Ionic Mechanism of Electrical Alternans

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Abstract

Although alternans of action potential duration (APD) is a robust feature of rapidly paced canine ventricle, currently available ionic models of cardiac myocytes do not recreate this phenomenon. To address this problem, we developed a new ionic model, using formulations of currents based on previous models and recent experimental data. Compared to existing models, $I_{K1}$ was decreased at depolarized potentials, the maximum conductance and rectification of $I_{Kr}$ were increased and $I_{Ks}$ activation kinetics were slowed. $I_{Ks}$ was increased in magnitude and activation shifted to less positive voltages and $I_{Ca}$ was modified to produce a smaller, more rapidly inactivating current. Finally, a simplified form of intracellular calcium dynamics was adopted. In this model, APD alternans occurred at cycle lengths = 150-210 ms, with a maximum alternans amplitude of 39 ms. APD alternans was suppressed by decreasing $I_{Ca}$ magnitude or calcium-induced inactivation and by increasing the magnitude of $I_{K1}$, $I_{Kr}$, or $I_{Ks}$. These results establish an ionic basis for APD alternans, which should facilitate the development of pharmacological approaches to eliminating alternans.

Action potential duration restitution, calcium current, potassium currents
Introduction

The duration of the cardiac action potential is determined in large part by the preceding diastolic interval. This relationship between action potential duration and diastolic interval, known as the action potential duration restitution relation, is an important determinant of cardiac dynamics (17). In particular, if the slope of the restitution relation is one or greater, an alternation of action potential duration, or electrical alternans, commonly develops during high frequency pacing (2,8).

It has been suggested that rate-dependent electrical alternans may be a precursor to the development of ventricular arrhythmias, particularly ventricular fibrillation (6,10,19,22). In support of this idea, several recent experiments have shown that when the slope of the restitution relation is one or greater, rapid pacing induces both alternans and fibrillation in isolated ventricles (5,11,23). If the slope of the restitution relation is reduced to less than one, neither electrical alternans nor fibrillation occurs (5,11,12,23). Unfortunately, the interventions used to date to suppress alternans and fibrillation (high dose calcium channel blockers (23), hyperkalemia (12) and bretylium (5)) have limited clinical utility. More effective means of suppressing alternans need to be identified, a process that would be facilitated by a more complete understanding of the ionic basis for alternans.

One approach to determining the ionic basis for alternans is to use a computer model, several of which have been developed. For example, Luo and Rudy, using data obtained primarily from guinea pig myocytes, developed a comprehensive ionic model (LR1) that subsequently was updated (LRd) to include formulations for the rapid and slow components of the delayed rectifier ($I_{Kr}$ and $I_{Ks}$, respectively) (15,16). Recently,
Winslow modified the LRd model (26) using data for ionic currents obtained from canine ventricular myocytes and a formulation for Ca dynamics developed originally in guinea pig myocardial cells (9). An alternative formulation for Ca dynamics has been proposed by Chudin et al (1) in their modification of the LR1 model.

Each of the models described above has limitations with respect to the study of the ionic basis for electrical alternans. The Winslow and LRd models do not produce sustained alternans at rapid pacing rates, whereas the Chudin model, which does generate electrical alternans, lacks formulations for repolarizing potassium currents likely to contribute importantly to alternans ($I_{Kr}$, $I_{Ks}$, and the transient outward potassium current $I_{to}$).

Given that a complete ionic model that generates electrical alternans is not currently available, we set out to develop such a model, guided by the results obtained from our experimental studies in canine ventricle (11,23). Our initial objectives were to develop an ionic model of the canine ventricular myocyte that exhibited stable electrical alternans and to use the model to identify the ionic currents responsible for alternans. Once the relevant ionic currents had been identified, we then manipulated these currents to eliminate alternans. Our expectation was that the same ionic manipulations that suppress alternans in the ionic model will suppress fibrillation in vivo, in which the case the results of the present study may suggest novel approaches to the prevention of ventricular fibrillation.
Materials and Methods

To study the ionic mechanism of electrical alternans in canine myocytes, we constructed a canine ventricular myocyte (CVM) model using appropriate formulations of ionic currents from the LRd, Winslow, and Chudin models, altered as necessary to fit experimental voltage clamp data from canine ventricular myocytes. It has been well established that cellular electrical properties in the canine ventricle vary, both between right and left ventricles and within a given ventricle, according to whether a cell resides in the epicardium, endocardium or mid-myocardium (13,14). Because the Winslow model is the only existing ionic model based on the electrical properties of canine ventricle, we elected to use that model as the basis for the CVM model. Consequently, the CVM model, like its predecessor, recreates the mid-myocardial or M cell action potential. Further alterations of various currents, including $I_{K_s}$, $I_{to}$ and $I_{NaCa}$, would be required to model the electrical activity of canine endocardial and epicardial myocytes (13,29).

