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Heart Failure Enhances Susceptibility to Arrhythmogenic Cardiac Alternans

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Abstract

Background—Although heart failure (HF) is closely associated with susceptibility to sudden cardiac death (SCD), the mechanisms linking contractile dysfunction to cardiac electrical instability are poorly understood. Cardiac alternans has also been closely associated with SCD, and has been linked to a mechanism for amplifying electrical heterogeneities in the heart. However, previous studies have focused on alternans in normal rather than failing myocardium.

Objective—To investigate the hypothesis that HF enhances susceptibility to arrhythmogenic cardiac alternans.

Methods—High-resolution transmural optical mapping was performed in canine wedge preparations from normal (n=8) and HF hearts (n=8) produced by rapid ventricular pacing.

Results—HF significantly ($p<.004$) lowered the heart rate (HR) threshold for action potential duration alternans (APD-ALT) from 236 ± 25 bpm to 185 ± 25 bpm. In dual optical mapping of action potentials and intracellular Ca experiments (n=16), HF lowered the HR threshold for Ca-ALT (beat to beat alternations of cellular Ca cycling) from 238 ± 35 to 177 ± 26 bpm ($p<.005$). Importantly 1) Ca-ALT always either developed at slower HR or simultaneously with APD-ALT in the same cells and 2) the magnitude of Ca-ALT and APD-ALT were closely correlated ($p<.05$). HF similarly lowered the HR threshold for Ca-ALT in isolated myocytes under non-alternating action potential clamp indicating that HF enhances susceptibility to cellular alternans independent of HF-associated changes in repolarization. Importantly, HF significantly ($p<.02$) lowered the HR threshold for spatially discordant arrhythmogenic alternans (different regions of cells alternating in opposite phase, DIS-ALT). Ventricular fibrillation (VF) was induced in 88% of HF preparations, but only 12% of normal preparations ($p<0.003$) and was uniformly preceded by development of DIS-ALT.

Conclusions—Heart failure increases the susceptibility to arrhythmogenic cardiac alternans which arises from HF-induced impairment in calcium cycling.

Keywords

heart failure; cardiac alternans; arrhythmias; ventricular fibrillation; calcium cycling; optical mapping

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Sudden cardiac death (SCD) is the most devastating manifestation of heart disease and accounts for at least 50% of deaths in patients with heart failure (HF).¹ The structural and electrophysiological changes that predispose to SCD in chronically diseased hearts have been studied extensively, yet surprisingly little is known of the complex sequence of events that incite malignant arrhythmias in some patients but not in others. It is well recognized that patients with ventricular contractile function (i.e. HF) have the highest incidence of SCD.² However, the mechanisms linking mechanical to electrophysiological dysfunction in the heart are unclear. Impaired Ca cycling is the most striking abnormality of failing myocytes, and is most directly responsible for contractile dysfunction; yet it remains unclear how this influences susceptibility to arrhythmias.³ Previous work has focused on a role of excitation-contraction (EC) coupling proteins as a potential source for triggering arrhythmias, but the role of abnormal EC coupling in modulating arrhythmia substrates is much more poorly understood.

Subtle beat-to-beat alternation of cardiac repolarization, manifested clinically as T wave alternans (T-ALT),⁴ is a highly sensitive marker of susceptibility to SCD in patients with HF. Remarkably, in the absence of T-ALT, patients with HF are, relatively speaking, resistant to SCD.⁵ Therefore, by elucidating the mechanisms of cellular alternans in HF, there may be an opportunity to understand why HF enhances susceptibility to arrhythmias⁶. Previously, we showed that alternans of cellular action potential duration (APD-ALT) is linked to a mechanism of arrhythmogenesis in normal hearts, where spatially discordant alternans between myocytes amplifies repolarization gradients to produce conduction block and reentrant excitation.⁷ Cellular alternans was found by us^{7,8} and others^{9,10} to be a consistent precursor to cardiac fibrillation in the mammalian heart. However, essentially all previous research on alternans has focused on normal hearts, rather than HF; hence the role of cellular alternans in the most common setting for SCD, i.e. HF, is unknown. Because susceptibility to cellular alternans is mechanistically related to impaired calcium cycling in normal hearts,¹¹⁻¹⁶ we hypothesize that: 1. HF enhances susceptibility to arrhythmogenic alternans and 2. Increased susceptibility to alternans is closely associated with HF-induced impairment of calcium cycling.

