Properties and ionic mechanisms of action potential adaptation, restitution, and accommodation in canine epicardium

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1Cardiac Bioelectricity and Arrhythmia Center, Department of Biomedical Engineering, Washington University in St. Louis; St. Louis, Missouri; 2Departments of Cardiology and Mathematics, Maastricht University, Maastricht, The Netherlands; 3Department of Pediatrics, University of Chicago, Pritzker School of Medicine; Chicago, Illinois; and 4Department of Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, Iowa

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Decker KF, Heijman J, Silva JR, Hund TJ, Rudy Y. Properties and ionic mechanisms of action potential adaptation, restitution, and accommodation in canine epicardium. Am J Physiol Heart Circ Physiol 296: H1017–H1026, 2009. First published January 23, 2009; doi:10.1152/ajpheart.01216.2008.—Computational models of cardiac myocytes are important tools for understanding ionic mechanisms of arrhythmia. This work presents a new model of the canine epicardial myocyte that reproduces a wide range of experimentally observed rate-dependent behaviors in cardiac cell and tissue, including action potential (AP) duration (APD) adaptation, restitution, and accommodation. Model behavior depends on updated formulations for the 4-aminopyridine-sensitive transient outward current (Ito1), the slow component of the delayed rectifier K+ current (IKs), the L-type Ca2+ channel current (ICaL), and the Na+-K+ pump current (INaK) fit to data from canine epicardium. We found that Ito1 plays a limited role in potentiating peak ICaL, and sarcoplasmic reticulum Ca2+ release for propagated APs but modulates the time course of APD restitution. IKs plays an important role in APD shortening at short diastolic intervals, despite a limited role in AP repolarization at longer cycle lengths. In addition, we found that ICaL plays a critical role in APD accommodation and rate dependence of APD restitution. Ca2+ entry via ICaL at fast rate drives increased Na+-Ca2+ exchanger Ca2+ extrusion and Na+ extrusion via inward INaK. APD accommodation results from this increased outward INaK. Our simulation results provide valuable insight into the mechanistic basis of rate-dependent phenomena important for determining the heart’s response to rapid and irregular pacing rates (e.g., arrhythmia). Accurate simulation of rate-dependent phenomena and increased understanding of their mechanistic basis will lead to more realistic multicellular simulations of arrhythmia and identification of molecular therapeutic targets.

Arrhythmia; cardiac electrophysiology; mathematical modeling; ion channels

CARDIAC ARRHYTHMIAS and sudden death involve complex myocardial activation patterns, including unidirectional block, reentry, and fibrillation. To understand the relations and transitions between these patterns, the ionic determinants of the response of healthy and diseased cardiac myocytes to complex patterns of excitation must be understood. The single-cell response to such excitation patterns depends on the complex interaction between ionic currents, intracellular ion concentrations, and membrane voltage. Computational cell models provide critical tools for exploring these interactions, allowing the development and testing of hypotheses about underlying ionic mechanisms based on careful integration of available experimental data (37). The dog is a common animal model for studying cell electrophysiology in a range of disease states. Our group and others have developed detailed mathematical models of the canine action potential (AP) (11, 20, 58). Although these models have been used to study arrhythmia mechanisms after heart failure (58) and myocardial infarction (5, 17), as well as ionic mechanisms of alternans (11, 26), they are limited in their ability to simulate important rate-dependent phenomena (6), including the dependence of steady-state AP duration (APD) on pacing cycle length (CL; i.e., APD adaptation), the dependence of APD on diastolic interval (DI; i.e., APD restitution), and the time course of the adjustment of APD to changes in rate (short-term memory (12) or APD accommodation (56)). These limitations extend to ionic models of other species, including the human (6). We hypothesized that canine epicardial APD adaptation, restitution, and accommodation could be simulated and understood on the basis of available descriptions of subcellular ionic processes. We incorporated updated and validated formulations of the 4-aminopyridine-sensitive transient outward current (Ito1), the slow component of the delayed rectifier K+ current (IKs), the L-type Ca2+ channel current (ICaL), and the Na+-K+ pump current (INaK) into a previously published model of the canine epicardial myocyte (17, 20, 26). Model behavior was examined in single-cell and multicellular (strand) simulations. Our work provides new insight into ionic mechanisms underlying important rate-dependent AP properties, including APD restitution, adaptation, and accommodation. Specifically, our studies highlight the importance of Ito1 and IKs in APD restitution and the role of ICaL and INaK in APD accommodation and rate-dependent APD restitution.

