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Hysteresis in DI Independent Mechanisms in Threshold for Transition between 1:1 and 2:2 Rhythms in Pigs*

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Abstract— **Previous studies have shown hysteresis in transition from 1:1 to 2:2 rhythms in action potential durations (APD) during decreasing and increasing cycle length pacing. As cycle lengths were constant, when alternans of APD occurred, so did of preceding diastolic intervals (DIs), which engaged the restitution mechanism, i.e. dependence of APD on preceding DI, making it impossible to determine whether the hysteresis originates from the DI independent or dependent mechanism. By using pacing with explicit control of DI, in the present study we investigated whether hysteresis in activation threshold exists in DI independent mechanism of alternans of APD. Transmembrane potentials were recorded from the left ventricles of 6 farm pigs, and the state transition was obtained from two pacing protocols where DIs decreased and increased in steps of 10 msec between 50 and 20 msec with 30 (15) beats at each DI level. Results showed hysteresis: for the 30 (15) beats protocol, onset and termination of alternans occurred at 27 (33) and 47 (49) msec DIs; average alternans amplitude was 22 (13) and 26 (15) msec for decreasing and increasing DIs. Because constant DI pacing was used, restitution dependent effect was eliminated, therefore, observation of hysteresis in our results suggests that restitution independent mechanisms such as activation memory also contribute to the hysteresis in this state transition.**

I. Introduction

Alternans of action potential duration (APD), also known as 2:2 rhythm which is beat-to-beat alternation of APDs, is believed to be mechanistically linked to ventricular fibrillation (VF) [1-3]. As activation rate increases, often there is a transition from 1:1 rhythm (no alternans) to either 2:1 (block) or 2:2 (alternans) rhythm. Both, block and alternans are thought to lead to electrical instability and arrhythmia. Restitution of APD, i.e. dependence of an APD on the preceding diastolic interval (DI), is a widely used mechanism to explain the initiation of alternans and arrhythmia [1, 3]. Briefly, it is hypothesized that at faster activation rates, the slope of restitution relationship becomes greater than 1, which leads to magnification of changes in alternating APDs from beat to beat, eventually resulting in failure of activation, i.e. block.

Previous studies have reported hysteresis in the transition between 1:1 rhythm and 2:1 or 2:2 rhythms [4-6], the transition from 2:1 or 2:2 rhythms back to 1:1 rhythm occurred at longer cycle length than the transition from 1:1 to 2:1 or 2:2 rhythm. In our previous study [7], we reported that alternans of APD could occur independent of changes in preceding DI, suggesting that other mechanisms besides restitution play an important role in occurrence of alternans. In the previous studies investigating hysteresis in the state transition [4-6], because pacing was performed using constant cycle lengths, when APDs changed, the preceding DIs changed too, making it impossible to determine whether hysteresis also exists in the restitution independent mechanism of alternans. We used a novel pacing protocol that permits explicit control of DI and recorded transmembrane potentials in pig ventricles. Our results showed hysteresis in the threshold for state transition even when DIs preceding each APD were constant. These results suggest that the hysteresis in the threshold for state transition may originate in the DI independent mechanisms of alternans.

II. Methods

All studies were approved by the Institutional Animal Care and Use Committee at the University of Kentucky. Data were collected from 6 farm pigs (18-21 kg). Animals were anesthetized first with a combination of Telazol (4-8 mg/kg), Ketamine (2-4 mg/kg), and Xylazine (2-4 mg/kg), followed by thiopental sodium (Pentothal, 10-11 mg/kg, IV). After anesthesia, hearts were quickly excised and placed in chilled Tyrode's solution. Tissue samples, approximately 20x10x5 mm, were excised from the mid to apical anterior-lateral region of left ventricle and were mounted in a plastic tissue chamber. The samples were superfused with gassed (95% O2 plus 5% CO2), warmed (36±1°C), modified Tyrode's solution. The composition of the Tyrode's solution (in mmol/L) was: 0.5 MgCl2, 0.9 NaH2PO4, 2.0 CaCl2, 137.0 NaCl, 4.0 KCl and 5.5 Glucose. NaHCO3 was added to the gassed solution until the pH of the solution was 7.35±0.05.

