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Rate-Dependent Action Potential Alternans in Human Heart Failure Implicate Abnormal Intracellular Calcium Handling

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Abstract

Background—Alternans in action potential voltage (APV-ALT) at heart rates <110 beats/min is a novel index to predict ventricular arrhythmias. However, the rate-dependency of APV-ALT and its mechanisms in failing versus non-failing human myocardium are poorly understood. It is hypothesized that APV-ALT in human heart failure (HF) reflects abnormal calcium handling.

Objective—Using a modeling and clinical approach, our objectives were to: (1) determine how APV-ALT varies with pacing rate, and (2) ascertain if abnormalities in calcium handling explain the rate-dependence of APV-ALT in HF.

Methods—APV-ALT was analyzed at several cycle lengths (CL) using a dynamic pacing protocol applied to a human left ventricle wedge model with various alterations in calcium handling. Modeled APV-ALT was used to predict APV-ALT in left ventricle monophasic action potentials recorded from HF (n=3) and control (n=2) patients with the same pacing protocol.

Results—Reducing the sarcoplasmic reticulum calcium uptake current $\leq 25\%$, the release current $\leq 11\%$, or the sarcolemmal L-type calcium channel current $\leq 43\%$ of control predicted APV-ALT to arise at $CL \geq 600\text{ms}$, then increase in magnitude by $>400\%$ for $CL < 400\text{ms}$. In HF patients, APV-ALT arose at $CL = 600\text{ms}$ then increased in magnitude by $>500\%$ at $CL < 350\text{ms}$. For all other model alterations and for control patients, APV-ALT occurred only at $CL < 500\text{ms}$.

Conclusions—APV-ALT shows differing rate-dependence in HF versus control patients, arising at slower rates in HF and predicted by models with abnormal calcium handling. Future studies should investigate whether APV-ALT at slow rates identifies patients with deranged calcium handling, including HF patients prior to decompensation or at risk for arrhythmias.

Keywords

Calcium Handling; Action Potential Alternans; Heart Failure; Computer Modeling

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Introduction

Sudden cardiac arrest from ventricular tachycardia (VT) or fibrillation (VF) is a leading cause of mortality in the U.S. that claims more than 300,000 lives annually, of whom many have a prior history of systolic heart failure (HF).¹ Mechanistically, beat-to-beat alternation (alternans) of action potential (AP) duration has been linked with VT/VF initiation in numerous animal,² in silico³ and human⁴ studies. However, translating these cellular and tissue observations into clinical practice, by using the ECG surrogate of T-wave alternans to predict VT/VF, has met with mixed success.^{4–6}

One explanation for this clinical dilemma is that AP duration restitution⁷ may not predict arrhythmic outcome in patients.⁸ Experimentally, very rapid rates are used to uncover alternans en route to VT/VF.^{3, 7} However, clinical VT/VF often arises from sinus rhythm⁹ at rates which AP duration would not be expected to oscillate.^{4, 10}

In HF patients, we recently showed that alternans in the novel index of ventricular AP voltage (APV-ALT) at the heart rate of 109 beats/min predicted ventricular arrhythmias, even when alternans in AP duration was not present.¹¹ We also used computational modeling to show that APV-ALT at this rate can arise from abnormal calcium handling, such as reduced calcium uptake into the sarcoplasmic reticulum (SR). However, it is unclear how APV-ALT varies with pacing rate, and whether there are rate-dependent differences for APV-ALT in patients with and without HF that can also be explained by abnormalities in calcium handling.

Thus, we hypothesized that APV-ALT differs between HF and control patients across a broad range of physiological and pathological heart rates, and that these differences can arise from abnormalities in calcium handling. In other words, disrupting calcium homeostasis by altered expression or function of calcium handling proteins leads to rate-dependent intracellular calcium alternans, which in turn produces rate-dependent APV-ALT via oscillations in sarcolemmal ion currents. To test this hypothesis, we first determined the rate-response of APV-ALT over the entire AP, as well as over phases II and III, for a wide range of pacing rates in a computer model of human left ventricle (LV) wedge that recapitulates alternans in calcium handling observed in HF.^{12, 13} We then validated these predictions against APV-ALT found in monophasic APs (MAP) recorded from the LV in a small group of patients with and without HF using the same pacing protocol.

