Restitution of Action Potential Duration During Sequential Changes in Diastolic Intervals Shows Multimodal Behavior

Runze Wu, Abhijit Patwardhan

Abstract—Restitution of action potential duration (APD) is thought to be critical in activation instability. Although restitution is used to predict APD during sequential changes in diastolic interval (DI), currently used protocols to determine restitution do not use sequential changes in DI. We explored restitution using a new pacing protocol to change DI sequentially and independently of APD. Transmembrane potentials were recorded from right ventricular endocardial tissue isolated from six dogs. We used three patterns of DIs: oscillatory, to demonstrate differences in APDs depending on previous activation history; random, to minimize effects of previous activation history; each DI preceding an APD had an equal probability of being short or long; and linear, to compare restitution relationship obtained during sequential changes in DI with those obtained using currently used protocols; DIs mimicked those that resulted using currently used protocols, except that they changed in sequence. During oscillatory DIs, restitution showed bimodal trajectory similar to hysteresis. Decrease in APD during decreasing DIs was faster than increase in APD during increasing DIs. When effects of previous activation history were minimized, we observed that for a given DI there were multiple values of APD. Restitution relationship obtained during sequential changes in DI was shallower than those obtained using currently used protocols. Our results show that the new pacing protocol may permit direct evaluation of effects of memory on APD. Sequential and explicit control of DI suggests that use of a unimodal relationship to predict APD when DIs change in sequence may not be appropriate. (Circ Res. 2004;94:634-641.)

Key Words: action potential duration | electrical restitution | arrhythmia | fibrillation

Cardiac electrical restitution is considered to importantly influence whether an electrical disturbance degenerates into a reentrant activation. Specifically, it is hypothesized that the slope of action potential duration (APD) restitution function, which relates diastolic interval (DI) and following APD, can predict stability of electrical activation. The hypothesized mechanism is that a slope equal to or greater than 1 can lead to alternans of APD and block propagation. Activation block, in turn, facilitates reentry, leading to arrhythmia. Conversely, for slopes less than 1, disturbances in APD get smaller, returning to a stable activation. Consistent with this view, in a few animal studies, drugs that decrease the slope of restitution have been shown to have antiarrhythmic properties. Therefore, it has been suggested that investigation of restitution relationship may prove helpful during development of treatments for arrhythmia or in evaluation of efficacy of antiarrhythmia drugs.

The APD restitution function is widely quantified using one of two pacing schemes. In one scheme, tissue is paced for several tens of beats at constant cycle length (S1) followed by a stimulus (S2) delivered at progressively shorter or longer intervals. The APD resulting from S2 is plotted against DI between the action potentials produced by last S1 and S2 stimuli. The process is repeated to obtain additional DI, APD pairs. This protocol is referred to as the standard protocol. Restitution curves resulting from this procedure differ depending on S1-S1 intervals and the number of S1 stimuli delivered to obtain a steady state. Furthermore, because S1-S1 intervals used typically tend to be longer than S1-S2 intervals, there are transitional effects. In order to address some of these limitations, a second approach has been recently proposed. In the newer scheme, tissue is also paced for several tens of beats (S1); however, instead of generating DI-APD pairs by using S2 stimulus, they are measured at the end of S1 stimulus train. Then the sequence of S1 stimuli is repeated by progressively shortening S1-S1 intervals. This protocol is referred to as the dynamic protocol.

We quantified restitution relationship using a previously developed feedback-based protocol that permits explicit control of DIs that can be changed sequentially in time. Motivation for using a feedback-based protocol was that although in restitution hypothesis the functional relationship is used to predict change in DI and APD for beats in sequence, the functional relationship is not quantified during sequential changes in DI in currently utilized protocols.

Materials and Methods
Six adult mongrel dogs (18 to 25 kg; LBL Kennels, Reelsville, Ind) were anesthetized (sodium pentobarbital, 30 to 40 mg/kg), and after
anesthesia, the hearts were rapidly excised and placed in chilled (4°C) modified Tyrode's solution. Endocardial tissue from the free wall of right ventricle was excised and mounted in a Plexiglas chamber. Tissue samples were approximately 20×10 mm, with thickness of about 5 mm. The tissue was superfused by modified Tyrode’s solution containing (in mmol/L) 0.5 MgCl2, 0.9 NaH2PO4, 2.0 CaCl2, 137.0 NaCl, 24.0 NaHCO3, 4.0 KCl, and 5.5 glucose. Solution was bubbled with 95% O2 and 5% CO2. The pH of the perfusate was kept at 7.3±0.05. Temperature was maintained at 36±1°C. All studies were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

Tissue samples were equilibrated for at least 60 minutes and paced at cycle length (CL)=500 ms. Pacing stimuli were 3 ms in duration, delivered through a bipolar platinum-iridium electrode. Transmembrane potentials were recorded using floating glass microelectrodes filled with 3 mol KCl. Distance between pacing and stimulation electrodes was about 5 mm.