METHODS

Dual Calcium-Voltage mapping in Canine Wedge Preparation and Isolated Myocytes

Experiments were carried out in accordance with Public Health Service guidelines for the care and use of laboratory animals. Details of the experimental procedure for optical mapping in the canine wedge preparation are provided elsewhere.¹⁷ Briefly, we designed a system capable of recording action potentials with high spatial (0.7-1.2 mm), temporal (0.5 ms), and voltage (0.5 mV) resolutions from cells spanning the entire left ventricular (LV) wall. This was achieved by applying a previously validated optical action potential mapping technique^{7,18} to the transmural surface of the arterially perfused canine wedge preparation.¹⁷ Canine wedge preparations were isolated from normal and HF dogs produced by 4-6 weeks of rapid right ventricular endocardial pacing as described previously.¹⁹ For these studies, all wedges were obtained from anterior or lateral base of the LV. Physiological stability of the preparations was insured by monitoring the volume-conducted electrocardiogram (ECG), coronary perfusion pressure (50-60mmHg), coronary flow, and perfusion temperature (35±0.2°C) continuously throughout each experiment. For voltage (Vm) mapping studies, wedges were stained with the voltage-sensitive dye, di-4-ANEPPS (15 µmol/L) and excitation of the voltage-sensitive dye's fluorescence was achieved using a 514 ± 5 nm light emitted by a 250 W tungsten-filament lamp while fluoresced light was longwave-pass filtered at 610 nm and focused onto a 256-element photodiode array with high numerical aperture tandem lens imaging. In all experiments, cytochalasin-D (6 µmol/L) was used to ensure that motion artifact was prevented.

In order to examine the relationship between HF-induced alterations in calcium handling and APD-ALT, dual Ca-Vm and ratiometric Ca mapping was performed in an additional set of

experiments. Wedges were initially stained with the calcium sensitive indicator Indo-1 AM (10 $\mu\text{mol/L}$). Excitation light was filtered at $350\pm 10\text{nm}$ and directed to the preparation. Fluorescent light from the preparation was collected by a tandem lens assembly. Ratiometric calcium transients were calculated by dividing the background-subtracted calcium transients at 405nm by the background-subtracted calcium transients at 485nm.²⁰ For every dual Ca-Vm mapping experiment, baseline Ca transient characteristics were then obtained at a variety of pacing heart rates (HRs). After initial ratiometric recordings, dual mapping of calcium and membrane voltage was performed as previously described.^{13,21} For Ca mapping experiments, calcium transients and simultaneous recordings of their corresponding optical action potentials were recorded from 128 sites across all myocardial layers of the transmural LV.

To investigate the effect of HF on beat to beat alternations of cellular Ca cycling (Ca-ALT), independent of HF induced changes on repolarization, we recently developed and validated a system for the simultaneous imaging of Vm and Ca in isolated myocytes (performed at 32°C).¹² The utility of this system is that it permits direct and quantitative comparison of Vm and Ca waveforms at the single-cell level, and permits assessment of Ca cycling properties of myocytes under voltage-clamp conditions. This is essential to our strategy for evaluating differences in Ca cycling properties between ventricular myocytes independent of any differences in action potential waveforms between myocytes in the same heart, and between normal and HF conditions.

Experimental Protocol and Data Analysis

For Vm mapping studies, (n=8 for both normal and HF), the APD of epicardial (EPI), mid-myocardial (MID), and endocardial (ENDO) cells across the transmural wall was assessed during steady-state endocardial pacing ($3\text{-}5 \times$ diastolic threshold) over a wide range of HR. APD-ALT was induced by decreasing pacing cycle lengths (CL) at 20ms intervals (thereby increasing HR) until failure to capture the preparation or VF was induced. Activation times, repolarization times, and action potential durations (APD) were measured directly from all optical action potentials using previously validated algorithms. Activation time was defined as the point of maximum positive derivative of the action potential upstroke, and repolarization time was defined as the point of maximum positive second derivative of the repolarization phase.¹⁸ Cells were classified as EPI, MID, or ENDO according to previously established criteria.¹⁷ APD-ALT was defined as the difference in APD between two consecutive beats and the APD-ALT threshold was defined as the slowest HR required to produce $\geq 10\text{ms}$ of APD-ALT during stable alternans.^{13,7}

As activation was homogeneous across the preparations while repolarization times were heterogeneous, we defined the conduction gradient as the average conduction gradient (inverse of conduction velocity) in the line of propagation across the transmural direction (endocardium to epicardium) of the preparation and defined repolarization gradients as the maximal local gradient of repolarization. Repolarization gradients were calculated by measuring repolarization time between each recording pixel and the neighboring pixels oriented in the transmural direction, divided by the distance between neighboring pixels to determine the local transmural repolarization gradient at each recording site.