Methods

Human LV Modeling Study: Description and Pacing Protocol

A human LV wedge model (Figure 1), as we have described previously,¹¹ was used to investigate the role of calcium handling dysfunction in APV-ALT. The LV wedge model included endocardial, M-cell, and epicardial regions to facilitate the comparison of endocardial APs “in silico” to endocardial MAPs “in vivo”.

The following modifications were made to the underlying membrane kinetics of the LV wedge model that incorporated the ten Tusscher-Panfilov model of the human ventricular myocyte;¹⁴ 1) SR calcium uptake current (I_{up}) scaled from 99 to 1% of baseline to simulate reduced SERCA2a expression¹⁵ and/or an increase in dephosphorylated phospholamban,¹⁶ 2) the sarcolemmal sodium-calcium exchange current (I_{NaCa}) scaled from 101 to 200% of baseline to simulate increased expression of the sodium-calcium exchanger,¹⁷ 3) the SR calcium-induced calcium release current (I_{rel}) scaled from 99 to 1% of baseline to simulate reduced ryanodine receptor expression,¹⁸ 4) the sarcolemmal L-type calcium current (I_{CaL}) scaled from 99 to 1% of baseline to simulate a reduction in peak I_{CaL} ,¹⁹ 5) the SR leakage

current (I_{leak}) and the transition rate (k_1') from the active and inactive resting states to the active and inactive open states of I_{rel} , scaled simultaneously from 101 to 200% of baseline to simulate PKA hyperphosphorylation of ryanodine receptors,²⁰ and 6) the transition rate (k_4) from inactive to active states of I_{rel} scaled from 99 to 1% of baseline to simulate a slow recovery of Ryanodine receptors from inactivation.²¹

For each of the conditions above applied separately to the entire LV wedge model, we paced the model endocardially at a CL of 650ms (92 beats/min) until steady state was achieved, which was determined when the model variables between subsequent beats converged. A dynamic pacing protocol was then applied, starting at a CL of 650ms and decreasing it in 50ms steps after every 100 beats until loss of 1:1 capture. AP data were collected at the center of the endocardium to detect and analyze APV-ALT.

Clinical LV Study: Patient Recruitment and Pacing Protocol

We studied patients (n=5) undergoing invasive programmed ventricular stimulation (HF patients; LV ejection fraction $\leq 45\%$) or ablation of atrial fibrillation (control patients; LV ejection fraction $>45\%$). We excluded patients within 30 days of an acute coronary syndrome or 6 weeks of coronary revascularization, with unrevascularized coronary ischemia, with decompensated HF or with permanent pacemakers. This study was approved by the joint Institutional Review Board of the Veterans Affairs and University of California Medical Centers, San Diego, and patients provided written informed consent.

All patients were studied in the post-absorptive state. Under conscious sedation with midazolam and fentanyl, a 7-F MAP catheter (EP Technologies, Sunnyvale, California) was advanced to the anterolateral LV either via the retrograde trans-aortic route or a trans-septal puncture. Patients were continuously monitored for oxygen saturation, blood pressure via cuff or intra-arterial recording (when arterial access was available) and for visible signs of distress, discomfort or fatigue.

We applied the identical pacing protocol as the modeling study. Patients were preconditioned with ventricular pacing at a CL of 650ms for >90 s. The dynamic pacing protocol then commenced at a CL of 650ms for 100 beats. To ensure patient safety, pacing CL was shortened in 50 ms decrements until 300ms (200 beats/min), where we paused between CL steps if needed, and routinely for CL ≤ 350 ms. The protocol was discontinued at any sign of discomfort. There were no adverse events in any patient.

MAPs were filtered at 0.05 to 500 Hz, digitized at 1 kHz from our electrophysiologic recorder (Bard Pro, Billerica, Massachusetts) and exported for offline analysis.

Analysis of Alternans in AP Voltage

APV-ALT was detected and analyzed spectrally using identical methods and validated software^{10,17} for computational modeling and clinical LV datasets. For each pacing CL, 64 successive APs were selected 36 beats after the onset of pacing, then aligned to phase 0 and baseline corrected to produce a 2-dimensional matrix $R(n,t)$, where n indicates the number of APs ($0 \leq n \leq 63$) and t the time sample. At each t , a fast-Fourier transform was performed to compute power spectra across all beats. APV-ALT magnitude was represented by the dimensionless K-score:

$$K - score = \frac{\sum T - \mu_{noise}}{\sigma_{noise}},$$

where ΣT is the spectral magnitude at 0.5 cycles/beat, and μ_{noise} and σ_{noise} are the mean and standard deviation of noise. The noise window was selected adjacent to alternans frequency (0.33–0.49 Hz) to avoid the 0.125–0.25 Hz respiratory peak. The mean voltage of alternation (V_{alt}), scaled by AP duration rather than noise standard deviation, was estimated as:

$$V_{alt} = \sqrt{\frac{\Sigma T - \mu_{noise}}{AP \text{ duration}}} \quad (\text{in mV}).$$