The CVM model contains the following ionic current formulations:

$$\frac{dV}{dt} = -(I_{stim} + I_{Na} + I_{K1} + I_{Kr} + I_{Ks} + I_{to} + I_{Kp} + I_{NaK} + I_{NaCa} + I_{Nab} + I_{Cab} + I_{pCa} + I_{Ca} + I_{CaK})$$

$I_{stim}$

The stimulus current used to drive the model is a square wave pulse consisting of $-80 \, \text{uA/uF}$ of current for 1 ms.

$I_{Na}$
The sodium current is the same as that used in the Winslow model (26), except that the discontinuities in the h and j gate formulations were removed.

\[
I_{Na} = \bar{G}_{Na} m^3 h j (V - E_{Na})
\]
\[
\frac{dm}{dt} = \alpha_m (1 - m) - \beta_m \quad \beta_m = 0.08 e^{-\frac{V}{11}}
\]
\[
\frac{dh}{dt} = \alpha_h (1 - h) - \beta_h \quad \alpha_h = 0.135 e^{\frac{V + 80}{23}}
\]
\[
\frac{dj}{dt} = \alpha_j (1 - j) - \beta_j \quad \beta_j = \frac{7.5}{1 + e^{-\frac{V + 10}{11}}}
\]
\[
E_{Na} = \frac{RT}{F} \ln \left( \frac{[Na^+]_o}{[Na^+]_i} \right)
\]
\[
\alpha_j = \frac{0.175 e^{\frac{V + 157}{23}}}{1 + e^{15(V + 79)}}
\]
\[
\beta_j = \frac{0.3}{1 + e^{-\frac{V + 32}{11}}}
\]

\[
I_{k1}
\]

\[I_{k1}\] was formulated to agree with data from Freeman et al (4). These data indicate a smaller outward current at depolarized potentials than is seen in the Winslow model.

\[
I_{k1} = \bar{G}_{k1} K_{1}^{\infty} \left( \frac{[K^+]_o}{[K^+]_i} + K_{mk1} \right) (V - E_K)
\]
\[
K_{1}^{\infty} = \frac{1}{2 + e^{\frac{1.62 F}{RT} (V - E_K)}}
\]
\[
E_K = \frac{RT}{F} \ln \left( \frac{[K^+]_o}{[K^+]_i} \right)
\]

\[
I_{kr}
\]

The rapid delayed rectifier current was fit to the data from Gintant (7). In particular, we reproduced the voltage clamp experiment used to generate figure 2 in his
paper. The Winslow formulation of the current was altered to increase rectification, slow kinetics at depolarized potentials and increase maximum conductance.

\[ I_{Kr} = \frac{\bar{G}_{Kr} R(V) X_{Kr}}{\left[ \frac{[K^+]_o}{4} (V - E_K) \right]} \]

\[ \frac{dX_{Kr}}{dt} = \frac{X_{Kr}^\infty - X_{Kr}}{\tau_{Kr}} \]

\[ R(V) = \frac{1}{1 + 2.5e^{10(V+28)}} \]

\[ \tau_{Kr} = 43 + \frac{1}{e^{5.495 \cdot 1691V} + e^{-7.67 \cdot 0.0128V}} \]

\[ X_{Kr}^\infty = \frac{1}{1 + e^{-2.182 \cdot 1819V}} \]

\[ I_{Ks} \]

The slow delayed rectifier current was fit to data from Varro et al (25), specifically the results shown in figure 2 of their paper. The Winslow model was altered to increase the magnitude of the current and shift activation to less positive voltages.

\[ I_{Ks} = \frac{\bar{G}_{Ks} X_{Ks}^2 (V - E_{Ks})}{\sqrt{\frac{\left[ K^+ \right]_o}{[K^+]_i} + 0.01833 [Na^+]_o}} \]

\[ E_{Ks} = \frac{RT}{F} \ln\left( \frac{\left[ K^+ \right]_o}{[K^+]_i} + 0.01833 [Na^+]_o \right) \]

\[ \frac{dX_{Ks}}{dt} = \frac{X_{Ks}^\infty - X_{Ks}}{\tau_{Ks}} \]

\[ X_{Ks}^\infty = \frac{1}{V - 16} \]

\[ \frac{1}{1 + e^{-13.6}} \]

\[ \tau_{Ks} = \frac{0.0000719(V - 10)}{1 - e^{-148(V - 10)}} + \frac{0.00131(V - 10)}{e^{0.0687(V - 10)} - 1} \]

\[ I_{to} \]