METHODS

Model. The Hund-Rudy dynamic model (Fig. 1) of the canine epicardial myocyte (17, 20, 26) serves as the basis for these simulations. Updates to intracellular Ca2+ ([Ca2+]i) handling in a recent study of APD and [Ca2+]i transient (CaT) alternans are included (26). Ion channel formulations, including ICaL, Ito1, IKs, and INaK, have been updated on the basis of additional data from canine ventricular myocytes. Model parameters were fit to experimental data from the literature (Fig. 2). Experimental data that represented the consensus of a wide range of experimental results or were obtained from the most complete available study were chosen. [See supplemental Figs. S2 (ICaL) and S4 (Ito1) in the online version of this article for comparison.

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A new Markov model of $I_{\text{Ca,L}}$ was formulated to reproduce a wide range of experimental data while maintaining computational tractability to facilitate long-term multicellular simulations. The state structure (see supplemental Fig. S1) of the model reflects the hypothesis that $\text{Ca}^{2+}$ binding to calmodulin removes a “brake” and speeds up $I_{\text{Ca,L}}$ voltage-dependent inactivation (28, 33). The model reproduces the $I_{\text{Ca,L}}$ current-voltage ($I-V$) relationship (36) (Fig. 2A), steady-state inactivation (48) (Fig. 2B), time constant of voltage-dependent inactivation (1), and recovery from inactivation (51) in canine ventricular myocytes. A model of $I_{\text{to1}}$ was developed and fit to the $I-V$ curve and time to peak (Fig. 2D), time constant of inactivation (27), and slow time constant of recovery from inactivation (25) in canine epicardial myocytes. A Markov model of $I_{\text{Ks}}$ (43) previously developed in our laboratory was adopted and fit to data from canine ventricular myocytes. The model accurately reproduces the kinetics of $I_{\text{Ks}}$ based on underlying voltage sensor transitions and has provided insight into the role of $I_{\text{Ks}}$ in APD adaptation in the guinea pig and humans (43). The model fits canine ventricular data on the time course of activation (Fig. 2E) (53) and voltage dependence of current accumulation (Fig. 2F) (47). $I_{\text{Ks}}$ density was scaled to fit data from canine epicardial myocytes (24). A recently developed formulation of $I_{\text{NaK}}$ based on data from canine ventricular myocytes (13) was also incorporated into the model. Model $I_{\text{NaK}}$ density matches epicardial myocyte data (Fig. 2G) and results in intracellular $\text{Na}^+$ concentration ($[\text{Na}^+]_i$) at rest and during pacing (CL = 0.5 s) consistent with experimental results (Fig. 2H).

Fig. 1. Hund-Rudy dynamic model of the canine epicardial myocyte. SR, sarcoplasmic reticulum; SS, steady state; CT,$\text{CaCl}_2$, $\text{NaCl}$ cotransporter; CT,$\text{CaCl}_2$, $\text{NaCl}$ cotransporter; $\text{Ca}^{2+}$, slowly activating late $\text{Na}^+$ current; $I_{\text{Ks}}$, limit $\text{Na}^+$ current; $I_{\text{Kab}}$, background $\text{Na}^+$ current; $I_{\text{KCl}}$, sarcolemmal $\text{Ca}^{2+}$ pump; $I_{\text{Iab}}$, 4-aminoopyridine-sensitive transient outward current; $I_{\text{Kib}}$, fast component of delayed rectifier $\text{K}^+$ current; $I_{\text{Ks}}$, slow component of delayed rectifier $\text{K}^+$ current; $I_{\text{Ks}}$, time-dependent $\text{K}^+$ current; $I_{\text{Kab}}$, $\text{Na}^+$/K$^+$ pump current; $I_{\text{Kab}}$, NSR leak current; $I_{\text{Kab}}$, ion diffusion, myoplasm-to-SR subspace; $I_{\text{Kab}}$, $\text{Ca}^{2+}$ transfer, NSR to JSR; $I_{\text{Kab}}$, JSR release current; $I_{\text{Kab}}$, ion diffusion, subspace-to-local $I_{\text{Ca,L}}$, subspace; $I_{\text{Kab}}$, $\text{Na}^+$/Ca$^{2+}$ exchanger (localized to SR subspace); $I_{\text{Kab}}$, L-type $\text{Ca}^{2+}$ current; $I_{\text{Kab}}$, $\text{Ca}^{2+}$-dependent transient outward $\text{Cl}^-$ current; SS(CaL), SS(SR), SR, subspace; PLB, phospholamban; SERCA, sarco(endo)plasmic reticulum $\text{Ca}^{2+}$-ATPase; CSQN, calsequestrin; CaMKII, $\text{Ca}^{2+}$/calmodulin-dependent kinase; NSR, network SR; JSR, junctional SR. (For additional model details, see Refs. 20 and 26, supplemental information in the online version of this article, and http://rudylab.wustl.edu/.)