In this study, AP duration in the equation for V_{alt} refers to the difference in time from the end of the alignment window (i.e. after phases 0-I) to 90% repolarization of the peak voltage of phase II amplitude. APV-ALT was analyzed for this entire interval, the first half of the interval (AP phase II) and the second half (AP phase III). APV-ALT was classified as positive when $V_{alt} \geq 0.05$ mV for patients, or $V_{alt} \geq 0.2$ mV for the LV wedge model, and alternans exceeded noise ($K\text{-score} > 0$).²² These thresholds for V_{alt} were chosen based on our previous work,¹¹ where we showed APV-ALT with $V_{alt} \geq 0.05$ mV in patients and $V_{alt} \geq 0.2$ mV in the LV wedge model to underlie T-wave alternans.

Ion Current and State Variable Clamping in Endocardial Myocytes

To provide insight into the mechanisms underlying APV-ALT in the endocardium of the LV wedge model, we studied how changes in the magnitude and time course of ion currents and state variables contribute to APV-ALT in endocardial myocytes with impaired SR calcium uptake or release. Specifically, we clamped each current and state variable one by one to the last odd and even beats for the CLs of 600ms and 300ms in the dynamic pacing protocol applied to a single endocardial myocyte. Pacing commenced for the clamped cell at each respective CL lasting for 100 beats, and APV-ALT was computed spectrally for the last 64 beats at the two pacing CLs. Clamped ion currents and state variables which reduced $V_{alt} > 99\%$ of baseline at both slow and fast pacing rates were considered the most essential for APV-ALT.

Results

APV-ALT in the LV Wedge Model

Of the modifications made to calcium handling in the LV wedge model, only reducing $I_{up} < 33\%$, $I_{rel} < 12\%$, or $I_{CaL} < 51\%$ produced APV-ALT at pacing CL ≥ 300 ms (pacing limit for patient study). Figure 2 shows the relationship of APV-ALT to pacing CL at the endocardium of the LV wedge model for various reductions of I_{up} , I_{rel} or I_{CaL} to simulate changes observed with progressive heart failure.¹⁵ Note, K-scores are not presented for the simulation studies since K-scores are always greater than zero in the presence of alternans due to the absence of noise intrinsic to the MAP recording techniques.

The longest pacing CL at which APV-ALT developed was 650 ms with $I_{up} \leq 25\%$ of control (maximum $V_{alt} = 1.8$ mV for the entire AP), then increased in magnitude by more than 400% (ratio of V_{alt}) at pacing CL < 400 ms (maximum $V_{alt} = 7.85$ mV). Conversely, when I_{up} was 26–100% of control, APV-ALT did not develop in the endocardium of the LV wedge model until pacing CL < 450 ms (maximum $V_{alt} = 10.74$ mV for the entire AP). APV-ALT with $I_{rel} \leq 11\%$ and $I_{CaL} \leq 43\%$ of control initially developed at the pacing CL of 600ms (maximum of $V_{alt} = 1.3$ mV and $V_{alt} = 0.5$ mV, respectively, for the entire AP), then increased in magnitude by more than 1700% for I_{rel} and 900% for I_{CaL} at pacing CL < 400 ms (maximum of $V_{alt} = 23.2$ mV and $V_{alt} = 4.7$ mV respectively). Conversely, when I_{rel} was 12–100% and I_{CaL} was 44–100% of control, APV-ALT did not develop in the endocardium of the LV

wedge model until pacing CL<450 ms (maximum of $V_{alt}=0.2$ mV and $V_{alt}=0.5$ mV, respectively, for the entire AP).

APV-ALT in Patients

V_{alt} and K-scores were determined in HF (n=3; LV ejection fraction $32\pm15\%$) and control (n=2; LV ejection fraction $58\pm13\%$) patients using the same dynamic pacing protocol as in the modeling study. The clinical characteristics of the patients studied are shown in Table 1.