The transient outward current in the model is the same as in the Winslow model.
\[ I_{to} = \bar{G}_{to} X_{to} Y_{to} (V - E_K) \]
\[ \alpha_{Xto} = 0.04516e^{0.3577V} \]
\[ \beta_{Xto} = 0.0989e^{-0.06237V} \]
\[ \frac{dX_{to}}{dt} = \alpha_{Xto}(1 - X_{to}) - \beta_{Xto} \]
\[ \alpha_{Yto} = \frac{0.005415e^{(V + 3.5)}}{V + 3.5} \]
\[ \beta_{Yto} = \frac{0.005415e^{(V + 3.5)}}{1 + 0.51335e^{(V + 3.5)}} \]
\[ \frac{dY_{to}}{dt} = \alpha_{Yto}(1 - Y_{to}) - \beta_{Yto} \]
\[ \alpha_{e} = 1 \]
\[ \beta_{e} = 1 + 0.51335e^{(V + 3.5)} \]

**I_{Kp}**

The plateau potassium current is the same as in the Winslow model.

\[ I_{Kp} = \bar{G}_{Kp} K_{kp} (V - E_K) \]
\[ K_{kp} = \frac{1}{7.488 - V} \]
\[ 1 + e^{5.98} \]

**I_{NaK}**

The sodium-potassium pump current is the same as in the LRd model.

\[ I_{NaK} = \bar{I}_{NaK} f_{NaK} \frac{1}{1 + (\frac{K_{mNa}}{[Na^+]})^{1.5}} \frac{[K^+]_o [Na^+]_i}{K_{Na} + K_{Na}} \]
\[ f_{NaK} = \frac{1}{1 + 0.1245e^{-\frac{1}{VT}} + 0.0365\sigma e^{-\frac{1}{VT}}} \]
\[ \sigma = \frac{1}{7} (e^{67.3} - 1) \]

**I_{Nab}, I_{NaCa}, I_{pCa}, I_{Cab}**

\[ I_{Nab} = \bar{G}_{Nab} (V - E_{Na}) \]
The sodium-calcium exchange current, sarcolemmal pump current, and calcium and sodium background currents are the same as in the Winslow model.

\[ I_{NaCa} = \frac{k_{NaCa}}{K_{mNa} + [Na^+]_o} \frac{1}{K_{mCa} + [Ca^{2+}]_o} \frac{1}{1 + k_{out}e^{\frac{VF}{RT}([Na^+]_o[Ca^{2+}]_o - e^{\frac{VF(g-1)}{RT}}[Na^+]_o[Ca^{2+}]_i)}} \]

\[ I_{pCa} = \frac{I_{pCa}}{K_{mPCa} + [Ca^{2+}]_i} \]

\[ I_{Cab} = \bar{G}_{Cab}(V - E_{Ca}) \]

\[ E_{Ca} = \frac{RT}{2F} \ln\left(\frac{[Ca^{2+}]_o}{[Ca^{2+}]_i}\right) \]

\[ I_{Ca} \]

The L-type calcium current in the model is a modified version of that found in the LRd model. A time-dependent, enhanced Ca-induced inactivation was used, as well as a decrease in the current magnitude. These changes produced a smaller more rapidly inactivating calcium current, in agreement with experimental observations by Zygmunt (personal communication).
\[ I_{Ca} = \bar{I}_{Ca} fdf_{Ca} \]

\[ \bar{I}_{Ca} = \frac{P_{Ca}}{C_{sc}} \frac{4VF^2 [Ca^{2+}]_o \frac{2VF}{e^{RT}} \cdot 0.341 [Ca^{2+}]_o}{e^{RT} - 1} \]

\[ f'^{\infty} = \frac{1}{1 + e^{\frac{V}{12.5}}} \]

\[ d'^{\infty} = \frac{1}{1 + e^{-6.24}} \]

\[ \tau_d = \frac{1}{1 + e^{-0.05(V+40)}} + \frac{0.07e^{-0.05(V+40)}}{1 + e^{-0.07V}} \]

\[ f'^{\infty} = \frac{1}{1 + \left(\frac{[Ca^{2+}]_o}{K_{mfc}}\right)^3} \]

\[ \tau_{fca} = 30 \]

\[ I_{CaK} \]

The potassium current through the L-type calcium channel is also a modified version of the LRd formulation.

\[ I_{CaK} = \frac{P_{CaK}}{C_{sc}} \frac{f_{df_{Ca}}}{1 + \frac{I_{Ca}}{I_{Cahalf}}} \frac{1000VF^2 [K^+]_o \frac{VF}{e^{RT} - [K^+]_o}}{e^{RT} - 1} \]

\[ Ca handling \]