In order to control DI explicitly, we used a custom program written in C language. In real-time, location of APD90 was identified, ie, time when transmembrane potential repolarized to 90% of its resting value. From the time of APD90, the code waited for a predetermined interval, at the end of which the next pacing stimulus was delivered. These predetermined time intervals became the DIs of next action potentials. Therefore, this procedure permitted control of DIs independent of APD. Pacing stimuli were two to four times stronger than the diastolic pacing threshold. Transmembrane potentials were digitized by a stand-alone commercial data acquisition system. All subsequent analyses were performed off-line using data recorded by the data acquisition system and not using those recorded by the real-time control program. By using offline analysis of data collected independently from the real time control program, we ensured correct assessment of DI and APD and validation of the performance of real-time control. Analyses were performed using custom programs developed in MATLAB.

We used three types of sequences where the DIs changed: (1) a stable oscillatory pattern, ie, DIs oscillated between a maximum and minimum value over a period of N beats; (2) a random pattern, where DIs changed in a random fashion; and (3) a linear pattern, where DIs changed from a maximum to minimum linearly and vice versa. In addition to these sequences, in two animals, we computed restitution relationships using the standard and dynamic protocols. For linear pattern, we selected DIs such that they reproduced those that resulted during standard and dynamic protocols.

**Results**

Figure 1A shows an example of transmembrane potential recorded during a trial. Points marked by asterisks (APD90) were identified in real time. After a predefined interval equal to DI0, a pacing stimulus was delivered to elicit the next activation. As an example of differences between previously used protocols and sequential control of DI, in Figure 1B, we schematically show the dynamic protocol and that used to obtain sequential and linear DI pattern. Figure 2 shows results from a trial of oscillatory changes in DI. During this trial, tissue was paced at a constant DI of 400 ms for 20 beats (Figure 2A), then the DIs oscillated between 700 to 100 ms with a period of 100 beats. Figures 2B and 2C show the APDs and restitution relationship that resulted during pacing with DIs as shown in Figure 2A. The restitution relationship clearly shows two trajectories, ie, hysteresis. Arrows in Figure 2C indicate beat (time) sequence, which shows a counterclockwise rotation of restitution relationship for successive beats. The restitution shows that for same DI, eg, DI of 400 ms, the resulting...
APDs can be different depending on prior activation history. For same DI preceding activations, APDs were shorter when DIs were increasing than APDs when DIs were decreasing. Figure 2C shows that restitution had a slope much smaller than 1 for all values of DI except for a very small region near the minimum and maximum values of DI. Data during constant DI pacing were excluded in plotting restitution. D, Overlay of DI and APD traces shows asymmetrical delay at peaks (X, Y) and nadirs (U, V).

The hysteresis type relationship occurred due to a delay between APD response and changes in DI. We overlaid the traces of APD and DI (scaled amplitudes) in Figure 2D. The vertical lines X and Y show that after DI reached its peak value, APD kept increasing and peaked after a delay of about 13 beats. Delay between APD and DI was not symmetrical: when the DIs were at a nadir, the APD reached the lowest level with a delay of only 3 beats (lines U and V). These results show that the increase in APD following increase in DI was considerably slower than the decrease in APD following a decrease in DI.

Figure 3 shows results from two trials in animals other than the one from which data in Figure 2 are shown. Figures 3A and 3B show that for these trials, the rate of change of DIs, ie, period of oscillation, mean value, and the range (maximum−minimum) of DIs were same; the initial phase of DI change was, however, different. The corresponding restitution relationships from these trials, in Figures 3C and 3D, show similar hysteresis type behavior. Although the difference between rates of decrease and increase in APDs was more pronounced in the trial shown in Figure 3B, the overall pattern of restitution relationship was similar among these trials and the one in Figure 2. These results indicate that whether DIs increased or decreased first did not affect the morphology of restitution relationship because both show dual trajectories.