Fig. 2. A and B: model $I_{\text{Ca,L}}$ current-voltage ($I-V$) relationship and steady-state inactivation fit to experimental data from canine epicardial myocytes (36, 48). C and D: model $I_{\text{to1}}$ $I-V$ curve and time to peak fit to data from canine epicardial myocytes (27). E and F: model $I_{\text{Ks}}$ activation and accumulation fit to data from canine ventricular myocytes (47, 53). G and H: model $I_{\text{NaK}}$ density and steady-state intracellular $\text{Na}^+$ concentration ($[\text{Na}^+]_i$) fit to data from canine ventricular myocytes (13). $V_{\text{test}}$, test potential; CL, cycle length.
Restitution results were obtained for an additional beat after pacing to steady state at a different CL (CLS2). Simulation protocols shift toward shorter APD, consistent with experimental measurements (Fig. 3B, right). The model also reproduces tissue AP restitution kinetics as a function of DI (see below; also see Fig. 6 and supplemental Fig. S5). Figure 3C (left) shows the time course of APD in a model cell and a strand after pacing to steady state at CLS1 = 1 s followed by sustained pacing at CLS2 = 0.5 s starting at time 0. APD at the new CL approaches steady state after several minutes of pacing, a process referred to as accommodation (56), consistent with experiments in canine ventricle (Fig. 3C, right) (39).

Previous simulation studies (6, 34) showed that direct stimulation in single cells and electrotonic loading in tissue can reproduce tissue AP restitution kinetics as a function of DI (see below; also see Fig. 6 and supplemental Fig. S5). Figure 3C (left) shows the time course of APD in a model cell and a strand after pacing to steady state at CLS1 = 1 s followed by sustained pacing at CLS2 = 0.5 s starting at time 0. APD at the new CL approaches steady state after several minutes of pacing, a process referred to as accommodation (56), consistent with experiments in canine ventricle (Fig. 3C, right) (39).
lead to different AP dynamics. Figure 4 compares cell and tissue steady-state APs and ion accumulation in the new model. A 80 µA/µF, 0.5-ms stimulus was used in single-cell simulations (Fig. 4A). In the strand, excitatory axial current in well-coupled tissue (Fig. 4A) consists of (1) an initial transient depolarizing current, (2) a transient repolarizing current as the cell supplies charge to depolarizing downstream cells, and (3) a small sustained repolarizing current (Fig. 4A, inset). The bi-phasic axial current in the tissue reduces AP upstroke velocity relative to its value in the single cell. (Fig. 4B). In addition, steady-state tissue APD is reduced slightly (Fig. 4C) as a result of the sustained repolarizing axial current (Fig. 4A, inset). APD adaptation in cell and strand simulations (Fig. 4C) is consistent with experimental results (25). Cell and strand simulations show an increase in Ca transient amplitude (CaTAMP, Fig. 4D) and maximum [Na] (Fig. 4E), with pacing rate, consistent with experimental results. Differences in CaTAMP and [Na] in cell and strand simulations are minimal. Subsequent results are from strand simulations unless otherwise specified.

