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A new approach to measure the contribution of restitution to repolarization alternans

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Abstract—**Several studies suggest link between repolarization alternans and arrhythmia. A potential target for minimization of alternans amplitude is pharmacological flattening of restitution function, which links a diastolic interval (DI) and subsequent action potential duration (APD). While our recent studies have shown that DI dependent restitution is not a necessary mechanism for alternans, in circumstances of nearly invariant activation intervals, restitution contributes to alternans. Determination of the degree to which restitution contributes to alternans during stable alternans, which requires determination of the gain between DI and APD, is not possible because it always is unity. We propose that the rate of change of alternans along the length of the tissue may provide an estimate of the degree to which restitution contributes to alternans amplitude. We conducted experiments with swine to demonstrate the above approach. In a linear strand of tissue, we paced such that DIs for successive activations were invariant at one end, which eliminates the restitution dependent mechanism of alternans at this end. Due to conduction delays, at the distal end, both restitution dependent and independent mechanisms manifest. Action potentials recorded from right ventricular endocardial tissue from swine (n=3) showed an average difference in amplitudes of alternans between the two ends to be 11.99, 25.49, and 39.37 msec. Rates of change of alternans amplitude as a function of distance, computed using linear interpolation, were 0.36, 1.69 and 0.97. We propose that this rate of change may provide an indirect measure of degree of contribution of restitution to alternans and thus may be useful in evaluating therapeutic approaches to minimize its amplitude.**

I. Introduction

SUDDEN cardiac death is caused by lethal arrhythmias. Despite extensive investigation, the mechanisms that lead to destabilizing of the rhythm into these arrhythmia remain unclear. A peculiar observation is that beat by beat alteration in action potential characteristics, which is manifest in the ECG as beat by beat alteration of T wave shape, is associated with incidence of sudden cardiac death, especially in those with systolic dysfunction, and possibly in other situations as well (1). This beat by beat change in shape of T wave is referred to as the T wave alternans (TWA), the cellular level origin of which is alternans of action potential duration (APD). The mechanisms underlying this behavior, which is period doubling bifurcation, have been the subject of extensive investigation. A putative mechanism is restitution of APD. Restitution, in its widely defined sense, is a function that relates an APD to the interval preceding this action potential when the cells are at resting potential, which is referred to as the diastolic interval (DI). An hypothesized predictor of alternans is a steep slope of the restitution function (2-4). Decreasing the slope of this restitution function has been proposed as an anti-arrhythmic target (2). Although the theory behind the predicted role of restitution in alternans of APD and in arrhythmia has received extensive attention, there have been studies which show that this link is not always observed in experiments. For example a recent study by Narayan et al (1) found that the slope of restitution was not predictive in terms of TWA and arrhythmia. We have previously shown that the mechanisms by which alternans of APD occur have restitution dependent and independent components, the restitution independent component can be separated and, importantly, the DI dependent restitution is not a necessary condition for alternans to exist (5). However, a change in DI does cause a change in APD, and thus it becomes important to determine the extent to which restitution contributes to alternans. One way to determine the contribution of DI dependent restitution to a change in APD is to use the slope, i.e. gain, of the function that relates an APD to preceding DI at the operating point. Virtually all previous investigations of stable alternans used protocols that employed constant cycle length pacing or combination thereof. Because the cycle lengths are constant, determination of the slope of restitution during stable alternans does not produce meaningful quantities because this slope is always equal to 1. We propose a new approach to determine the contribution of restitution to alternans of APD. We have previously developed a pacing protocol which allows one to explicitly control DI during pacing (6, 7). When this protocol is used to pace such that DIs for successive beats are invariant, the restitution dependent mechanism is eliminated. If a linear strand of tissue is paced such that at one end the DIs are maintained invariant, then the train of activations that travel from the pacing site to the other end, i.e., distal site, will experience effects of conduction delay which will bring about a graded change in DI as a function of increasing distance from the pacing site. Thus the DI dependent component of restitution will contribute to the amplitude of alternans in a graded fashion, with being eliminated at the pacing end and maximal at a location where alternans have the maximal amplitude. We consider that the rate of change of alternans amplitude as a function of distance, therefore, should provide a measure of the degree to which restitution contributes to alternans. In the present study, we demonstrate the feasibility of the proposed approach.