To verify reproducibility, we repeated oscillatory DI trials in four animals. All trials had a mean DI of 400 ms, a period of 100 beats, and a range of DI between 700 to 100 ms. In each trial, tissue was stimulated for two periods of oscillatory DI. Average of the restitution relationships from four animals is shown in Figure 4A as mean values with standard error represented by vertical bars. In order to account for differences in baseline APDs among animals, APDs were normalized within each animal with respect to the maximum and minimum APD recorded in each trial. The normalized APDs were averaged across animals. Figure 4A shows hysteresis type morphology of restitution relationship, similar to the results in Figures 2 and 3.

In order to determine whether rate with which the DIs changed affected restitution relationship, we used oscillatory DIs with different periods. Figure 4B shows restitution relationship obtained during two trials of oscillatory DIs. In both trials, mean and range of DIs were same (400 ms and 700 to 100 ms); however, the periods of oscillation were 100 and 200 beats. Figure 4B shows that the restitution relationship was similar for both periods of oscillation of DIs.

The DI APD relationship that we explored using oscillatory DI with mean and a range equal to 400 ms and 700 to 100 ms corresponded to a range of activation rates between 60 and 180 activations per minute. In order to determine the restitution relationship for higher activation rates, we conducted oscillatory DI trials with a mean DI of 80 ms and a range between 150 and 20 ms. Results from a
trial of these shorter oscillatory DI are shown in Figure 5. We paced the tissue at constant DI of 80 ms for 20 beats and then changed the DIs in an oscillatory pattern with a period of 200 beats (Figure 5A). The APDs (Figure 5B) decreased during pacing at constant DI. During oscillatory DIs, the change in APD was bicomponent; there was a monotonic decrease in APD superimposed on an oscillatory change. The restitution relationship displayed hysteresis type looping with each trajectory displaced downward due to monotonic decrease in APD. We removed the effects of monotonic decrease in APDs by fitting a linear function to the nadirs of APD curve, as shown in Figure 5B. We then subtracted the linear function from APDs. The relationship between the resulting "corrected" APDs and oscillatory DIs (Figure 5C) showed hysteresis type behavior, similar to that observed during oscillatory DIs with longer means. The cycle lengths that resulted during this trial are shown in Figure 5D. Figure 5D shows that the tissue was activated at considerably faster rates during this trial, ranging between 160 to 350 activations per minute.

The relationship between an APD and preceding DI is governed by restitution and memory effects. As described by Koller et al., Fox et al., and Hall et al., memory effects can be modeled as accumulative and dissipative, during activated and recovered phases of transmembrane potential. A similar construct for memory suggests that during oscillatory change in DI, there would be accumulation of memory during upswing of DI and dissipation during downswings. In order to explore the restitution relationship when cumulative effects of memory would be expected to be minimal, we paced the tissue using a sequence where the DIs were random and uncorrelated. The random DI sequence had a mean value of 80 ms and a range between 160 and 25 ms. Probability distribution of DIs between these limits (160 to 25) was uniform, ie, the DI preceding any activation had an equal probability of being short or long. We paced the tissue at constant DI of 80 ms for 50 beats, followed by random DIs. Random DIs and resulting APDs from a trial are shown in Figures 6A and 6B, resulting cycle lengths, ie, $APD_n \div DI_{n-1}$, are shown in Figure 6D. Figure 6D shows that the tissue activated at rates between 160 and 300 activations per minute. Restitution, in Figure 6C, shows that the relationship was not unimodal, ie, there was no one DI to one APD relationship. Instead of well-defined trajectories, as observed during oscillatory changes in DI, we obtained a cluster of points. Results in Figure 6C show, that for a single value of DI, eg, DI=100 ms, the APDs were
dispersed in a range from about 180 to 200 ms. The overall tilt or slope of the cluster of points was shallower than 1.