APV-ALT in endocardial MAPs presents different rate-dependence in HF versus control patients, arising at slower rates in HF (Figure 3). In HF patients, APV-ALT was first identified at the pacing CL of 600 ms with mean $V_{alt}=3.71$ mV and K-score=1.18 (for the entire AP); APV-ALT then increased in magnitude by more than 500% at CL of 300 ms (mean $V_{alt}=22.09$ mV and K-score =2.61). APV-ALT was not found in control patients until CL was shortened to 450 ms (mean $V_{alt}=0.74$ mV and K-score=1.09 for the entire AP). Overall, the CL threshold for APV-ALT was higher in control patients (CL=450 ms) as compared to HF patients (CL=600 ms).

APV-ALT in AP Phases II and III: Clinical Observations

APV-ALT in the LV wedge model most accurately predicted the rate-dependence and magnitude of clinical APV-ALT in HF patients when I_{up} was reduced to 23%, I_{rel} to 10%, and I_{CaL} to 43% of control (compare rate-responses in Figure 2 to that in Figure 3). Thus, we compared APV-ALT in AP phases II and III from the endocardium of the LV wedge model with each of these modifications to MAPs recorded from the LV endocardium in patients.

Figure 4 shows AP alternans in the LV of a 66 year old male with LV ejection fraction 31%. At moderate (CL=600 ms) and fast (CL=300 ms) pacing rates, APV-ALT was visible in AP phases II and III for both the model (columns 1–3, Figure 4) and this HF patient (column 4, Figure 4). At the CL of 300 ms, the model with reduced I_{up} and this individual exhibited greater V_{alt} in phase II than that in phase III, as illustrated by the arrows in Figure 4. Notably, at the slower pacing CL of 600 ms the model with reduced I_{CaL} and this patient exhibited V_{alt} in phase II greater than in phase III.

Using Modeling to Link APV-ALT with Abnormal Calcium Handling

In the endocardium of the LV wedge model, alternans were present in cytosolic calcium ($[Ca]_i$), dyad subspace calcium ($[CaSS]$) and SR calcium ($[CaSR]$) concentrations during phase II and III APV-ALT (Row 1, Figure 5). Alternans in these three calcium concentrations (CA-ALT) and APV-ALT were also present in isolated endocardial myocytes with reduced SR calcium uptake or release (Results shown for reduced I_{up} in Row 2, Figure 5), indicating that APV-ALT in the LV wedge model is linked to CA-ALT in failing myocytes. To determine the relationship between CA-ALT and APV-ALT, we clamped each ion current and state variable in an endocardial myocyte with reduced I_{up} , I_{rel} , or I_{CaL} .

Only when clamping I_{rel} or $[CaSS]$ were 99% of APV-ALT eliminated at both slow and fast pacing rates for all reductions of I_{up} , I_{rel} or I_{CaL} in endocardial myocytes (Results shown for reduced I_{up} to 30% of control in Tables 2 and 3). In particular, clamping $[CaSS]$ also caused CA-ALT to cease (Row 3, Figure 5), which in turn eliminated alternans in sarcolemmal ion currents. Notably, when membrane voltage was clamped, CA-ALT still persisted in the endocardial cell with impaired SR calcium uptake (Row 4, Figure 5).

Discussion

The present study examines potential mechanisms underlying alternans in action potential voltage (APV-ALT) in HF patients, which has recently been shown to be a novel index for stratifying patients at risk for ventricular arrhythmias. Using a combined clinical and modeling approach, we reveal differences in APV-ALT between HF and controls across a wide range of heart rates, and show that these differences can largely be ascribed to abnormalities in calcium handling. Specifically, computational modeling showed that altering SR calcium uptake or release by reducing I_{up} , I_{rel} , or I_{CaL} produced APV-ALT that predicted rate-dependent APV-ALT in HF patients. These findings mechanistically link APV-ALT to abnormalities in intracellular calcium handling found in human HF, and suggest APV-ALT as a potential index of calcium handling dysfunction in HF patients.

Reduced SR Calcium Uptake and Release as a Mechanism for CA-ALT in HF

Alternations in calcium homeostasis are considered a fundamental cellular abnormality in HF and a mechanism for arrhythmogenesis.²³ Computational²⁴ and experimental²⁵ studies attribute CA-ALT to a steep relationship between SR calcium release and SR calcium content. Therefore, altered expression and/or function of calcium handling proteins during HF could steepen this relationship and potentially generate CA-ALT.