$I_{\text{tol}}$, peak $I_{\text{Ca,L}}$, and CaTAMP in cell and strand. Experimental (38) and computational (14) studies have suggested that $I_{\text{tol}}$, by controlling phase 1 repolarization, is an important modulator of $I_{\text{Ca,L}}$ activation and sarcoplasmic reticulum (SR) Ca$^{2+}$ release. We examined differences in the effect of $I_{\text{tol}}$ on $I_{\text{Ca,L}}$ activation and SR Ca$^{2+}$ release in cell and strand simulations. Figure 5A shows that although blockade of $I_{\text{tol}}$ leads to a significant reduction in CaTAMP in cell simulations, reduction of CaTAMP after $I_{\text{tol}}$ blockade in tissue simulations is minimal. The interplay between membrane potential ($V_m$), $I_{\text{tol}}$, $I_{\text{Ca,L}}$, and SR Ca$^{2+}$ release in cell and a strand is examined in Fig. 5, B–E. We note several important differences between simulated APs in the strand and in the isolated cell. These unique features of the strand AP include (1) reduced peak upstroke voltage (Fig. 5B), (2) reduced $I_{\text{tol}}$ activation (Fig. 5C), and (3) minimal dependence of peak $I_{\text{Ca,L}}$ on $I_{\text{tol}}$ activation (Fig. 5D). Reduced peak upstroke voltage in the strand leads to a reduction in peak $I_{\text{tol}}$ due to the approximately linear dependence of $I_{\text{tol}}$ activation on $V_m$. This reduction in $I_{\text{tol}}$ activation leads to a reduction in phase 0 repolarization. Arrows in Fig. 5B denote $V_m$ at the peak of $I_{\text{Ca,L}}$ activation in a cell and a strand, respectively. In cell simulations, a large $I_{\text{tol}}$ repolarizes $V_m$ (Fig. 5C) toward the peak of the $I_{\text{Ca,L}}$-V relationship (Fig. 5B), promoting $I_{\text{Ca,L}}$ activation (Fig. 5D) and SR Ca$^{2+}$ release (Fig. 5E). When $I_{\text{tol}}$ is blocked in the cell, $I_{\text{Ca,L}}$ activation occurs at a $V_m$ that is far from the peak of the I-V curve (Fig. 5B), decreasing peak $I_{\text{Ca,L}}$ (Fig. 5D) and SR Ca$^{2+}$ release (Fig. 5E). For a propagating action potential, however, increased load decreases peak up-
stroke voltage, so that it is near the peak of the $I_{\text{CaL}}$-V curve (Fig. 5B, ~10 mV). Maximal $I_{\text{CaL}}$ activation occurs shortly after the peak upstroke $V_m$, such that the depth of the phase 1 notch plays little role in determining $V_m$ as $I_{\text{CaL}}$ is peaking. Consequently, blockade of $I_{\text{to1}}$ in the strand has little effect on peak $I_{\text{CaL}}$ (Fig. 5D) and SR Ca$^{2+}$ release (Fig. 5E). Furthermore, reduced phase 1 repolarization (Fig. 5B) due to reduced $I_{\text{to1}}$ activation (Fig. 5C) also suggests a diminished importance of $I_{\text{to1}}$ in the strand relative to the cell.

$I_{\text{to1}}$ and APD restitution. Although our results suggest a limited role for $I_{\text{to1}}$ in regulation of peak $I_{\text{CaL}}$ and SR Ca$^{2+}$ release in multicellular strands, $I_{\text{to1}}$ was found to play an important role in APD restitution. Figure 6A shows that the time course of APD restitution is consistent with experiments in epicardial tissue (42). (See supplemental information and Fig. S5 in the online version of this article for comparison of model restitution kinetics with experiments for a range of CLS1.) In canine epicardium, APD gradually increases as DI increases beyond 0.3 s. After 100% $I_{\text{to1}}$ block, the increase in APD with increasing DI is more abrupt, as observed in canine endocardium (23), consistent with the absence of $I_{\text{to1}}$ in this tissue. Figure 6, B–D, examines the role of $I_{\text{to1}}$ in APD restitution time course. At short CLS2, $I_{\text{to1}}$ has not fully recovered from inactivation (Fig. 6C), resulting in minimal phase 1 repolarization (Fig. 6B). As CLS2 increases, notch depth increases as $I_{\text{to1}}$ recovers from inactivation. Since activation of the fast component of the delayed rectifier K$^+$ current ($I_{\text{Ks}}$) is slower at more negative voltages, increasing notch depth slows $I_{\text{Ks}}$ activation (Fig. 6D). The gradual increase in APD during restitution follows the slow time course of $I_{\text{to1}}$ recovery and the suppression of $I_{\text{Ks}}$ activation due to increasing notch depth. When $I_{\text{to1}}$ is absent, increase in CLS2 beyond 0.3 s results in minimal phase 1 voltage change (Fig. 6B, overlapping gray traces), constant $I_{\text{Kr}}$ activation (Fig. 6D), and minimal APD increase (Fig. 6B inset, gray traces). This small increase in APD at CLS2 >0.3 s results in a restitution curve resembling that recorded in endocardium (Fig. 6A) (23).