For dual Ca-Vm and ratiometric Ca mapping experiments (n=8 for both normal and HF), alternans was induced in the same manner as for Vm mapping studies. Ca-ALT was defined as the difference in amplitude (systole minus diastole) between two consecutive Ca transients divided by the amplitude of the larger Ca transient and the Ca-ALT threshold was defined as the slowest HR required to produce a $\geq 10\%$ difference in amplitude.¹³ The rate of decrease of intracellular calcium transient (which includes both SR Ca reuptake via SERCA and Ca extrusion via NCX), was used as a measure of Ca reuptake kinetics, the decay portion of the calcium transient (from 70% to the end of the decline phase) was measured by the time constant

(τ) of a single exponential fit.¹¹ To compare magnitude and thresholds of APD-ALT, Ca-ALT, Ca transient characteristics and arrhythmia susceptibility between normal and HF, levels of significance were determined using repeated measures ANOVA, Student's t-test, and χ^2 test where appropriate. When statistical significance between the two groups was found for any variable by ANOVA, statistical significance between any two individual means was determined by a Neuman Keuls post-hoc test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Heart Failure Increases Susceptibility to Action Potential Duration Alternans

The effect of HF on susceptibility to APD-ALT is shown in Figure 1. Optically recorded action potentials spanning the transmural wall of HF and normal left ventricular wedge preparations were recorded while alternans was induced. In HF, there is a leftward shift in the APD-ALT to HR relationship, indicating greater susceptibility to APD-ALT in HF preparations. There was a significant ($p < .004$) reduction in HR threshold required to induce APD-ALT (from 236 ± 25 bpm to 185 ± 25 bpm.). This was true for all cell types, but, as demonstrated previously in normal hearts^{11,12,22} ENDO and MID cells were most susceptible to APD-ALT compared to EPI cells ($p < .001$) in HF (Figure 1).

Susceptibility to Action Potential Duration Alternans in HF arises from Alternans of Calcium Cycling

The canine pacing-induced HF model produces significant alterations in calcium handling. Consistent with other reports, in HF myocytes we observed a significant decrease in calcium transient amplitude (by $72 \pm 4\%$), increase in time to peak of the calcium transient (by $135\% \pm 9\%$) and calcium transient duration prolongation (by $60 \pm 5\%$, all $p < .01$) when compared to normal myocytes.³

Dual Ca-Vm mapping experiments were performed in the canine wedge preparation to evaluate the relationship between Ca-ALT and APD-ALT in HF. As was observed for APD-ALT, increased susceptibility to Ca-ALT in HF was evident by the significant ($p < .005$) reduction in HR threshold required to induce Ca-ALT (from 238 ± 35 bpm to 177 ± 26 bpm). Figure 2 (panel A) demonstrates the very close relationship between the development of Ca-ALT and APD-ALT as HR is increased in a representative HF wedge preparation. The inset demonstrates the generalizability of these results as very similar HR thresholds for both Ca-ALT and APD-ALT were observed across all experiments, in which Ca-ALT either occurred at a slower HR or simultaneously with APD-ALT. In a subset of these preparations ($n=6$), a detailed analysis of the magnitude of both Ca and APD-ALT in ENDO, MID and EPI cells over multiple HRs was performed. Again, Ca-ALT and APD-ALT were both highly rate-dependent (Figure 2, panel B, $p < .05$). Moreover, cells which exhibited the greatest magnitude of APD-ALT (ENDO and MID, Figure 2, panel B, upper $p < .03$), also exhibited the greatest magnitude of Ca-ALT (Figure 2, panel B, lower $p < .02$) again suggesting that cellular susceptibility to Ca-ALT is closely associated with susceptibility to APD-ALT in HF. An additional set of experiments performed in isolated endocardial myocytes from HF and normal hearts under current clamp conditions revealed similar results; i.e., the HR threshold for Ca-ALT was significantly lower ($p=0.01$) in HF (137 ± 22 bpm, $n=7$) compared to normal (230 ± 32 bpm, $n=6$) myocytes, reaffirming that HF induces a similar leftward shift of the Ca alternans-HR threshold (data not shown).