The present study implicates reduced SR calcium uptake or release as enhancing the susceptibility of HF patients to CA-ALT across a broad range of heart rates. Several studies have documented significant reductions in the expression of SERCA2a^{12, 13} and phosphorylated phospholamban¹⁶ in failing human myocytes, thus supporting the large reductions in SR calcium uptake ($I_{up} \leq 25\%$) that predicted rate-dependent APV-ALT in HF patients. As further evidence for a lowered SERCA2a function in HF patients, I_{up} reduction led to a smaller amplitude and slower rate of decay in the $[Ca]_i$ transient (Figure 5), which is in agreement with $[Ca]_i$ transients observed in failing tissue¹⁰ and isolated myocyte preparations.^{26, 27} With respect to the large reductions in SR calcium release ($I_{rel} \leq 11\%$ or $I_{CaL} \leq 43\%$) that predicted rate-dependent APV-ALT in HF patients, Northern Blot experiments have shown significant reductions in the mRNA encoding the L-type calcium channel and Ryanodine receptor in failing human myocytes.¹² Although, it is uncertain whether these reductions in mRNA levels translate to a lower expression of I_{rel} and I_{CaL} channels in failing ventricular myocytes, and ultimately, a significant reduction in SR calcium release.

CA-ALT as a Mechanism for Phase II and III APV-ALT

There is debate as to whether CA-ALT lead to APV-ALT, or vice versa. In our studies, we found clamping $[CaSS]$ to eliminate APV-ALT (Row 3, Figure 5), but clamping membrane voltage did not eliminate CA-ALT (Row 4, Figure 5). This is in agreement with the studies by Chudin et al.²⁸ Furthermore, oscillations in sarcolemmal ion currents still persisted during voltage clamping, resulting from the presence of CA-ALT. Therefore, the following mechanisms can potentially explain how CA-ALT leads to APV-ALT in human HF for the pacing CLs used in this study.

At slow and fast heart rates, we showed that alternans in $[CaSS]$ are essential for APV-ALT (Tables 2–3, Row 3 Figure 5). At slow heart rates, phase II APV-ALT may arise due to the high sensitivity of I_{CaL} to changes in $[CaSS]$. When the peak $[CaSS]$ is larger than for the previous beat, the calcium-dependent inactivation of I_{CaL} is increased. As a result, the amount of inward I_{CaL} during the plateau of the AP is lessened and the amplitude of AP phase II potential is reduced. Conversely, if the peak $[CaSS]$ is smaller than for the previous beat, less calcium dependent inactivation of I_{CaL} occurs, resulting in more inward I_{CaL} and

greater amplitude of the AP phase II potential. Phase III APV-ALT may arise at slow heart rates from oscillations in I_{NaCa} due to changes in $[Ca]_i$. Normally, when $[CaSS]$ is larger than for the previous beat, the decrease in I_{CaL} from stronger calcium-dependent inactivation would be expected to decrease AP phase III voltage and shorten AP duration. However, less I_{CaL} occurs when SR calcium released into the dyadic subspace is larger than the previous beat, resulting in more calcium flow into the cytosol, with more inward I_{NaCa} in AP phase III. This can lead to delayed activation of repolarizing potassium currents and an increase in AP phase III voltage and prolongation of AP duration.

At fast heart rates, APV-ALT is amplified. Larger APV-ALT magnitude may be a direct result of alternans in the voltage dependent activation or inactivation of sarcolemmal currents due to the shortened diastolic interval between beats. The results in Table 3 suggest the latter by showing that clamping the voltage dependent activation or inactivation of I_{CaL} , I_{Kr} and I_{Ks} at fast pacing rates significantly reduced V_{alt} from baseline. Thus, at faster pacing rates the voltage dependent activation and/or inactivation of sarcolemmal ion currents likely contribute significantly to APV-ALT magnitude.