In two animals, we computed restitution relationship using standard and dynamic protocols. Details of pacing protocols used to assess these two restitution relationships were similar to those described by Koller et al. After analysis of data collected using these protocols was completed offline, in a subsequent animal, we determined restitution relationship when the DIs changed linearly in sequence. That is, we explored restitution relationship when the DI axis (abscissa) was traversed sequentially in time, without the steady state effects produced by pacing at 20 or 50 beats at constant cycle lengths S1-S1. For linear change in DIs, first, we paced the tissue at a constant DI of 140 ms for 20 beats. After 20 activations, DI was changed for each successive activation (Figure 1B), values of these DIs were those that resulted during the standard protocol. At an APD90 threshold, restitution of conduction velocity can make it difficult to pace using very short, near zero, DIs (unless the threshold is changed to APDX, X). Therefore, we used 20 ms as the shortest DI; once this value of DI was obtained, successive values of DIs increased until we reached the initial value of longest DI. These DIs are shown in Figure 7A. Resulting APDs (Figure 7B) showed a monotonic decrease superimposed on a decrease and increase in APD corresponding to the change in DI. Figure 7B also shows presence of APD alternans during the first 20 beats when the DIs were constant. The restitution relationship that resulted during this linear and sequential change in DI is shown in Figure 7C. The solid lines in Figure 7C show that the restitution relationship during sequential activation resulted in APDs that were longer than those that resulted during either standard or dynamic protocols. In addition, slopes of the two trajectories of restitution curves were shallower than slopes of either standard or dynamic curves. We eliminated the monotonic decrease in APD, using a scheme similar to that used for data shown in Figure 5, and adjusted for the difference in the starting value of APD between sequential pacing and standard protocol. The resulting restitution relationship (inset in Figure 7C) shows that even after the monotonic decrease in APDs and a higher initial value of APD was factored out, the restitution relationship during sequential activation still resulted in longer APDs and a shallower slope. As expected from previous results of Koller et al., the restitution obtained using the dynamic protocol showed markedly steeper relationship for DIs shorter than about 60 ms than the relationships obtained using standard protocol or sequential pacing.

Discussion

We explored the relationship between DI and APD when DIs changed sequentially. Motivation in our study for using sequential change in DI, instead in pacing cycle length, was based on the following: as illustrated schematically in Figure 8, restitution relationship between DI and APD is used to explain and predict changes in dynamics of activation via iteration of a perturbation around an operating point (eg, asterisk in Figure 8A, produced at the intersection of restitution relationship and a line that describes cycle length, CL). The restitution hypothesis states that a transient change in DI (disturbance) can oscillate (alternans), degenerate into block, or “spiral back” to operating point depending on slope of restitution around the operating point. Figure 8A illustrates the process with a slope of restitution of 1, which is a case of transition between stable and unstable behavior. A perturbation such as an abrupt shortening of DI (point 1 on abscissa) results in a stable oscillation between long-short APD and DI (1, 3... and 2, 4...). If the slope >1, these
Hysteresis in restitution of APD has been previously reported by Elharrar et al. Using an abrupt change in CL, they observed that when CL changed from 500 to 1500 ms, increase in APD was slower and more distributed over successive beats, with a steady state reached in about 50 beats (Figure 4 of Elharrar et al\textsuperscript{21}). In contrast, when CL decreased from 1500 to 500 ms, decrease in APD was more rapid in the first premature beat, distributed less over successive beats, and reached a steady state in 20 beats. These investigators used the relationship between restitution and steady state activation curves to predict this hysteresis in adaptation to a new cycle length. Results in Figure 2D demonstrate this hysteresis type behavior more directly. Hall et al\textsuperscript{16} in bullfrog cardiac muscle, and Yehia et al\textsuperscript{23} in isolated rabbit ventricular cells, also observed hysteresis type bimodal trajectory of APD (eg, Figures 2 and 4 in Hall et al\textsuperscript{16}). It is noteworthy, however, that in these studies the bimodal trajectories resulted during a bifurcation in activation from a 1:1 to 2:1 type behavior. Our results clearly demonstrate hysteresis in restitution during 1:1 region of activation.

Yehia et al\textsuperscript{23} used iteration of a finite-difference equation based on restitution to predict bistability and 1:1 to 2:1 transition in single isolated cells. Our results, however, suggest that use of a unimodal restitution relationship is unlikely to adequately describe activation dynamics during 1:1 and 2:2 behavior. This interpretation is further supported by results of Banville and Gray\textsuperscript{24} who showed that there was no direct link between restitution relationship and activation dynamics in intact perfused hearts. A different approach to visualization of multimodal restitution was recently proposed by Tolkacheva et al.\textsuperscript{27} In a simulation study, they proposed a modification of dynamic protocol to obtain a multidimensional restitution surface that permits visualization of different aspects of restitution such as steady state and transient responses. Results obtained using the protocol proposed by these investigators should provide additional insight into the dynamics of restitution.