In addition to impaired Ca cycling, HF is associated with remodeling of the sarcolemmal ion currents governing repolarization which could potentially contribute to the increased susceptibility to APD-ALT.^{3,13,23,24} To eliminate the effect of HF-induced changes in the

action potential, additional experiments were performed in isolated myocytes where Ca transients were measured with repetitive, non-alternating action potential clamp waveforms, using the method previously developed by Chudin et al.²⁵ In Figure 3 (panel A), the non-alternating action potential clamp protocol is shown in the upper panel. At this pacing HR, minimal Ca-ALT is observed in the normal myocyte (middle panel), while in the HF myocyte, significant Ca-ALT is observed. In Figure 3, panel B, the Ca alternans-HR relationship for isolated myocytes under action potential clamp are shown, demonstrating that HF induces a similar leftward shift of the Ca alternans-HR threshold even under conditions where APD-ALT is prevented (Figure 3, panel B). In these experiments, the HR threshold for Ca-ALT was lower ($p < .003$) in HF (168 ± 3 bpm, $n=4$) compared to normal (233 ± 41 bpm, $n=4$) myocytes, reaffirming the findings observed in the intact heart preparation. Therefore, enhanced susceptibility to alternans at the level of the myocyte could not be solely attributed to differences in action potential waveforms between HF and normals; rather, these data suggest that intracellular Ca cycling is centrally involved in the genesis of cellular alternans in HF.

Optical mapping in the canine wedge preparation allowed us to compare Ca handling properties between myocytes exhibiting different susceptibilities to alternans. This strategy was used to gain insight into cellular properties which underlie alternans, as described previously.¹¹⁻¹³ Cells (optical recording sites in the canine wedge preparation) were defined as either alternans susceptible or resistant if the magnitude of APD-ALT at a HR of 230 bpm were in the top or bottom quartile of APD-ALT, respectively. There was reduction of SR Ca reuptake kinetics in alternans susceptible cells (typically in MID and ENDO cells) compared to alternans resistant cells (typically EPI) within both HF (by $30 \pm 16\%$, $p < .02$) and normal (by $13 \pm 6\%$, $p < .02$) wedge preparations (Figure 4). Importantly, HF alternans susceptible cells exhibited significantly slower Ca reuptake kinetics compared to normal alternans susceptible cells (by $30 \pm 5\%$, $p < .01$, Figure 4) suggesting HF-induced abnormalities in Ca reuptake kinetics is mechanistically related to increased susceptibility to APD-ALT in HF. To evaluate the potential role of Ca release in mechanisms of HF-induced susceptibility to alternans, we compared the kinetics of Ca transient rise time between these same alternans susceptible and resistant cells within both HF and normal wedge preparations. Ca transient rise time was significantly longer in alternans susceptible vs. resistant cells within both HF (by $40 \pm 9\%$, $p < .003$) and normal (by $40 \pm 7\%$, $p < .05$) preparations. The difference in rise time between alternans prone cells in HF and normal wedge preparations were not significant (longer by $25 \pm 10\%$, $p = .08$). Taken together, these data from intact failing myocardium suggest that alternans susceptibility in HF is related to HF-induced abnormalities in calcium cycling.

Effect of HF on Discordant Alternans and Susceptibility to Arrhythmias

Figure 5 (panel A) shows representative volume conducted ECGs and action potentials from EPI and ENDO cells in normal (left panel) and HF (right panels) hearts. In normal hearts at a pacing HR of 180 bpm, both EPI and ENDO cells alternate in phase, with each alternating in a long-short-long pattern (concordant alternans, CON-ALT). However at the identical HR in HF, EPI and ENDO cells alternate in opposite phase (discordant alternans, DIS-ALT). Note that T-Wave alternans is also observed in the ECG. The magnitude and phase of APD-ALT across the transmural LV wall of normal (left) and HF (right) wedges are illustrated by iso-alternans maps in Figure 5 (panel B). At the slower HR, only small regions of normal LV ENDO myocytes are alternating (left, red contours), while in HF, at the identical stimulation rate, significant alternans is observed across the transmural wall. When HR is increased, APD-ALT increases (red contours) as expected in the normal wedge, and all cells are alternating with identical phase (CON-ALT). However, in HF, the same HR induced DIS-ALT (Figure 5, panel B) where APD-ALT of cells within different regions of myocardium was opposite in phase (red versus blue shading). Interestingly, DIS-ALT was typically oriented in the endocardial-epicardial direction. When considering all experiments, DIS-ALT occurred at

significantly slower pacing HRs in HF (Figure 5, panel C, left) and although DIS-ALT was observed in 100% of HF preparations, it was observed in 63% of normal preparations (5/8, $p=0.06$) (Figure 5, panel C, right).