$I_{\text{Ks}}$ and APD adaptation. The role of $I_{\text{Ks}}$ in canine APD adaptation is examined in Fig. 7. As shown in Fig. 7A, 100% block of $I_{\text{Ks}}$ leads to a modest prolongation of APD at steady state. Percent APD prolongation after $I_{\text{Ks}}$ blockade decreases as CL decreases (“use-dependent” block), except at short CL, where percent prolongation begins to increase again. The biphase effect of $I_{\text{Ks}}$ block on APD is consistent with the rate dependence of $I_{\text{Ks}}$ activation shown in Fig. 7B. $I_{\text{Ks}}$ activation in Fig. 7B is smallest at CLS1 = 0.5 s (Fig. 7B), corresponding to the smallest percent prolongation of APD after $I_{\text{Ks}}$ block in Fig. 7A. $I_{\text{Ks}}$ activation at CLS1 = 0.25 and 1 s is increased relative to CLS1 = 0.5 s, leading to a relative increase in percent APD prolongation after $I_{\text{Ks}}$ block at these rates. Figure 7, C–E, examines the rate dependence of $I_{\text{Ks}}$ state occupancy, following previously published analysis in the guinea pig (43). The 17-state $I_{\text{Ks}}$ model can be divided into two open states, five zone 1 closed states that can activate rapidly, and 10 “deep” zone 2 closed states, which require slow transitions to zone 1 before activation is possible. At CLS1 = 0.5 and 1 s, all channels reside in zone 2 at the start of the AP (Fig. 7C) and activate slowly upon depolarization (Fig. 7B). Slow activation from zone 2 and small peak density in the dog dictate that $I_{\text{Ks}}$ plays a limited role in APD repolarization at longer CL. At CLS1 = 0.25 s, the DI is insufficient for activated channels to return to zone 2. Channels activated during the previous beat remain in zone 1 (Fig. 7D) and the open state (Fig. 7E) as the next stimulus is applied, resulting in more rapid channel activation (Fig. 7B) and an increased role for $I_{\text{Ks}}$ in APD shortening at fast rate.

$I_{\text{Ks}}$ and APD restitution. We also examined the role of $I_{\text{Ks}}$ in APD restitution (Fig. 8). Simulation results show that the percent increase in APD after 100% $I_{\text{Ks}}$ block is largest at the shortest CLS2 (Fig. 8B). At CLS2 = 0.26 and 0.3 s, a significant

Fig. 7. A: percent increase in steady-state APD after 100% $I_{\text{Ks}}$ block as a function of CLS1. B–E: $I_{\text{Ks}}$ current density and state occupancy of zone 2, zone 1, and open state at steady-state for CLS1 = 0.25, 0.5, and 1 s.

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fraction of channels activated during the previous AP remain in the open state (Fig. 8D), while an additional fraction occupies zone 1 to the open state to yield prominent $I_{Ca,L}$ activation (Fig. 8C). Peak activation of $I_{Ca,L}$ at $CL = 0.26$ s is markedly increased relative to peak density during the S1 beat. This prominent $I_{Ca,L}$ activation due to accumulation in the open state and zone 1 is required to achieve APD shortening at short DI consistent with experimental results (42). APD at $CL = 0.26$ s is increased by $\sim 7.5\%$ after $I_{Ks}$ block (Fig. 8B). As DI increases, channels move from zone 1 and the open state to zone 2, resulting in less prominent $I_{Ks}$ activation for more mature stimuli and reduced ($\sim 2.5\%$) APD prolongation after $I_{Ks}$ block.

**APD accommodation.** We sought to gain insight into the ionic mechanism of APD accommodation by examining underlying ionic currents and concentrations. The transition from steady state at $CL = 1$ s to steady state at $CL = 0.5$ s is examined in Fig. 9. APD accommodation exhibits several properties consistent with experimental observations. Equilibration of APD after a change in CL occurs after several minutes of pacing, consistent with experiments in canine ventricular tissue (39). Consistent with the effect of nisoldipine on rabbit ventricular myocytes (50), 100% block of $I_{Ca,L}$ in the model diminishes APD accommodation. In addition, accommodation persists after 100% block of SR $Ca^{2+}$ uptake (uptake current) and release (release current), consistent with effects of ryanodine and thapsigargin on rabbit myocytes (50). Figure 9B shows changes in $[Na^+]_i$ during APD accommodation. In control and when SR release and uptake are blocked, accumulation of $[Na^+]_i$ occurs with a time course similar to that of APD shortening. When $I_{Ca,L}$ is blocked, APD shortening and $[Na^+]_i$ accumulation are diminished. When $[Na^+]_i$ is clamped to its steady-state value at $CL = 0.5$ s, APD accommodation is eliminated. Figure 9C demonstrates that, in control, $[Na^+]_i$ accumulation leads to a large increase in outward $I_{NaK}$ from the first beat to the steady-state beat after a change in CL. After $I_{Ca,L}$ block, there is a smaller difference in $I_{NaK}$ from the first beat to the steady-state beat. Collectively, these simulations suggest that $I_{Ca,L}$ is responsible for the increases in $[Na^+]_i$ and outward $I_{NaK}$ that underlie APD accommodation.