Clinical Implications

The current study linking calcium abnormalities to alternans of AP voltage, and numerous studies linking AP alternans with arrhythmias, motivates better clinical approaches to detect AP alternans. Indeed, T-wave alternans on the ECG has been shown to predict the risk for VT/VF in a variety of populations,⁴ despite some recent negative studies.^{5, 6} Notably, clinical T-wave alternans testing is most predictive of arrhythmic vulnerability at heart rates < 110 beats/min ($CL > 550ms$);^{4, 22} this is in agreement with the results of this study since only HF patients exhibited APV-ALT at $CL > 450ms$ with magnitudes large enough to produce T-wave alternans ($V_{alt} > 0.05 mV$). Second, our computational studies suggest that APV-ALT at $CL > 550ms$ reflect impaired SR calcium uptake and/or release in HF patients. Thus, APV-ALT may be a useful index for grossly detecting calcium handling abnormalities in HF patients, where it is possible that a surrogate for phase II and III APV-ALT could be obtained from implanted device leads via the unipolar activation-recovery interval.²⁹ Lastly, recent reports of intracardiac repolarization alternans at rapid rates captured by an implanted device en route to VT/VF³⁰ are consistent with our data: at pacing CL of 300 ms, mean V_{alt} in HF patients was significantly larger than that in control patients (V_{alt} of 22.09mV and 6.56mV respectively).

Study Limitations

The first limitation of the *computational study* is that the LV wedge model lacks realistic geometry and fiber orientation. Although such complexity was not essential to establish the mechanistic link between cellular calcium and AP dynamics in the LV wall here, a structurally more complex (heterogeneous) model would be necessary to investigate the mechanisms by which the spatial distribution of APV-ALT may lead to the initiation and/or maintenance of VT/VF in HF patients with impaired calcium handling. Secondly, other potential mechanisms for rate-dependent APV-ALT may also exist, such as the interplay of I_{CaL} and the transient outward current during ischemia,³¹ or altered fibroblast-myocyte coupling³² and gap junction remodeling³³ during HF. Modifying the LV wedge model to appropriately test these scenarios should be addressed in future studies. Third, differences between APV-ALT magnitudes in the LV wedge model compared to clinical data (Figure 4) likely reflect differences in the shape of modeled APs versus MAPs. Furthermore, larger magnitude of APV-ALT in patients may be due to noise, such as produced by catheter movement, which may be enhanced at rapid pacing rates. Fourth, the present study only shows correlation of model results to clinical data, not causality. Thus, future studies should determine which components of APV-ALT are directly linked to VT/VF by performing a

similar study in a model of the entire human ventricles. Lastly, clamping model parameters to odd or even beat waveforms to describe the contribution of ionic currents and state variables to V_{alt} magnitude may miss important information regarding alternans dynamics. Future studies could explore the use of robust dynamical systems approaches for analyzing action potential stability around a fixed point^{34, 35} in order to provide further insight into the mechanisms of rate-dependent alternans in myocytes with abnormal calcium handling.

There were several limitations of the *clinical study*. First, this validation population was small because of the very aggressive pacing required to maintain parallel data collection with the modeling-derived protocol. Second, cardiac motion, respiratory artifact or baseline wander could theoretically influence our analyses. However, our MAPs had consistent shape on raw superimposed tracings, and artifacts should not alternate. Third, cellular mechanisms could be influenced by medications and blood chemistry, yet these factors were essentially normal and similar between groups (Table 1). Lastly, long term follow-up on a larger population would provide useful information.

Conclusion

APV-ALT differs between patients with and without HF, and is predicted by quantitative reductions in SR calcium uptake or release. HF patients exhibited APV-ALT at moderate and fast pacing rates, while control patients exhibited APV-ALT only at fast rates. Modeling suggests that APV-ALT in HF can be explained by alternans in intracellular calcium concentrations generating alternans in sarcolemmal ionic currents. Future studies should identify whether rate-dependent APV-ALT provides a clinical index of deranged calcium handling in HF patients that may help guide therapeutic efforts to prevent HF decompensation or arrhythmia initiation.

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Glossary of Abbreviations

AP	Action Potential
APV-ALT	Action Potential Voltage Alternans
CA-ALT	Calcium Concentration Alternans
$[Ca]_i$	Cytosolic Calcium Concentration
[CASR]	Sarcolemmal Calcium Concentration
[CASS]	Dyadic Subspace Calcium Concentration
CL	Cycle Length
HF	Systolic Heart Failure
I_{up}	Sarcolemmal Calcium Uptake Current
LV	Left Ventricle
MAP	Monophasic Action Potential

SR	Sarcoplasmic Reticulum
V_{alt}	Absolute voltage of alternation
VF	Ventricular Fibrillation
V_m	Membrane Voltage
VT	Ventricular Tachycardia

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Figure 1. LV Wedge Model Setup

Top: Location in the ventricles of the LV wedge model and the stimulus site. Bottom: Dimensions of the LV wedge model, based on a study by dos Santos et al.³⁶

Figure 2. APV-ALT in the LV Wedge Model

Shown are V_{alt} computed over the entire AP, over phase II and over phase III in the endocardium of the LV wedge model to illustrate the rate-dependence of APV-ALT to progressive reductions in either I_{up} , I_{rel} , or I_{CaL} .