A memory model developed previously by others\textsuperscript{15,13,16,25,26} considers memory to accumulate and dissipate during depolarized and repolarized states and predicts that an APD following a long APD would be longer than that predicted by preceding DI and vice versa.\textsuperscript{17,21} We hypothesize that differences in rates of change in APD during increasing and decreasing phases of DI, in Figures 2 through 4, reflect effects of memory. It is likely that the continual increase in APD for several beats even after DIs have started decreasing (Figures 2B and 2D) is a result of accumulation of memory due to increasing APDs during preceding activations. If this change is indeed due to memory, then our results suggest that this effect is more pronounced when the APDs are increasing than when they are decreasing.

In Figure 8B, we consider the effect that hysteresis may have on evolution of a perturbation. As in Figure 8A, we again consider trajectory 1 to have a slope of 1. A shortening of DI (1, abscissa) produced by perturbation of operating point (asterisk) results in a short APD (2, ordinate) predicted using trajectory 1 (decreasing DI). The
increased DI that precedes subsequent activation (2, abscissa), projected on trajectory 2 (increasing DI), predicts next APD (3, ordinate). Trajectory 2 being below trajectory 1, however, APD for beat 3 will be shorter than that for beat 1. Absence of hysteresis, as in Figure 8A, predicts APD for beat 3 to be as long as that for beat 1, resulting in stable APD alternans. Continued iteration in Figure 8B shows a complex pattern of APD and DI around intersections of new cycle length line and the trajectories. Amplitudes of these changes, however, are smaller than those in Figure 8A. If the slope of trajectories >1, it is possible to get unstable evolution of a perturbation even with hysteresis; iterations in Figure 8, however, suggest that memory tends to buffer activation instability. This interpretation is consistent with that of a study by Chialvo et al,20 which was one of the first to demonstrate that memory suppressed complex dynamics by flattening restitution.

Consistent with the observations of Elharrar et al21 and Watanabe et al22 for shorter DIs we observed a bicomponent change in APDs, ie, a monotonic decrease in APD, even when DIs were constant. Interestingly, Figure 7A shows that alternans of APD (although of small amplitude) resulted even when there were no (or very minimal) changes in DI. We do not think that these alternans resulted from amplification of small changes in DI as the restitution slope in this region of DIs was less than 1. The observation that alternans can result independent of restitution mechanism, ie, when there are no changes in DI, is consistent with the finding of Saitoh et al.28 Saitoh et al used interpolated beats to show that alternans could not be suppressed in ventricular tissue even if they could make two successive beats to have same preceding DI. The use of explicit control of DI in our study permits us to more directly demonstrate behavior of APD alternans independent of changes in DIs.

We consider that a corollary of the memory accumulation and dissipation model is that when the changes in preceding APD (and DI) are least correlated with succeeding APD (and DI), the resulting restitution relationship will have minimal effect of cumulative memory. When effects of previous activation history were minimal, the relationship between DI and APD (Figure 6C) was multimodal and shallow (slope less than 1). We compared restitution relationships quantified using standard, dynamic, and our feedback protocol. Linear and sequential change in DIs (Figure 7) also resulted in shallower restitution, more similar to that obtained using the standard protocol. It is interesting to note that restitution quantified using random DIs and linearly changing DIs overlapped in the APD-DI space (Figure 6C). It is possible that short duration of linear change in DIs (for about 10 beats) did not permit significant accumulation of effects of memory and thus produced a restitution relationship similar to that seen during random DI.

Our study has limitations. We did not to explore restitution relationships for very short DIs, ie, <20 ms. In comparing results from our study with those in the literature, it should be noted, however, that some previous investigations10,12 have used APD90, resulting in shorter DIs than those obtained using APD50. Nevertheless, the fastest rates of activation in our study, about 300 activations per minute, are slower than typical mean activation rates observed in dogs during first minute or so of ventricular fibrillation. We consider, therefore, that results from our study do not address the role of restitution in maintenance of fibrillation. Our results are more relevant to explore the role of restitution in activation instability during rhythmic activity such as genesis of alternans. We did not obtain information about restitution relationship during compromised cellular function such as during ischemia. Our approach of obtaining data from nonischemic tissue is similar to that utilized in the large number of studies reported in literature, making comparison of our results with those from earlier studies valid. However, whether multimodal restitution relationship exists in case of diseased, ischemic hearts, is unclear.

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References


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