A modified form of the intracellular calcium dynamics from Chudin et al. (1) was used. We included buffering from calmodulin in the cytoplasm and calsequestrin in the sarcoplasmic reticulum (SR), omitted spontaneous release of calcium from the SR, and combined the concentrations of calcium in the junctional sarcoplasmic reticulum (JSR) and the non-junctional sarcoplasmic reticulum (NSR) into a single variable.
\[ \frac{d[Ca^{2+}]_{s}}{dt} = \beta_{s}(J_{rel} + J_{leak} - J_{up} - \frac{A_{Cap}C_{sc}}{2F_{myo}}(I_{Ca} + I_{CNa} + I_{NaCa} - 2I_{NaCa}) \]

\[ \beta_{s} = (1 + \frac{[CMDN]_{tot}K_{CMDN}^{CMDN}}{(K_{m}^{CMDN} + [Ca^{2+}]_{i})^{2}})^{-1} \]

\[ J_{rel} = \bar{P}_{rel} \int df_{Ca} \frac{\mu[Ca^{2+}]_{s} - [Ca^{2+}]_{i}}{V} \]

\[ \gamma = \frac{1}{1 + (\frac{2000}{[Ca^{2+}]_{s}})^{3}} \]

\[ J_{up} = \frac{V_{up}}{1 + (\frac{K_{mup}}{[Ca^{2+}]_{s}})^{2}} \]

\[ J_{leak} = \bar{P}_{leak}([Ca^{2+}]_{s} - [Ca^{2+}]_{i}) \]

\[ \frac{d[Ca^{2+}]_{s}}{dt} = \beta_{sr}(J_{up} - J_{leak} - J_{rel}) \frac{V_{max}}{V_{s}} \]

\[ \beta_{sr} = (1 + \frac{[CSQN]_{tot}K_{m}^{CSQN}}{(K_{m}^{CSQN} + [Ca^{2+}]_{s})^{2}})^{-1} \]

Numerical Methods

The equations listed above were solved using parameter values and initial conditions found in the Appendix. The simulations were run on Macintosh G3 and G4 computers using a program written in C. The numerical integration scheme was similar to that used in Luo and Rudy (15,16) and in Rush and Larsen (24). Briefly, the time steps of integration were made small enough so that the changes in voltage and in calcium concentrations remained below maximum values, \( \Delta V_{\text{max}} \) and \( \Delta Ca_{\text{max}} \). If the changes in voltage and calcium concentration were below a minimum value (\( \Delta V_{\text{min}} \) and \( \Delta Ca_{\text{min}} \)), the time step was increased. By keeping the changes in voltage small, we could solve the linear gate variable equations exactly during each time step. We used \( \Delta V_{\text{max}} = .8 \) mV,
$\Delta V_{\text{min}} = 0.2$ mV, and $\Delta C_{a_{\text{max}}} = 1.067 \times 10^{-2}$ uM, $\Delta C_{a_{\text{min}}} = 2.67 \times 10^{-3}$ uM. See \cite{15,16} and \cite{24} for more details. The other time dependent variables in the model were solved using an adaptive fourth order Runge-Kutta method \cite{21}. The errors were normalized as described in Jafri et al \cite{9}. We used a maximum error of $1e-6$, a minimum time step of $0.005$ ms, and a maximum time step of $0.5$ ms. During the stimulus, the step size was fixed at $0.005$ ms.

To further increase computational speed, lookup tables were used to avoid repeatedly calculating exponentials and other computationally expensive functions. The lookup tables were calculated once before each simulation for 15,000 values of voltages ranging from $-100$ mV to $+100$ mV. Values of voltages lying between the indices of the lookup table were calculated using linear interpolation. To check that these numerical techniques did not affect the accuracy of the simulation, simulations also were run using no lookup tables, with a maximum time step of $0.1$ ms. The action potential durations throughout a pacedown from a pacing cycle length of $400$ ms to a cycle length of $90$ ms differed by less than $1\%$ between the two simulations.

Restitution relations were generated using the procedure described in Koller et al \cite{11}, where action potential duration was expressed as a function of the preceding diastolic interval. The magnitude of action potential duration alternans was defined as the difference in action potential duration between two consecutive action potentials. Action potential duration was measured to $95\%$ of repolarization.
Results

Action potential and ionic currents

Figure 1 illustrates the action potentials, ionic currents and Ca\(^{2+}\) transients generated by the CVM model at a pacing cycle length of 400 ms. The action potential (figure 1a) was characterized by the familiar spike and dome morphology of canine mid-myocardial cells. I\(_{Ca}\) (figure 1b) was of smaller magnitude and inactivated more rapidly than I\(_{Ca}\) in previous models, in agreement with the recent experimental observations of Zygmunt (private communication). The time course and magnitude of [Ca\(^{2+}\)]\(_i\) (figure 1d) was similar to experimental results reported previously (1,26), indicating that the simplified calcium handling in the CVM model generated realistic calcium transients.

As shown in figure 1f, I\(_{Kr}\) increased significantly toward the end of plateau, in good agreement with the data from Gintant (7). In contrast, I\(_{Ks}\) was too small to contribute significantly to repolarization at this cycle length (figure 1g; note current scale compared to 1f), primarily because of its very slow recovery from deactivation (25).