Previously we demonstrated in normal, non-failing myocardium that the development of DIS-ALT amplifies spatial gradients of repolarization, leading to conduction block and reentrant excitation.⁷ Therefore, we sought to determine whether DIS-ALT also serves to amplify gradients of repolarization in HF. Figure 6 illustrates the magnitude of gradients of repolarization in the transmural axis (i.e. endocardial to epicardial direction). The plots show conduction and repolarization gradients during 4 phases of the stimulation protocol; 1. At relatively slow HR, well before alternans initiated, 2. At the fastest HR without alternans, 3. At the fastest HR which initiated spatially concordant alternans without inducing DIS-ALT, and 4. At the fastest HR which initiated DIS-ALT without inducing VF. In HF (figure 6A, $n=8$), the development of DIS-ALT, was also associated with a marked (>7 fold, $p<.003$) increase in the maximum repolarization gradient. Increasing HR to induce DIS-ALT was associated with similar slowing of conduction in both HF and normal preparations (decreased by $28 \pm 4\%$ and $23\% \pm 10\%$, respectively, each $p<.007$). Although conduction slowing might potentially enhance repolarization gradients, as shown in figure 6A, during induction of DIS-ALT, there is only a small increase in conduction gradient, as opposed to the marked increase in the repolarization gradient. Therefore, the relative contribution of conduction slowing to the local repolarization gradients was small compared to the contribution induced by DIS-ALT of APD. Such marked amplification of repolarization gradients were not observed in normal controls (Figure 6A, $n=5$). Moreover, in HF preparations, the development of steep gradients of repolarization during DISALT was closely associated with susceptibility to arrhythmogenesis, as VF was only induced after DIS-ALT appeared. VF was inducible in 7/8 HF preparations, but in only 1 normal preparation (Figure 6, panel B), suggesting that arrhythmia inducibility was dependent on the marked repolarization gradients induced by DIS-ALT in HF, as these large gradients were not observed in normal preparations.

DISCUSSION

Heart Failure Increases Susceptibility to Alternans

T-ALT has been associated with ventricular arrhythmias in diverse experimental and clinical settings. Subtle (microvolt-level), and visually unapparent T-ALT is actually quite common (approximately 50% incidence) in patients at highest risk for SCD; i.e., those with left ventricular dysfunction^{4,5,26} These observations imply that understanding mechanisms of T-ALT can provide fundamentally new insights to the pathophysiology of SCD. There is compelling evidence^{7,8,27} that T-ALT results from beat-to-beat alternation in the time course of action potential duration at the cellular level (APD-ALT)⁷ and APD-ALT is mechanistically related to the development of VF.⁷ However, essentially all prior research into mechanisms underlying APD-ALT has been performed in normal hearts. In the present study, we demonstrate for the first time, that HF increases susceptibility to APD-ALT, and does so at the level of cellular Ca-cycling. Importantly, the increased susceptibility to APD-ALT in HF promoted arrhythmogenic DIS-ALT, which markedly increased repolarization gradients and was associated with an increased incidence of inducible VF.

Increased Susceptibility to APD ALT is closely associated with Ca-ALT in Heart Failure

In normal hearts, there are convincing data for a primary role of sarcoplasmic reticulum (SR) calcium cycling in the mechanism of APD-ALT.^{13,28,29} In the present study, HF lowered the HR threshold for APD-ALT and Ca-ALT. Importantly, the magnitude of both Ca and APD-ALT were very closely associated in HF (Figure 2). These data are consistent with observations in normal hearts, in which alternans of Ca and APD are also closely associated¹³ and suggest

that impairment of calcium cycling is fundamentally related to the increased susceptibility to alternans observed in HF.

