltage.

**SR Fluxes**

CaMKII dependence of $I_{ap}$ was set to up $\Delta I_{ap,CMK} = 0.9$.

**Model of SR Ca2+ Release and SR Fluxes for Lrd Model**

Numerical values for $I_{rel}$ and $I_{rel}$ are provided in Table 2. $I_{rel}$ time constant $\tau_{rel}$ was set to 120 ms.

Table 4 provides documentation for the electrophysiological data used for the canine model validation. Table 5 contains CaMKII data used in the simulations.

### Table 2. Parameters values of SR Ca2+ release model

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<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>$\beta_0$</td>
<td>4.75</td>
<td>Minimal value of $\beta_r$</td>
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<tr>
<td>$K_0$</td>
<td>0.28 mmol/l</td>
<td>Half saturation coefficient for $\Delta \beta_{Ca,CMK}$</td>
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<td>$\Delta \beta_0$</td>
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<td>Maximal CaMKII-dependent change for $\beta_r$</td>
</tr>
<tr>
<td>$\alpha_{rel}$</td>
<td>$\kappa \beta_r$ mmol$^{-1}$ s$^{-1}$</td>
<td>Amplitude coefficient for $I_{rel}$</td>
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<td>$K_{rel}$</td>
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<td>$h_{rel}$</td>
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<td>Hill coefficient for $I_{rel}$</td>
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<td>Hill coefficient for $I_{ap}$</td>
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<td>$\kappa$</td>
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<td>Time-constant of $I_{Ca}$</td>
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<tr>
<td>$\Delta I_{ap,CMK}$</td>
<td>0.9 mmol/l</td>
<td>Maximal CaMKII-dependent change for $I_{ap}$</td>
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</table>

### Table 3. Definitions of Icalc model parameters

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>$d_{Ca}$</td>
<td>Steady-state fast voltage-dependent activation gate of $I_{Ca}$</td>
</tr>
<tr>
<td>$I_{Ca}$</td>
<td>Fast Ca2+-dependent inactivation gate of $I_{Ca}$</td>
</tr>
<tr>
<td>$I_{Ca,ss}$</td>
<td>Steady-state value of $I_{Ca}$</td>
</tr>
<tr>
<td>$\tau_{Ca}$</td>
<td>Time constant of $I_{Ca}$</td>
</tr>
<tr>
<td>$\Delta I_{ap,CMK}$</td>
<td>Maximal CaMKII-dependent change for $I_{ap}$</td>
</tr>
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</table>

### Table 4. Canine ventricular myocyte electrophysiological data for model validation

<table>
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<tr>
<th>Source</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Song et al. (63), Fig. 2E (adapted)</td>
<td>35°C, canine, ventricular myocyte, whole cell patch clamp</td>
</tr>
<tr>
<td>Sipido et al. (60), Fig. 2B</td>
<td>37°C, dog, myocyte, perforated patch clamp</td>
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<td>Hua and Gilmour (23), Fig. 1B</td>
<td>37°C, dog, myocyte, perforated patch clamp</td>
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<tr>
<td>Hua et al. (24), Fig. 2A</td>
<td>37°C, dog, myocyte, perforated patch clamp</td>
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### Table 5. CaMKII data

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<th>Source</th>
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<tr>
<td>DeSantiago et al. (12), Fig. 18 (adapted)</td>
<td>35°C, mouse, isolated myocyte</td>
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<tr>
<td>Wehrens et al. (69), Fig. 3A (adapted)</td>
<td>37°C, rabbit, whole heart</td>
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<tr>
<td>De Koninck and Schulman (10), Fig. 3B and Fig. 4A (adapted)</td>
<td>Room temperature, in vitro CaMKII phosphorylation</td>
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<tr>
<td>Diaz et al. (13), Fig. 2B (adapted)</td>
<td>Room temperature, perforated patch clamp, rat</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

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