Figure 3. APV-ALT in Patients

Mean V_{alt} (row 1) and K-scores (row 2) computed over the entire AP, over phase II and over phase III in the endocardium of HF (n=3) and control (n=2) patients, plotted as a function of the same pacing CLs from the modeling study.

Figure 4. Modeled and Clinical APV-ALT at Moderate and Fast Rates

Columns 1–3: APs from the endocardium of the LV wedge model with I_{up} reduced to 23%, I_{rel} reduced to 10%, and I_{CaL} reduced to 43% of control. V_{alt} for APV-ALT at moderate (600ms) and fast (300ms) pacing rates is given for each trace. Column 4: endocardial MAPs from a 66 year old male patient with coronary artery disease and LV ejection fraction of 31% who exhibited APV-ALT at the same pacing rates. Modeled APs and MAPs are superimposed to show APV-ALT between odd (blue) and even (red) beats.

Figure 5. Voltage and Calcium Alternans in Endocardial Myocytes

For the CL of 300ms in our dynamical pacing protocol, the membrane voltage (V_m), cytosolic calcium ($[Ca]_i$), SR calcium ($[CaSR]$), and calcium in the dyad subspace ($[CaSS]$) are superimposed to show alternans between odd (blue) and even (red) beats in the endocardium of the LV wedge model with I_{up} reduced to 24% of control (Row 1), and a single cell endocardial myocyte with I_{up} reduced to 30% of control (Rows 2–4). Rows 1 and 2 show alternans in both V_m and the subcellular calcium compartments for tissue and single cells with I_{up} reduced. When $[CaSS]$ is clamped to the last even $[CaSS]$ waveform in row 2, all alternans are abolished (Row 3). When V_m is clamped to the last even V_m waveform in row 2, alternans in calcium persisted (Row 4).

Table 1**Baseline Clinical Characteristics**

	LV Dysfunction (n=3)	Preserved LV (n=2)
Age, yrs	68±3	62±2
Ejection Fraction, %	32±15	58±13
Coronary disease, n	3	1
Hypertension, n	1	2
Diabetes Mellitus, n	2	1
Serum Na ⁺ , mmol/l	137±4	137±2
Serum K ⁺ , mmol/l	4.4±0.3	4.6±0.1
Serum Ca ²⁺ , mg/dl	9.3±0.8	9.3±0
Serum Mg ²⁺ , mmol/l	2.3±0.2	2.0±0
Serum HCO ₃ ⁻ , mmol/l	24±3	27±6
Plasma BNP, pg/ml	305±329	321±0
Medication Use, n		
Beta Blockers	2	2
ACE inhibitors/ARB	3	2
Spironolactone	1	0
CCB	0	0
Digoxin	1	1
Amiodarone	0	0
Statins*	3	0

Key: *3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. ACE =Angiotensin Converting Enzyme; ARB = Angiotensin Receptor Blockers; BNP = B-Type Natriuretic Peptide concentration; CCB = Calcium Channel Blockers; Statins, HMG-CoA reductase inhibitors

Table 2

Clamping I_{rel} Eliminates APV-ALT in Endocardial Cells with Reduced SR Calcium Uptake at Slow and Fast Pacing Rates