However, in clinical and experimental HF, APD prolongation is also a consistent finding.²⁴ Modeling data have suggested that restitution properties of myocytes have been implicated as a mechanism of APD-ALT, which has been taken as evidence that sarcolemmal ion channels drive APD ALT.^{9,10} As HF affects the action potential, it is possible that restitution plays a more prominent role in the mechanism of increased susceptibility to alternans in HF. For this reason, we performed action potential clamp experiments to isolate the effects of HF on Ca-ALT independent of the effect HF has on the action potential. We observed that in HF Ca-ALT is similarly induced under current-clamp (where APD ALT occurs) and voltage-clamp (i.e. where APD ALT is prevented) conditions suggesting that Ca-ALT is not dependent on APD ALT, and strongly supports the notion that cellular alternans primarily arises from SR Ca cycling, and is not dependent on HF induced remodeling of the action potential.

Potential Mechanism of Increased Susceptibility to Alternans in Heart Failure

A fundamental cellular abnormality in HF is impaired Ca cycling. As the mechanism of cardiac alternans in normal myocytes is related to impaired Ca cycling, we hypothesized that HF myocytes are intrinsically more susceptible to alternans. Interestingly, more than a century ago, Traube described pulsus alternans, now recognized as a manifestation of Ca-ALT, in patients with severe HF.³⁰ Multiple HF-induced changes in Ca cycling that are directly responsible for contractile dysfunction are consistently observed in experimental and human HF. A common observation in human and experimental HF is significant blunting of SR calcium reuptake, caused by reduced expression of the SR Ca ATPase (SERCA2a).³ Expression of the SERCA2a regulatory protein phospholamban may also be reduced. In HF, SR calcium content may be reduced due to decreased SERCA2a function and expression as well as increased NCX expression, both leading to less cytosolic calcium being taken up into the SR.³¹ In addition, faster stimulation rates result in decreased SR calcium uptake in HF versus normal hearts, indicating a decreased ability for calcium cycling to keep up with increasing HRs.³²

We observed that in HF, the cells most susceptible to Ca and APD-ALT were predominantly located in the endocardium and subendocardial/mid-myocardium (Figures 1 and 2), consistent with prior work from our laboratory^{11,12} and others.²² A human study, using activation-recovery interval recordings of TWA, suggested that epicardium may be more susceptible to cardiac alternans. However in that study, endocardial alternans was only measured from the RV and not LV, so a direct comparison of transmural LV alternans susceptibility was not performed.³³ We utilized the regional differences in cellular susceptibility to APD and Ca-ALT in HF to further investigate potential mechanisms underlying increased susceptibility to APD-ALT in HF. We observed that in HF, alternans susceptible cells had significantly slower calcium reuptake kinetics than alternans susceptible normal cells. Importantly, in HF cells most susceptible to Ca-ALT also demonstrated slower calcium reuptake kinetics when compared to alternans-resistant HF cells. Although from our data we cannot determine the specific mechanism for enhanced alternans susceptibility in HF, impaired Ca reuptake in alternans susceptible myocytes may be attributed to one of several mechanisms that impaired cytosolic diastolic Ca clearance, including delayed reuptake via SERCA2a due to altered expression or function, changes in SERCA2a regulation by phospholamban, or alterations in NCX function.³ In addition to rate-dependent properties of SR calcium reuptake, SR Ca release has also been implicated in the mechanism of Ca and APD-ALT in normal hearts.^{14-16,34} Our data also suggest that HF-induced abnormalities of SR Ca release may also play a role in the increased susceptibility to alternans we observed in HF. Overall, these data suggest that slowed calcium cycling kinetics in HF may be directly responsible for the increased susceptibility to alternans,

and therefore, related to a mechanism of arrhythmogenesis in HF. Importantly, these data suggest that cellular alternans may be a mechanism linking calcium cycling and electrical dysfunction in HF.

Heart Failure Increases Susceptibility to Discordant Alternans and Ventricular Fibrillation