All tissues were paced at basic cycle length of 500 msec for at least 60 minutes before recording any data. The pacing stimuli were biphasic, duration 3 msec, delivered by bipolar platinum-iridium electrodes. Glass capillary microelectrodes filled with 3 mol KCl were used for recording transmembrane potentials (TMPs) from both the endocardial and epicardial side of the tissue samples. The TMPs were digitized using a commercial data acquisition system at a rate of 10000 samples/second. After low-pass filtering the digitized TMPs with a cut-off frequency of 1000 Hz, APDs were computed as the duration from the start of an action potential to the time point when the membrane repolarized to 90 % of the amplitude of the action potential. All analysis of TMPs recorded from the data acquisition system was

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conducted using a custom developed program written in Matlab (Mathworks, Natick, MA).

Two feedback-based pacing protocols were used. In both protocols, DIs decreased first from 50 to 20 msec in steps of 10 msec, and then increased back to 50 msec with the same step change. In one protocol, the tissue was paced for 30 beats at each level of DI, while in the other, there were 15 beats at each DI level. Figure 1 shows an example of the pacing protocol. We note an important difference between our and previous studies, we used a real-time control system to time the pacing stimuli at predefined intervals, i.e. the value of DI for the control. Briefly, a customized Labview program was used to detect the end of an AP, i.e. 90% repolarization, and from then the program waited a pre-set time interval equal to the target DI before delivering the next stimulus. Therefore, unlike cycle lengths being the independent variable in previous studies, we independently controlled DI in this study. All analyses were conducted on data that were collected by the stand-alone acquisition system and processed off line. Therefore, the DIs computed off line differed slightly from the target DI for the control. We note that for the purposes of our study, it was not very important that a particular value of DI was met, rather, it was keeping DI invariant from one activation to next. The above approach ensured that the DIs that were computed off-line and are reported in the results section were the actual DIs experienced by the myocyte from which TMPs were obtained. As figure 1B shows, due to conduction delays, the resulting DIs were slightly different than the target DIs. However, the figure also shows that the important criterion of minimizing beat by beat changes in DI was met. Alternans of APD was considered to occur when beat-to-beat difference in APDs (i.e. in long-short-long or short-long-short pattern) was ≥ 4 msec for at least 5 consecutive beats. The criterion of using 4 msec to define occurrence of alternans is the same as that used by Laurita et al [8]. When alternans occurred, the following parameters were computed, which were then averaged: 1) the rate threshold of alternans onset and termination, i.e. the value of DI when alternans started, and the value of DI when alternans stopped; 2) the average amplitude of alternans (absolute value of the average difference between long and short APD) when DI was decreasing and when DI was increasing; 3) the average APD and the average cycle length at each level of DI. Differences between the measures of onset and termination of alternans, or between the descending and ascending phase of DI were tested for statistical differences using paired t-test. Significant difference was accepted at $p \leq 0.05$.