To understand the link between $I_{Ca,L}$, $[Na^+]_i$, and accommodation, we examined the sarcolemmal $Ca^{2+}$ and $Na^+$ flux (µM/S) via currents, pumps, and exchangers after a change from $CL = 1$ s to $CL = 0.5$ s. Fluxes were calculated at steady state at $CL = 1$ s and for each subsequent beat after a change to $CL = 0.5$ s. At steady-state CL $= 1$ s, $Ca^{2+}$ influx (primarily $I_{Ca,L}$) and efflux (primarily $I_{NaCa}$) are balanced (Fig. 10A). After a change to $CL = 0.5$ s, the shorter DI does not provide sufficient time for $I_{NaCa}$ to extrude $Ca^{2+}$ that entered via $I_{Ca,L}$. Excess $Ca^{2+}$ influx leads to $[Ca^{2+}]_i$ accumulation after the first beat and with continued pacing at $CL = 0.5$ s (Fig. 10B). $[Ca^{2+}]_i$ accumulation provides feedback [autoregulation (8)] by decreasing $[Ca^{2+}]_i$ influx (through $Ca^{2+}$-dependent inactivation of $I_{Ca,L}$) and increasing $[Ca^{2+}]_i$ efflux (through forward-mode $I_{NaCa}$). This feedback promotes decreased net $Ca^{2+}$ entry.
per cycle, so that \([\text{Ca}^{2+}]_i\) approaches equilibrium within the 1st minute of paceing at the new CL.

\(\text{Na}^+\) influx exceeds efflux after change from CL\(_s1\) = 1 s to CL\(_s2\) = 0.5 s (Fig. 10C). As with \(\text{Ca}^{2+}\), the shortened DI provides insufficient time for complete extrusion of \(\text{Na}^+\) that entered during the AP, primarily via forward-mode \(\text{INaC}_s\) and the fast \(\text{Na}^+\) current (\(\text{INa}\)). Increased forward-mode \(\text{INaC}_s\) as \([\text{Ca}^{2+}]_i\) accumulates further increases \(\text{Na}^+\) influx. The imbalance of \(\text{Na}^+\) influx and efflux drives \([\text{Na}^+]_i\) accumulation (Fig. 9B) until \(\text{Na}^+\) efflux via \(\text{INaK}\) increases to match influx after minutes of pacing (Fig. 10C). The time course for equilibration of \(\text{Na}^+\) influx and efflux in our simulations (on the order of minutes) agrees with the slow time course of \(\text{Na}^+\) accumulation seen experimentally (13, 34). The increasingly outward \(\text{INaK}\) as \(\text{Na}^+\) influx and efflux approach equilibrium is the primary cause of APD shortening during accommodation. This ionic mechanism by which \(\text{ICaL}\) drives \([\text{Na}^+]_i\) accumulation and \(\text{INaK}\) increase via \(\text{INaCa}\) is consistent with effects of nisol- dipine and ryanodine/thapsigargin on rabbit ventricular myocytes (50). Block of \(\text{ICaL}\) (nisol-dipine) eliminates a critical driving force for \([\text{Na}^+]_i\) accumulation (greater forward-mode \(\text{INaCa}\), which promotes \(\text{Ca}^{2+}\) extrusion and \(\text{Na}^+\) entry). After \(\text{ICaL}\) blockade, \(\text{Ca}^{2+}\) (Fig. 10A) and \(\text{Na}^+\) (Fig. 9C) influx barely exceeds \(\text{Ca}^{2+}\) and \(\text{Na}^+\) efflux after a change to CL = 0.5 s. \([\text{Ca}^{2+}]_i\) (Fig. 10B) and \([\text{Na}^+]_i\) (Fig. 9B) accumulation after \(\text{ICaL}\) blockade is diminished, resulting in diminished APD accommodation. Block of SR \(\text{Ca}^{2+}\) release (ryanodine) and SR \(\text{Ca}^{2+}\) uptake (thapsigargin) eliminates SR \(\text{Ca}^{2+}\) cycling, but not in multicellular tissue.