Electrical alternans

The CVM model generated electrical alternans at physiologically relevant pacing cycle lengths. Figure 2 shows the action potential and selected plateau currents at a cycle length of 180 ms, where the CVM model produced stable alternans of large magnitude. Note that I\(_{Ca}\), f\(_{Ca}\), and the calcium transient were significantly different between the long and short action potentials, whereas peak I\(_{Kr}\) and peak inward I\(_{NaCa}\) were not. I\(_{Ks}\) varied in magnitude between the long and short action potentials, but the peak current magnitude remained small.
Figure 3 shows the relationship between action potential duration and the pacing cycle length over the range of cycle lengths that produced electrical alternans (400 to 90 ms; panel a) and over a wider range of cycle lengths (8000 to 90 ms; panel c). Action potentials generated at several different pacing cycle lengths are shown in panel d. The model generated electrical alternans over a wide range of pacing cycle lengths, in association with a region of the restitution relation having slope = 1, (figure 3b). At cycle lengths less than 150 ms, alternans was absent. The initial increase in alternans magnitude as the pacing cycle length was shortened, followed by a subsequent decrease in alternans magnitude with further shortening of the cycle length, is in good agreement with experimental data (11).

Role of plateau Na\(^+\) and Ca\(^{2+}\) currents in alternans

The large difference in I\(_{\text{Ca}}\) between the long and short action potentials shown in figure 2 suggests that I\(_{\text{Ca}}\) contributes significantly to the development of alternans. Experiments using calcium channel blockers also have indicated that I\(_{\text{Ca}}\) may mediate alternans (23). To simulate the effects of a generic calcium channel blocker in the model, we decreased the magnitude of I\(_{\text{Ca}}\) by 20%. Figure 4 shows the action potential and plateau currents in the decreased I\(_{\text{Ca}}\) model at a pacing cycle length of 180 ms. No alternans of I\(_{\text{Ca}}\) or action potential duration occurred at this, or any other, pacing cycle length. As expected, the restitution relation lacked a region of slope = 1 (figure 5a).

The elimination of alternans in the reduced I\(_{\text{Ca}}\) model was mediated primarily by alterations of calcium induced-inactivation of I\(_{\text{Ca}}\) and the resultant changes in action potential duration (figure a). After a long diastolic interval, Ca-induced inactivation
recovered to a nearly maximal value, which resulted in a large $I_{Ca}$ during the next action potential and a correspondingly long action potential duration. Because of the long action potential duration, the next diastolic interval was shortened. Consequently, the Ca-induced inactivation gate did not recover fully by the time the next stimulus was applied. The subsequent $I_{Ca}$ was smaller, causing a shorter action potential duration. A long diastolic interval followed the short action potential duration, and the cycle repeated.

When $I_{Ca}$ was diminished action potential duration was shortened, resulting in a prolongation of diastolic interval (figure b). The longer diastolic interval allowed for complete recovery of $f_{Ca}$. Consequently, $I_{Ca}$ was constant for each action potential, although reduced in magnitude. As the pacing cycle length was shortened, both the diastolic interval and action potential duration were reduced. However, the diastolic interval remained sufficiently long to allow for complete recovery of $f_{Ca}$, until the cycle length became so short that 2:1 conduction block occurred.

According to the scenario described above, not only should a reduction of $I_{Ca}$ decrease alternans magnitude, but an increase in $I_{Ca}$ should increase alternans magnitude. To test this hypothesis, the magnitude of $I_{Ca}$ was varied and the resultant magnitude of action potential duration alternans was measured. As shown in figure 7, alternans magnitude was proportional to the magnitude of $I_{Ca}$. In addition, alternans magnitude could be altered predictably by varying the time constant for Ca-induced inactivation ($\tau_f$), where decreasing $\tau_f$ eliminated alternans of $I_{Ca}$ and action potential duration, secondary to a reduction in the magnitude of $I_{Ca}$ and increasing $\tau_f$ had the opposite effects.

The magnitude of action potential duration alternans also could be altered by changing the magnitude of $I_{Na}$ and $I_{NaCa}$ (figure 7). As $I_{Na}$ was increased (by increasing
G(\text{Na}), alternans magnitude increased, secondary to increased plateau Na\textsuperscript{+} current. Conversely, alternans magnitude was decreased following a reduction of \(I_{\text{Na}}\). Both increases and decreases of \(I_{\text{NaCa}}\), secondary to alterations of \(k_{\text{NaCa}},\ reduced the magnitude of action potential duration alternans.

### Role of repolarizing K\textsuperscript{+} currents in alternans

The effects of altering \(I_{\text{to}}, I_{\text{p}}, I_{\text{K1}}, I_{\text{Kr}},\) and \(I_{\text{Ks}}\ on alternans also were determined (figure 7). The magnitude of each of the currents was increased individually until alternans no longer occurred during pacing at any cycle length. Elimination of alternans occurred after increasing \(I_{\text{to}}\) by 10% or more, \(I_{\text{K1}}\) by 7% or more or \(I_{\text{Kr}}\) by 62% or more. A substantially greater increase in the magnitude of \(I_{\text{Ks}}\ or I_{\text{Kp}}\ was required to eliminate alternans. The suppression of alternans after increasing \(I_{\text{K1}}\, I_{\text{Kr}},\) or \(I_{\text{Ks}}\ was associated with a reduction of the maximum slope of the action potential duration restitution relation to < 1 (figure 5). Decreasing the magnitude each of the K\textsuperscript{+} currents increased the magnitude of action potential duration alternans, with the exception of \(I_{\text{to}},\ where decreasing the magnitude of the current decreased alternans magnitude.