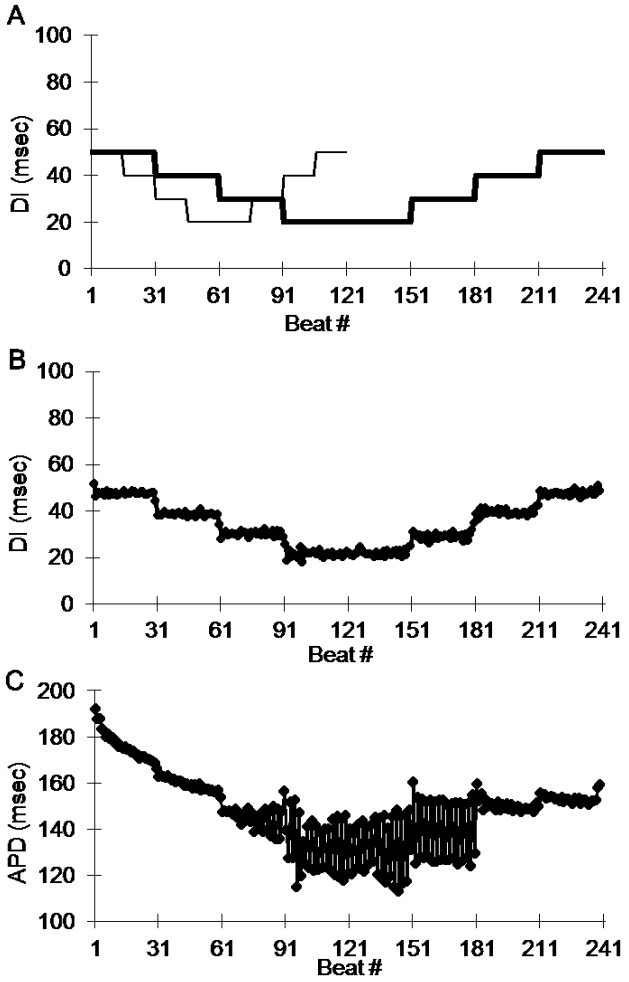

Fig. 1 Illustration of stepwise constant DI protocol. A: The target trace of DI for a 30 beats (thick line) at each level of DI, and for a 15 beats (thin line) at each level of DI protocols. B: The averaged DI trace from trials with 30 beats at each level of DI. C: The averaged trace of APD resulting from the DI protocol shown in panel B.

III. Results

In the protocol with 30 beats at each level, alternans was observed in 4 out of the 6 pigs. This incidence of APD alternans is consistent with results of previous studies in pigs [9]. In the other 2 pigs, decrease of DI lead to 2:1 block, with no transition to 2:2 rhythm. In all trials when alternans occurred, hysteresis between the onset and termination of alternans was observed, transition from 2:2 rhythm, i.e. alternans of APD, back to 1:1 rhythm occurred at longer DI than the transition from 1:1 to 2:2 rhythm, i.e. initiation of alternans. Figure 2 shows an example of the hysteresis of alternans from one trial. The figure shows that alternans occurred at DI equal to 34 msec during the DI descending phase, and persisted until DI increased to 53 msec. On average, alternans started at 27±6 msec DI during the descending phase and persisted until 47±6 msec DI during the ascending phase. The cycle lengths corresponding to these thresholds were 162±32 msec and 182±32 msec (both changes had $p<0.05$). Figure 3A shows a plot of averaged APD alternans for the 30 beats DI protocol. The figure shows that at the same level of DI, the average amplitude of alternans was greater during DI ascending phase than the DI descending phase. The average amplitude of alternans was 22±9 msec when DIs were decreasing, and was 26±11 msec when DIs were increasing. The average area of the hysteresis loop was 197 $msec^2$. A summary of these results is shown in table I.

During the protocol with 15 beats at each level of DI, alternans occurred in 3 out of 6 pigs. Similar to the protocol with 30 beats, hysteresis was consistently observed whenever alternans occurred. Overall, the difference between onset and termination threshold for alternans was smaller than the 30