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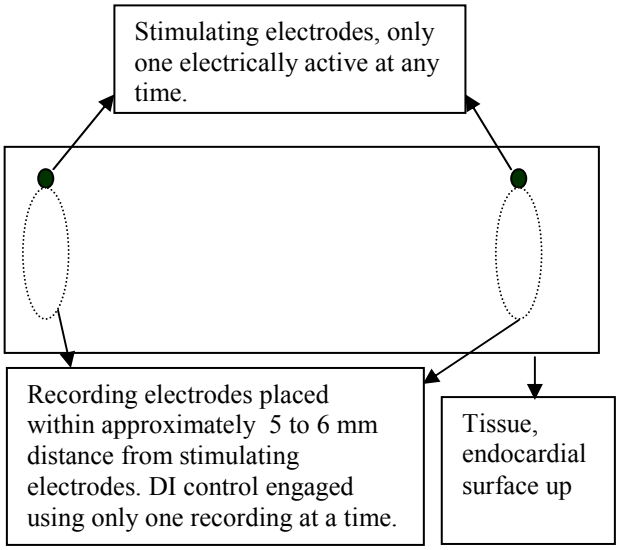

Figure 1: Schematic of a linear strand of tissue showing the arrangement of stimulating and recording electrodes.

II. METHODS

A. Diastolic Interval Control:

We implemented a previously developed DI control protocol in a custom program written in Labview™. Details of the control protocol are described elsewhere (6, 7), briefly: the transmembrane potentials were sampled on-line and using a threshold crossing method, end of an action potential was determined on-line in real-time. Upon detection of end of an action potential (defined as recovery to 90% of total depolarizing potential), a timer was started to wait a pre-defined interval, at the end of which the next stimulating pulse was delivered. Thus, these pre-defined intervals become the DI for the subsequent activations.

B. Experimental Setup

Animal experiments were conducted after obtaining approval from the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky. A narrow strip of right ventricular tissue from swine (n=3) was placed in a tissue chamber and superfused with modified Tyrodes solution bubbled with 95% O_2 + 5% CO_2 gas mixture (7). Standard glass microelectrodes were used to record transmembrane potentials (TMP) from the endocardial side of the tissue. Two micro-electrodes were impaled at the two ends of the tissue. Figure 1 shows a schematic of the experiment. In each trial, the tissue was electrically stimulated from one end and DI control was engaged using a feed-back based pacing protocol using the TMP recorded from that end. The DI was controlled to be beat by beat invariant for 150 beats, while TMPs were recorded from both electrodes. In subsequent trials, stimulation was switched to pace the tissue from the other end with the TMP from the electrode closest to the stimulating electrode used in feedback control. The DI control protocol was then repeated. The TMP from both electrodes were continuously digitized at a rate of 10000 samples/second using a commercial data acquisition system. All data were digitally filtered by using a low-pass filter with a cut-off frequency of 500 Hz. From the digitized and filtered data APD were calculated as the duration within which the membrane potential returned to 90% (i.e. APD_{90}) of the maximal change in potential from the start of that action potential using a fixed threshold. Amplitude of alternans was computed as the absolute difference between APD_n and APD_{n-1}. A digital image was used to quantify approximate distance between impalements.

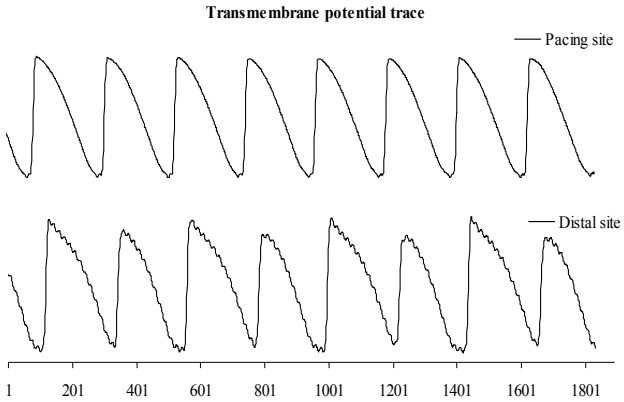

Figure 2: Example of TMP recorded from the two ends of a linear strand of tissue. The DI was made nearly invariant for successive beats at the pacing site. Alternans of larger amplitude are clearly seen at the distal site.

III. RESULTS

Figure 2, shows an example of TMPs recorded from two ends of tissue. The TMPs show that the DI was controlled for successive beats at the pacing site. The figure shows that while at the pacing site the DIs preceding each action potential were nearly invariant, at the distal site, the oscillations in DI were large. The amplitude of alternans of APD at the pacing site was small compared to that at the distal site indicating increased DI dependent contribution of restitution.