Increasing \(I_{\text{K1}}, I_{\text{Kr}},\) or \(I_{\text{Ks}}\ reduced action potential duration from a control value of 220 msec to 211, 211 and 197 msec, respectively, at a pacing cycle length of 1000 msec. Despite the reduction in action potential duration, the magnitudes of \(I_{\text{Ca}}\ and the calcium transient were minimally affected, both at short pacing cycle lengths (compare figures 2 and 8) and at a cycle length of 1000 msec: peak \(I_{\text{Ca}}\ magnitudes for control and elevated \(I_{\text{K1}}, I_{\text{Kr}},\) \(I_{\text{Ks}}\ were -1.57, -1.57, -1.57 and -1.58 \text{pA/pF}, respectively and peak [Ca\textsuperscript{2+}]i magnitudes were 2.15, 2.10, 2.12 and 2.04 \text{µM}, respectively.
Discussion

We have developed an ionic model of the canine ventricular muscle cell that generates physiologically realistic action potential duration alternans, characterized by a large magnitude and a wide range of pacing cycle lengths over which they appear. Action potential duration alternans was caused primarily by an alternans of $I_{Ca}$, where the latter resulted from the time-dependent behavior of the calcium-induced inactivation gate, $f_{Ca}$. Alternans was suppressed by reducing the magnitude of $I_{Ca}$, as well as by increasing the magnitude of selected repolarizing $K^+$ currents. Although the CVM model has some limitations, as discussed below, it is the first ionic model of the canine ventricular myocyte that reproduces physiological alternans at rapid pacing rates. As such, it provides a useful simulation tool for studying the complicated interactions of cardiac membrane currents.

Role of $I_{Ca}$ in alternans

The development of action potential duration alternans required that: 1) the duration of the action potential have a sensitive dependence on $I_{Ca}$, and; 2) the recovery of $I_{Ca}$ have a sensitive dependence on diastolic interval. The first condition applied so long as there was a relative balance of repolarizing $K^+$ current and $I_{Ca}$ during the action potential plateau. The second condition was manifest during pacing at short cycle lengths, where partial recovery of $I_{Ca}$ after short diastolic intervals resulted in short action potential durations, followed by long diastolic intervals. Nearly complete recovery of $I_{Ca}$ after long diastolic intervals produced action potentials with long durations, followed by
short diastolic intervals. By this mechanism, a self-perpetuating sequence of long-short action potential durations was established. A similar mechanism likely contributed to action potential duration alternans in previously published ionic models (1,22), although alternans of $I_{Ca}$ was not specifically reported in those studies.

After reducing the magnitude of $I_{Ca}$ by decreasing $P_{Ca}$ or increasing Ca-induced inactivation, the balance of $I_{Ca}$ and repolarizing $K^{+}$ currents was shifted in favor of the repolarizing currents, resulting in shorter action potential durations. The resultant longer diastolic intervals allowed for complete recovery of $I_{Ca}$, albeit to a lesser magnitude, during pacing at cycle lengths that induced alternans in the control model. At even shorter pacing cycle lengths, diastolic intervals were too short to allow full recovery of $I_{Ca}$. However, because of the reduced magnitude of $I_{Ca}$ and rate-dependent accumulation of incompletely deactivated $K^{+}$ current, the dependence of action potential duration on $I_{Ca}$ was minimized and action potential durations remained consistently short. A similar mechanism accounts for the attenuation of alternans in the control model at very short pacing cycle lengths (figure 3).

**Role of repolarizing $K^{+}$ currents in alternans**

Beat-to-beat alterations of $I_{K1}$, $I_{Kr}$, and $I_{Ks}$ appeared to play a minor role in mediating alternans. As expected, $I_{K1}$, which has no time-dependence, displayed no beat-to-beat variations in magnitude, whereas the beat-to-beat changes in $I_{Ks}$ were too small to affect action potential duration appreciably at short pacing cycle lengths. Total $I_{Kr}$ also alternated during alternans, yet peak $I_{Kr}$ did not, suggesting that alternation of $I_{Kr}$ resulted from alternans of action potential duration, rather than vice versa.
Although the beat-to-beat variations of $I_{K1}$, $I_{Kr}$, and $I_{Ks}$ did not contribute appreciably to alternans, increasing any one of these currents sufficiently suppressed alternans. The mechanism for this effect was analogous to that described above for the suppressant effects of reducing $I_{Ca}$ on alternans. With elevation of $I_{K1}$, $I_{Kr}$, or $I_{Ks}$, the balance of repolarizing $K^+$ currents and $I_{Ca}$ during the action potential plateau was skewed, resulting in consistently short action potential durations. Consequently, the pattern of action potential duration and diastolic interval was similar to that shown in figure 6, except that $I_{Ca}$ not only recovered fully, it achieved a larger magnitude.