In the present study, when APD-ALT was first initiated, it occurred with identical phase in all cells of a particular region of ventricular myocardium (i.e. spatially concordant alternans). Above a critical HR threshold we observed that consistently in HF, APD-ALT switches phase in some cells but not others, such that some cells undergo a prolongation of action potential duration (APD) while other populations of cells undergo APD shortening on the same beat (i.e. DIS-ALT).^{7,8} Previously, this was found to be a key mechanism linking alternans and cardiac arrhythmogenesis in non-failing hearts.⁷ In this study, using the canine wedge preparation, we determined that HF enhances susceptibility to DIS-ALT (Figure 5). As we have previously demonstrated in normal hearts, the transformation from concordant alternans to DIS-ALT has significant consequences on the spatial organization of repolarization across the ventricle. During discordant alternans, marked spatial dispersion of repolarization emerges of sufficient magnitude to produce conduction block and reentrant excitation resulting in VF.^{7,8} In this study, inducible VF was almost exclusively observed in HF, and importantly, the development of VF was preceded by DIS-ALT and marked increase in repolarization gradients in HF preparations. Additional aspects of HF may have contributed to the increase in susceptibility to DIS-ALT, including intracellular uncoupling, changes in the extracellular matrix, and HF-induced APD prolongation. In normal preparations, we did not observe as marked amplification of repolarization gradients during DIS-ALT as had been observed previously in other models, which may be related to species and model differences and perhaps the relatively higher HRs required to induce DIS-ALT in normal vs. HF preparations. Although it is also possible that HF-induced conduction slowing also played a role in the increased susceptibility to VF observed in HF, the amplified repolarization gradients observed were almost certainly caused by DIS-ALT and not conduction gradients associated with slow conduction (Figure 6A).

Limitations

The canine pacing-induced HF model may not be generalizable to other models of experimental HF or human HF and the use of tachypacing may itself induce changes in calcium handling independent of HF. For example, the HR threshold to induce APD alternans was significantly higher than that observed in clinical TWA testing in humans. However, our threshold data in wedge preparations are consistent with prior data in intact normal canine hearts in which the HR required for induction of TWA is significantly higher than in man.³⁵ Importantly, this model allows us to take advantage of the powerful approach of transmural optical mapping to simultaneously assay the topography and contribution of various cell types that reside within the ventricular wall, which is not feasible in smaller animal models of HF. In addition, the resolution of our recordings allows us to characterize differences in susceptibility to Ca and APD-ALT among these distinct cell types and relate them to differences in cellular Ca cycling.

We cannot exclude that during AP clamp studies; there was beat to beat alternation in current flow via sarcolemmal ion channels that could potentially influence Ca-ALT. However, as the same AP clamp was used in both HF and normal myocytes, we do not believe that this would have significantly affected the observed differences in Ca-ALT between the HF and normal myocytes. The threshold for Ca-ALT measured from the wedge preparation differed somewhat in absolute terms from that measured from isolated myocytes. A number of different experimental conditions could have contributed to the lower HR threshold for Ca-ALT in isolated myocytes compared to the wedge, including temperature, comparison of uncoupled to coupled conditions, the process of cell isolation, among others.

HF produces complex Ca cycling and electrophysiologic remodeling, and although we excluded an exclusive role of sarcolemmal ionic currents, it is certainly possible that additional aspects of HF-induced remodeling contribute to the increase in alternans susceptibility observed. These might include HF-induced alterations in ion channel expression or function, additional aspects of Ca cycling including RyR dysfunction, changes in myocardial energetics, tissue architecture, connexin expression and function, cellular uncoupling, and potentially others. The substrates for arrhythmias in HF are equally complex, so it is unlikely that alternans is a sole arrhythmogenic mechanism, but is rather an important contributor to arrhythmogenesis in HF. Although we did not definitively observe triggered activity in these experiments during rapid pacing, it is possible that triggered activity independently, or interacting with discordant alternans, may have contributed to the increased arrhythmogenesis observed in these experiments.³⁴

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Glossary of Abbreviations

APD-ALT, action potential duration alternans
 Ca-ALT, calcium transient alternans
 DIS-ALT, spatially discordant alternans
 EC coupling, excitation-contraction coupling
 ENDO, endocardial
 EPI, epicardial
 HF, heart Failure
 HR, heart rate
 LV, left ventricle
 MID, mid-myocardial
 SCD, sudden cardiac death
 T-ALT, T wave alternans
 VF, ventricular fibrillation
 Vm, membrane voltage

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Figure 1. HF increases the susceptibility to APD-ALT

Summary data for all Vm optical mapping experiments demonstrating the HR-alternans relationship from normal and HF hearts (each n=8). In HF, more APD-ALT is observed at each HR tested and HF produced a profound left-ward shift in APD-ALT to HR relationship, depicted by the arrow showing the shift to slower threshold HRs (depicted by the blue shading) when APD-ALT was first detected (red dashed lines). These data demonstrate that HF preparations have greater susceptibility to alternans. In addition, there is transmural heterogeneity in susceptibility to APD-ALT, with ENDO and MID cells exhibiting more APD-ALT relative to EPI cells in both normal and HF.