Ion Current	Entire AP		Phase II		Phase III	
	600ms	300ms	600ms	300ms	600ms	300ms
Sarcolemmal						
I_{Na}	+4(-1)	-27(-13)	+11(-1)	-54(-55)	+4(-1)	-26(-12)
I_{to}	-4(-2)	+7(-25)	-6(-2)	+14(-55)	-3(-5)	+6(-24)
I_{CaL}	-37(-45)	-88(-94)	-65(-71)	-34(-66)	-35(-44)	-96(-99)
I_{Kr}	-22(-31)	-35(-76)	+16(-9)	-1(-41)	-25(-33)	-36(-78)
I_{Ks}	-9(+21)	-50(+80)	-8(-24)	-27(+99)	-9(+23)	-51(+77)
I_{K1}	-32(-88)	-68(-99)	+19(-51)	+40(-96)	-37(-97)	-79(-99)
I_{NaCa}	-99(-99)	+99(+99)	-98(-98)	+99(+99)	-99(-99)	+99(+99)
I_{NaK}	+3(+1)	+20(+61)	-5(-6)	+23(+96)	+4(+1)	+20(+59)
I_{bNa}	0(-4)	+6(-4)	-6(-9)	+7(-13)	0(-3)	+6(-4)
I_{bCa}	+1(+3)	+11(+11)	-5(-10)	+12(+6)	+2(+3)	+11(+11)
I_{pCa}	+8(+9)	+8(-1)	-3(+7)	+11(-9)	+8(+9)	+8(-1)
I_{pK}	+23(+3)	+96(+44)	+79(+92)	+99(+99)	+19(-6)	+91(+37)
SR						
I_{up}	+13(+7)	+6(-22)	+24(+68)	+8(+28)	+13(+2)	+6(-24)
I_{sfer}	-5(-85)	-65(-99)	+80(-92)	-14(-99)	-14(-85)	-68(-99)
I_{leak}	-13(+4)	-17(-19)	+46(+18)	-13(+25)	-18(+3)	-17(-21)
I_{rel}	-99(-99)	-99(-99)	-99(-99)	-99(-99)	-99(-99)	-99(-99)

All values are % reduction (-) or increase (+) of V_{alt} from control and X(Y) = odd(even) beats.

Clamping [CaSS] and I_{rel} Open Channel Probability Eliminated APV-ALT in Endocardial Cells with Reduced SR Calcium Uptake at Slow and Fast Pacing Rates

Table 3

State Variables	Entire AP		Phase II		Phase III	
	600ms	300ms	600ms	300ms	600ms	300ms
<i>Concentrations</i>						
[Na]i	-1(-3)	+3(-23)	0(0)	+3(+13)	-1(-4)	+3(-25)
[K]i	-4(-1)	+2(-22)	-5(-6)	+3(+16)	-4(-1)	+2(-24)
[Ca]i	-69(-53)	-28(-86)	+24(+86)	+43(-26)	-94(-89)	-31(-95)
[CaSR]	-99(-99)	-27(-99)	-99(-99)	+34(-99)	-99(-99)	-30(-99)
[CaSS]	-99(-99)	-99(-99)	-99(-99)	-99(-99)	-99(-99)	-99(-99)
<i>Gating</i>						
D	-53(-59)	-84(-76)	+38(+3)	-62(-35)	-67(-66)	-85(-78)
F	+14(+25)	-51(-98)	-2(-9)	-43(-94)	+15(+27)	-51(-99)
F2	-5(0)	-58(-82)	-15(-16)	-31(-29)	-4(+1)	-59(-87)
FCaSS	+15(+11)	+99(+99)	-61(-57)	+99(+99)	+19(+14)	+99(+93)
M	-35(-5)	-35(-12)	+25(-4)	-42(-24)	-41(-6)	-35(-12)
H	+5(-4)	+3(-13)	+8(+5)	+2(-24)	+5(-4)	+3(-13)
J	+2(-2)	-23(-20)	+6(-4)	-53(-54)	+2(-2)	-23(-19)
O	-99(-99)	-99(-99)	-99(-99)	-99(-99)	-99(-99)	-99(-99)
R	0(+99)	-55(-41)	-10(+83)	-38(-34)	0(+99)	-55(-41)
S	-2(+2)	+8(-25)	-6(-1)	+14(-55)	-1(+2)	+7(-24)
Xr1	-8(-2)	+3(-25)	-14(-7)	+3(+13)	-8(-2)	+3(-26)
Xr2	-2(-5)	+9(+23)	-7(-8)	+7(+40)	-2(-5)	+9(+22)
Xs	-61(-49)	-82(-83)	-46(-2)	-44(-61)	-62(-53)	-85(-85)
Xs	-34(-33)	-66(-93)	-25(+1)	-37(-60)	-34(-35)	-67(-98)
<i>Voltage</i>						
Vm	-100(-100)	-100(-100)	-100(-100)	-100(-100)	-100(-100)	-100(-100)

All values are % reduction (-) or increase (+) of V_{alt} from control, and X(Y) = odd (even) beats