Implications

Decreasing the magnitude of $I_{Ca}$, either experimentally (23) or in an ionic model (22), has been shown to eliminate alternans and to convert ventricular fibrillation into a periodic rhythm. However, this approach clearly is not useful clinically because decreasing $I_{Ca}$ decreases the Ca transient, thereby reducing contractile force. Using the CVM model to explore other methods for eliminating alternans, we found that alternans was suppressed by increasing the magnitude of three repolarizing potassium currents: $I_{K1}$, $I_{Kr}$, and $I_{Ks}$.

Given that increasing $I_{K1}$, $I_{Kr}$, and $I_{Ks}$ decreased action potential duration, we determined whether such shortening truncated $I_{Ca}$, in which case increasing $K^+$ conductance might have the same clinical limitation as decreasing $Ca^{2+}$ conductance. However, $I_{Ca}$ was minimally affected both at short and at long pacing cycle lengths, as was the calcium transient. Consequently, it is possible, at least in the CVM model, to
increase K⁺ conductance to the point of suppressing alternans without reducing contractility.

These simulation results suggest a novel strategy for treating ventricular tachyarrhythmias. Previous attempts at treatment of such arrhythmias with pharmacological agents have been largely unsuccessful. In particular, class III anti-arrhythmic drugs, which are designed to block potassium currents, have been shown to be pro-arrhythmic (20). The CVM simulations suggest that a new class of drugs designed to increase the magnitude of selected outward currents may be useful in preventing alternans, and therefore in preventing the development of arrhythmias such as ventricular fibrillation. It should be emphasized, however, that only those K⁺ channel agonists that reduce the slope of the action potential duration restitution relation are expected to suppress VF. Drugs such as the I_{KATP} agonists, which markedly increase outward K⁺ current and shorten action potential duration, increase the slope of the restitution relation and, presumably by that mechanism, facilitate the induction of VF (27).

Limitations

While the CVM model successfully reproduces alternans, it has several limitations. First, the formulation of I_{Ca} is based solely on the qualitative characteristics of I_{Ca}. To improve the model, I_{Ca} should conform to the results of quantitative voltage clamp experiments, where the latter ideally have been conducted under circumstances that preserve the native behavior of I_{Ca} during pacing at short cycle lengths (e.g., no buffering of [Ca²⁺]i, or washout of the intracellular space). Second, although the simplified calcium handling in the model reproduces physiological calcium transients, it
ignores several of the details of calcium release from the sarcoplasmic reticulum. Further work needs to be done to incorporate detailed calcium handling mechanisms such as those in the Winslow model (26). Third, the model does not include the late sodium current, which may contribute significantly to plateau duration (28). A formulation of this current that agrees with voltage clamp experiments also needs to be included to complete the model. Finally, it has been shown that transmural heterogeneity of the heart is caused by differences in $I_{to}$, $I_{Ks}$, $I_{NaCa}$, and the late sodium current in endocardial, mid-myocardial and epicardial canine heart cells (13,14,27,29). We hope in the future to develop specific models for canine endocardium, mid myocardium and epicardium cells that will take these differences into account.

Acknowledgments

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Appendix

Parameters:
Initial conditions:

\[
\begin{align*}
t &= 0.0 \text{ms} & h &= .99869 \\
V &= -94.7 \text{mV} & j &= .99887 \\
[Ca^{2+}]_o &= .0472 \mu\text{mol} & fca &= .942 \\
[Ca^{2+}]_o &= 320 \mu\text{mol} & xkr &= .229 \\
f &= .983 & xks &= .0001 \\
d &= .0001 & xto &= 3.742 \times 10^{-5} \\
m &= 2.4676 \times 10^{-4} & yto &= 1
\end{align*}
\]

References


**Figure Legends**

Figure 1. Action potentials, ionic currents and Ca\(^{2+}\) transients generated by the CVM model after 50 beats at a cycle length of 400 ms. a, Action potential. b, L-type calcium current, I\(_{\text{Ca}}\). c, Intracellular Ca\(^{2+}\) concentration, [Ca]\(_i\). d, Rapid component of the delayed rectifier current, I\(_{\text{Kr}}\). e, Slow component of the delayed rectifier current, I\(_{\text{Ks}}\).

Figure 2. Action potentials, ionic currents and Ca\(^{2+}\) transients generated by the CVM model after 50 beats at a cycle length of 180 ms. a, Action potentials. b, I\(_{\text{Ca}}\). c, [Ca]\(_i\). d, I\(_{\text{Kr}}\). e, I\(_{\text{Ks}}\).