A **B**

Figure 2. Development of repolarization and calcium alternans are closely associated in heart failure

Panel A. APD-ALT (solid line) and Ca-ALT (interrupted line) from a representative HF heart measured over multiple HRs. As HR increases, both APD-ALT and Ca-ALT similarly increase. Inset shows the HR threshold for development of APD-ALT in normal and HF hearts in which dual Ca-Vm mapping was performed (each n=8). Importantly, APD-ALT and Ca-ALT are closely associated suggesting that increased susceptibility to APD-ALT in HF is related to an increased susceptibility to Ca-ALT. **Panel B.** Regional susceptibility to APD-ALT (upper panel) is also closely associated with Ca-ALT (lower panel), in which more APD-ALT susceptible cells (MID and ENDO) are also the most susceptible to Ca-ALT.

Figure 3. Heart failure increases susceptibility to alternans independent of effects on the action potential

Panel A. The upper tracing shows the command action potential clamp waveform and a representative example of Ca transients from normal (middle tracing) and HF (lower tracing) myocyte are shown at a HR of 200 bpm. In the HF, but not in the normal myocyte, Ca-ALT was induced under constant action potential clamp conditions, independent of action potential alternans. **Panel B.** Data over a range of HRs for normal and HF myocytes are shown. HF induced a similar leftward shift of the HR/Ca-ALT relationship as was observed in the intact heart (i.e. the alternans threshold was lower, and the amplitude of Ca-ALT was greater in HF compared to normal) under non-alternating action potential clamp conditions.

Figure 4. Slower calcium reuptake kinetics in HF predicts susceptibility to action potential alternans

Panel A. Representative calcium transients from normal and HF hearts from alternans resistant and susceptible cells are shown. Tau (τ), the time constant of decay of the calcium transient, is slower in alternans susceptible cells in both normal and HF hearts. **Panel B.** Summary data of τ in alternans resistant (R, gray bars) and alternans susceptible (S, black bars) in both HF (right) and normal (left) hearts at a baseline pacing HR of 100 bpm. Importantly, τ is slowest in the most alternans susceptible HF cells, suggesting that HF-induced slowing of calcium reuptake promotes alternans. Similar results were also observed at a baseline pacing HR of 30 bpm (data not shown).

Figure 5. Heart failure enhances susceptibility to spatially discordant APD-ALT

Panel A. Volume averaged ECG and representative epicardial and endocardial optically recorded action potentials from a normal and HF heart at a pacing HR of 180 bpm. L signifies a long AP, S a short AP, and APD is shown below in ms. In HF, alternans which is out of phase between EPI and ENDO cells (DIS-ALT) is observed at this HR, but not in normals. **Panel B.** Transmural maps of APD-ALT magnitude (contour intensity) and phase (contour color, red

is +phase, blue is -phase). At lower HR (top) concordant alternans (i.e. one color) is extensively distributed across the transmural surface in HF but not in normals. At faster HR (bottom), concordant alternans is transformed to DIS-ALT in HF only, as myocytes near the EPI and ENDO surfaces begin alternating with opposite phase, depicted by the presence of both red and blue contours. **Panel C. Left.** Summary data from all Vm experiments, demonstrating that HF lowers the HR threshold for the development of DIS-ALT (increases susceptibility to DIS-ALT) and that DISALT was observed more frequently in HF (**Right**).

Figure 6. Discordant alternans promotes dispersion of repolarization in HF, which is closely associated with the development of VF

Panel A. Maximal local repolarization and conduction gradients are shown: 1) In the absence of alternans (at baseline HR, i.e. 60 bpm and at the fastest HR prior to the development of APD-ALT) 2) During CON-ALT (at the fastest HR prior to onset of DIS-ALT) and 3) during DIS-ALT (at the fastest HR prior to development of 15 VF or failure to capture the preparation) in both HF (n=8, upper panel) and normal hearts (n=5, lower panel). The development of DIS-ALT is associated with a marked amplification of repolarization gradients in HF ($p < .003$), while conduction slowing is minimal relative to the developed repolarization gradient. **Panel B.** The development of DIS-ALT and increased DOR in HF was associated with a higher incidence of inducible VF in HF than in normal hearts.