To determine contribution of DI dependent restitution at the pacing and the distal sites we computed instantaneous slopes, i.e. ratios of beat-by-beat changes in APD divided by changes in preceding DI. Effective DI control was considered when absolute change in beat by beat DI was less than 4 msec for 10 or more successive beats. Similarly, an absolute change greater than 4 msec in amplitude of APDs for 10 or more successive beats was considered as occurrence of APD alternans. It is technically difficult to explicitly control short DIs for large number of beats, therefore, we selected sections of 10 or more successive beats meeting the above criteria in any trial to compute alternans amplitudes and instantaneous slopes. Results were first averaged from multiple trials within each animal, which includes pacing from either end, and then were averaged across the 3 animals.

Figure 3 shows histograms of instantaneous slopes (ΔAPD/ΔDI) computed from the TMPs recorded at the pacing and the distal sites. When computing slopes, if the change in DI was numerically zero we set those slopes equal to the maximum range used in computing these histograms, which was ± 10. The figure shows that the slopes at the distal end were all concentrated in the bin with a center value of 1, very similar to the situation observed during constant cycle length pacing. The slopes at the pacing end were more widely distributed which reflects the fact that contribution from restitution was effectively abolished at this end, as expected. The peaks in the histograms within the two bins with center values of ± 1 are a result of ratios of two similar small numbers, i.e., a small change in APD divided by a nearly equal small change in DI.

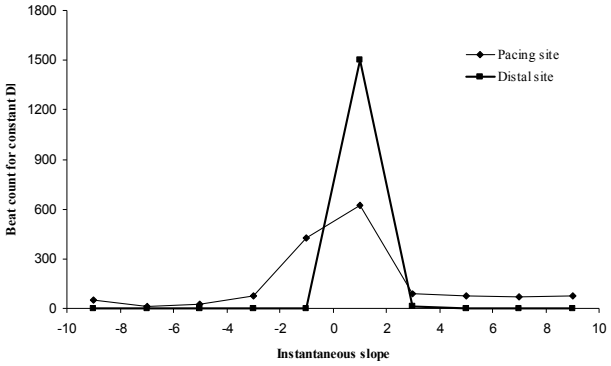

Figure 3: Histogram of instantaneous restitution slopes. The data points are plotted at the central value of each bin. At the pacing site, the instantaneous slopes were distributed over a wide range whereas at the distal site, the slopes were distributed over a much narrower range centered at 1.)

The average difference in alternans amplitude between the paced and the distal ends was 11.99, 25.49, and 39.37 msec for the three animals. The approximate distances between the impalements were 33, 15 and 35 mm. Assuming a linear change in alternans, we computed the rates of change in alternans amplitude as a function of distance to be 0.36, 1.69 and 0.97 msec/mm, with an average value of 1.01 msec/mm.

IV. CONCLUSIONS

Restitution of APD contributes to alternans of APD, i.e. to the period doubling bifurcation. Although the presence of restitution independent component suggests it may not be possible to completely eliminate alternans by flattening restitution, it may be possible to minimize its amplitude. In order to determine how much decrease is likely to result from altering restitution, it becomes important to determine how much restitution contributes to alternans. We posit that the rate of change of alternans amplitude as a function of distance may provide such an estimate. While further studies are required to determine whether this rate of change does provide contribution of restitution, our results suggest that it may do so and show that the approach is feasible. The change in alternans amplitude as a function of distance has been previously reported by Fox et al (8) using constant cycle length pacing. It is possible to obtain rate of change of amplitude using constant cycle length pacing also, an advantage of the proposed approach is that it also provides an estimate of the non-restitution dependent contribution to alternans. In constant cycle length pacing approach, there are nodes along the distance where alternans of APD (and thus of DI) are eliminated i.e. amplitude of alternans increases and decreases along the distance. If these nodes are stationary in time and space, then it may be possible to obtain rates of change similar to the proposed approach. Because we just had two impalements, it is not possible to determine which phase of the alternans change the recordings were made, which may have contributed to the differences in the slopes (msec/mm) that we estimated. Obtaining more TMP measurements along the tissue will be needed to fully address this issue. However, the marked difference in the histograms of slopes at the two ends suggest that the rate of change of alternans amplitude has the potential to be useful in determining contribution of restitution and thus may be helpful in evaluating potential therapeutic approaches.

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