Figure 3. Action potentials generated by the CVM model at pacing cycle lengths of 8000 ms to 90 ms. 2:1 block occurred at a cycle length of 80 ms. a, Action potential duration (APD) plotted as a function of the basic cycle length (BCL) of pacing over a BCL range of 90 to 400 msec. b, APD restitution, where APD is plotted as a function of diastolic interval (DI) for DI < 210 ms. The solid line has a slope of 1. Note that alternans occurred where the slope of the restitution relation was 1 or greater. c, APD as a function of BCL over a BCL range of 90 to 8000 msec. d, Examples of action potentials at BCL = 300, 500, 1000, 2000, 4000 and 8000 ms. Over this range of BCL, resting membrane potential = -94 mV, action potential amplitude = 139 mV, overshoot = 45 mV and dV/dt\(_{\text{max}}\) = 278-280 V/sec.
Figure 4. Action potentials, ionic currents and Ca\textsuperscript{2+} transients generated by the reduced I\textsubscript{Ca} CVM model at a pacing cycle length of 180 ms.  

a, Action potentials.  
b, I\textsubscript{Ca}.  
c, [Ca\textsubscript{i}].  
d, I\textsubscript{Kr}.  
e, I\textsubscript{Ks}.

Figure 5. Relationship between action potential duration (APD) and diastolic interval (DI) in the CVM model after reducing P\textsubscript{Ca} by 20\% (a), increasing G\textsubscript{K1} by 7\% (b), increasing G\textsubscript{Kr} by 62\% (c) and increasing G\textsubscript{Ks} by 14.3 fold (d).  Solid line has slope = 1.

Figure 6. Relationship between the kinetics of the calcium-induced inactivation gate, f\textsubscript{Ca}, action potential duration (APD), diastolic intervals (DI) and the time course of I\textsubscript{Ca} in the normal CVM model (a) and in the reduced I\textsubscript{Ca} model (b) at a pacing cycle length of 180 ms.  See text for discussion.

Figure 7. Dose-response relationships between ionic current magnitude and alternans magnitude in the CVM model.  Each panel shows the maximum magnitude of action potential duration alternans as a function of a particular model parameter.  The open circle in each panel is the control parameter value.  

Left panels (top to bottom): Maximum Na\textsuperscript{+} current conductance, Na-Ca exchange current, maximum Ca\textsuperscript{2+} current permeability, time constant for the Ca-induced inactivation gate and maximum conductance for the transient outward K\textsuperscript{+} current.  

Right panels (top to bottom): Maximum conductance for the rapid component of the delayed rectifier, the time constant for the rapid component of the delayed rectifier, maximum conductance for the slow
component of the delayed rectifier, maximum conductance for the inward rectifier and maximum conductance for the plateau $K^+$ current.

Figure 8. Intracellular $Ca^{2+}$ concentration ($[Ca]_i$; left panels) and L-type calcium current ($I_{Ca}$; right panels) in the CVM model after increasing $I_{K1}$ (a,b), $I_{Kr}$ (c,d), or $I_{Ks}$ (e,f).
Figure 1

a. Membrane potential (mV)

b. $I_{Ca}$ (pA/pF)

c. $I_{C_{s}}$ (pA/pF)

d. $[Ca]_{i}$ (uM)

e. $I_{NaCa}$ (pA/pF)

f. $I_{Kr}$ (pA/pF)

g. $I_{Ks}$ (pA/pF)
Figure 2

a. Membrane potential (mV)

b. $I_{Ca}$ (pA/pF)

c. $f_{Ca}$

d. $[Ca]_i$ (uM)

e. $I_{NaCa}$ (pA/pF)

f. $I_{K}$ (pA/pF)

g. $I_{K_{solv}}$ (pA/pF)
Figure 3
Figure 4

(a) Membrane potential (mV)

(b) $I_{Ca}$ (pA/pF)

(c) $I_o$

(d) $[Ca^{2+}]_{i}$ (uM)

(e) $I_{NaCa}$ (pA/pF)

(f) $I_{Kr}$ (pA/pF)

(g) $I_{ks}$ (pA/pF)
Figure 5

a. Decreased $P_{Ca}$
b. Increased $G_{K1}$
c. Increased $G_{Kr}$
d. Increased $G_{Ks}$
Figure 6
Figure 7

a. Available Ca current

state of $f_{Ca}$ gate

<table>
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<th>DI</th>
<th>APD</th>
<th>DI</th>
<th>APD</th>
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<td>closed</td>
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b. Available Ca current

state of $f_{Ca}$ gate

<table>
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<th>APD</th>
<th>DI</th>
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<tr>
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<td>fully open</td>
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| closed |
Figure 8

- Increased $I_{K1}$
- Increased $I_{Kr}$
- Increased $I_{KS}$