**Dependence of APD restitution on pacing rate.** Several experimental studies have shown that the APD restitution curve shifts to shorter APD as basic pacing CL decreases (shorter CL\(_{s1}\)) (4, 6, 9, 15). Consistent with these experimental observations, our model reproduces the dependence of restitution on the rate of S1 pacing (Fig. 11). These simulations also provide insight into the underlying ionic mechanism. Fast pacing (short CL\(_{s1}\); Fig. 11A) is accompanied by \([\text{Na}^+]_i\) accumulation (Fig. 11B), as described above in the case of accommodation (Fig. 9). \([\text{Na}^+]_i\) accumulation, in turn, promotes an increase in repolarizing \(\text{INaK}\) (Fig. 11C), which decreases APD, thereby shifting the APD restitution curve to shorter APDs.

**DISCUSSION**

The rate-dependent phenomena examined in this study are thought to play an important role in the dynamics of arrhythmia (2, 22, 29). The model presented here reproduces experimentally observed APD adaptation, restitution, and accommodation in cell and tissue. Although the rate dependence of model APD, \(\text{CaT}\), and \([\text{Na}^+]_i\) accumulation is similar in cell and strand, our simulation results show that the effects of \(\text{I}_{\text{Ca}}\) block on ion accumulation differ. Other simulation results (34) have suggested that APD restitution kinetics and the transition to APD alternans and more complex excitation patterns in cell and tissue also differ. Taken together, these results suggest that experimental findings in isolated myocytes should be extrapolated to the multicellular tissue with caution.

Recent studies have emphasized limitations in our mechanistic understanding of the rate dependence of APD (6). The detailed, physiologically based mathematical descriptions of critical ionic currents, pumps, and exchangers incorporated into the model lead to novel insight into underlying ionic mechanisms. Specific mechanistic insights generated by our study include the following. 1) \(\text{I}_{\text{Ca}}\) potentiates \(\text{I}_{\text{CaL}}\) and SR \(\text{Ca}^{2+}\) release during early AP repolarization in isolated cells, but not in multicellular tissue. 2) \(\text{I}_{\text{Ca}}\) plays an important role in APD restitution because of its slow recovery kinetics. As DI increases, \(\text{I}_{\text{Ca}}\) recovery and phase 1 notch depth increase, suppressing \(\text{I}_{\text{Kr}}\) activation and lengthening APD. 3) \(\text{I}_{\text{Kr}}\) plays a limited role in repolarization of paced APs but plays an important role in APD shortening during premature stimuli. 4) \(\text{I}_{\text{CaL}}\) plays a critical role in APD accommodation and memory. As a response to \(\text{Ca}^{2+}\) entry via \(\text{I}_{\text{CaL}}\) at fast rates, forward-mode \(\text{INaC}_s\) increases, leading to increased \(\text{Ca}^{2+}\) efflux and \(\text{Na}^+\) influx. \(\text{Na}^+\) accumulates and repolarizing...
I_{NaK} increases, leading to APD shortening during accommodation. 5) I_{Ca,L}-dependent increase in [Na\(^+\)], and I_{NaK} at fast rates is responsible for the shift in APD restitution curves toward shorter APD.

Previous experiments and simulation studies have suggested that I_{to1}, by increasing phase 1 repolarization, increases peak I_{Ca,L} (the trigger for SR release) and, consequently, SR Ca\(^{2+}\) release (14, 38). These studies showed enhanced I_{Ca,L} and SR Ca\(^{2+}\) release when phase 1 repolarization increases the I_{Ca,L} driving force. Although this scenario is relevant to an isolated, directly stimulated myocyte, model simulations indicate that it does not apply to the situation in vivo, where electrotonic loading from neighboring cells weakens the AP upstroke. Our simulations suggest that, for propagating APs, I_{to1}-dependent phase 1 repolarization has little effect on peak I_{Ca,L} and SR Ca\(^{2+}\) release. In addition, simulations show that the weakened AP upstroke in the tissue decreases I_{to1} activation and phase 1 repolarization. I_{to1} has been accorded a role in conduction (16, 55) and in electrophysiological remodeling in various diseased states (21, 27, 60). Our results suggest that predictions of the effect of I_{to1} and I_{to1} block based on single-cell simulations and experiments may not apply to the in vivo situation. In contrast, model simulations predict that I_{to1} plays a significant role in APD restitution. The interplay between I_{Ks} activation and the slow recovery kinetics of I_{to1} leads to a gradual increase in APD during restitution, whereas I_{to1} block results in a more abrupt APD increase. This result provides a mechanistic explanation for the correlation between notch depth and APD observed in canine ventricular tissue (23). The time course of APD restitution is thought to play an important role in the stability of ventricular arrhythmias (22). Our results suggest that experimentally observed heterogeneity in I_{to1} density and recovery kinetics (30, 32) may play a role in the stability of arrhythmias in different species, regions of tissue, or pathophysiological states. Canine experiments have shown transmural heterogeneity in I_{NaCa} (61), I_{to1} (25), I_{Kr} (46), and I_{NaCa} (62). The epicardial model accurately reproduces endocardial restitution kinetics after blocking only I_{to1}, suggesting that I_{to1} is the dominant determinant of heterogeneity in restitution between the two cell types.

Experimental measurements of the effect of I_{Ks} block on APD in canine ventricle have ranged from near 0 (47) to as much as 30% (41). Varro et al. (52) report a modest prolongation (3–7%) and biphasic CL dependence after chromanol block, consistent with our simulation results. Although the role of I_{Ks} in repolarization remains controversial, LQT mutations linked to genes for I_{Ks} α- and β-subunits (46) argue for an important role in repolarization. Our simulations demonstrate that slow activation and relatively low density limit the role of I_{Ks} in AP repolarization at physiological heart rates in the absence of β-adrenergic stimulation, consistent with our previous modeling studies (20). Simulations with a detailed Markov model of I_{Ks} predict an important role for I_{Ks} in APD restitution. During deactivation, channels accumulate in closed states (zone I), where rapid activation in response to a premature stimulus is possible, leading to an important role for I_{Ks} in APD restitution at short DI. A role for I_{Ks} in APD shortening at fast CLs1 via accumulation in closed states has been shown in the guinea pig and humans (43). Despite major differences between I_{Ks} density and kinetics in the guinea pig and dog, the mechanism for enhanced participation of I_{Ks} at fast CLs1 during adaptation and at short DI during restitution are similar in the two species. Importantly, although large density in the guinea pig ensures an important role for I_{Ks} at all rates, we find that I_{Ks} plays a major role in canine restitution at short DI, despite a limited role at longer physiological CL. Although the effect of I_{Ks} block on paced APs has been studied experimentally, no studies on the role of I_{Ks} in restitution have been reported. I_{Ks} is also likely to play an important role in repolarization in the presence of β-adrenergic stimulation (53) or for abnormally prolonged APD (52).

Recent simulations have addressed the role of APD accommodation and short-term memory in the dynamics of arrhythmia (2, 29). Simulations (2) and experiments (50) have emphasized uncertainty about the ionic mechanisms underlying this phenomenon. The results presented here predict a major role for I_{Ca,L}-driven [Na\(^+\)] accumulation in this phenomenon. A role for [Na\(^+\)] and I_{NaK} in gradual APD changes during long-term pacing has been proposed (4, 10, 19). Similarities between the effect of block of I_{Ca,L} and SR function in simulations and experiments (50) support for this hypothesis. Although a similar time course of APD accommodation after a CL change has been observed in a range of preparations, APD accommodation in other species and preparations is often more rapid (12, 50, 57). This suggests that other mechanisms may play a role or, alternatively, that additional experiments are required to improve our understanding of equilibration of [Na\(^+\)] and [Ca\(^{2+}\)] during long-term pacing. A role for I_{Ca,L} in the dynamics of arrhythmia has been proposed by many investigators, usually through effects on APD restitution (4, 35). Our simulations suggest that the effect of I_{Ca,L} on [Na\(^+\)] and APD accommodation should also be considered. The importance of [Na\(^+\)] in determining the rate dependence of APD also points to an important limitation of simplified computational models, where intracellular ion concentrations are often held constant.

The predictive value of multicellular simulations of arrhythmia depends on the ability of cell models to reproduce experimental observation. Despite variability across species, cell type, and preparation, APD adaptation, restitution, and accommodation are qualitatively similar in many species. Our model examines the ionic basis of these rate-dependent phenomena in the dog but may provide insight into ionic mechanisms in other species, including humans. In addition, our model will provide a valuable tool for linking initiation and maintenance of arrhythmia to underlying cellular processes and ionic currents through multicellular simulations.

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