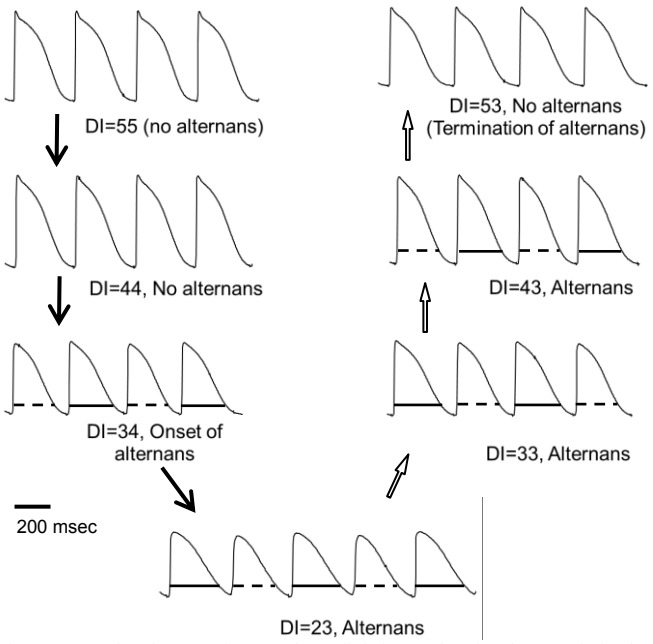

Fig. 2 Example of TMPs from endocardial tissue showing hysteresis in the state transition. The figure shows that during the descending phase of DI (black arrows), the transition from 1:1 rhythm to 2:2 (alternans) rhythm, i.e. onset of alternans, occurred at DI = 34 msec, with an amplitude of 5~6 msec. The APD alternans persisted at DI = 23 msec, with an increased amplitude of about 25 msec. During DI ascending phase (white arrows), alternans of APD persisted at DI = 33 and 43 msec with decreased amplitudes of 8~9 msec and 5~6 msec respectively, and terminated (transition back to 1:1 rhythm) at DI = 53 msec. Solid and dashed lines on the AP traces represent long and short APDs, respectively.

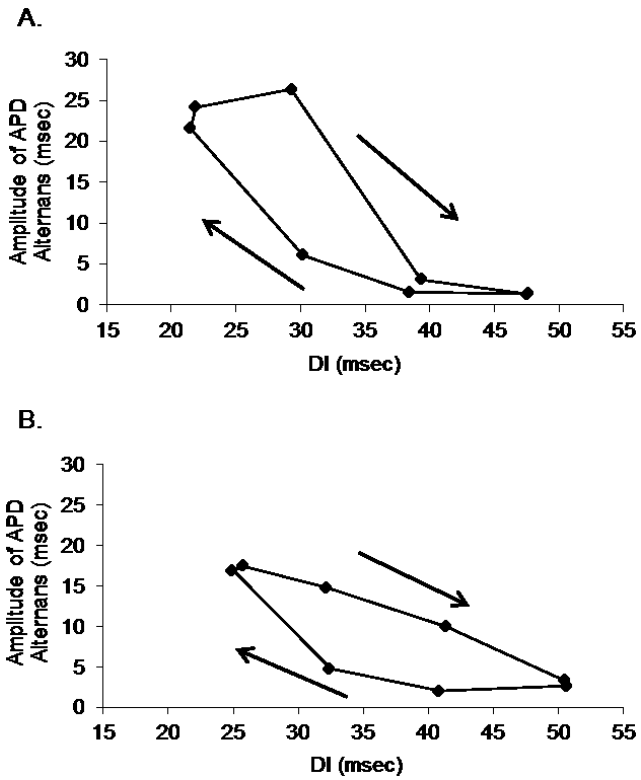

Fig. 3 Averaged amplitude of alternans of APD for 30 beats (A) and 15 beats (B) DI protocols. Each black dot represents mean amplitude of APD alternation at that level of DI. Arrows refer to directions of changes in DI. The figure indicates that at the same level of DI, the amplitude of APD alternans was larger at DI ascending phase than DI descending phase during both protocols.

beats protocol, and significant difference was obtained only between the DIs at the initiation and termination of alternans. On average, alternans started at 33±6 msec DI (175±37 msec cycle length) and stopped at 49±5 msec DI (194±38 msec cycle length). Same as during the 30 beats protocol, figure3B shows an averaged hysteresis plot during 15 beats protocol. The alternans amplitude, although generally smaller than that during the 30 beats protocol, was still larger during DI ascending phase compared to descending DIs. The average amplitude of alternans was 13±3 msec when DIs were decreasing, and was 15±4 msec when DIs were increasing. Compared to the 30 beats protocol, the loop area was smaller (160 msec^2). These results are summarized in table I.

IV. Discussion

In the current study, we eliminated the restitution-dependent mechanism of alternans by explicitly controlling the DI using a feedback-based protocol, and still observed hysteresis in the transition from 1:1 to 2:2 (alternans) and back to 1:1 rhythms. These results suggest that the mechanisms underlying this hysteresis may be distinct from the DI dependent restitution of APD.

Previous studies [5, 6] have reported hysteresis in the transition between 1:1 and 2:2 rhythms. A possible explanation for the hysteresis that is observed during constant cycle length pacing can be visualized based on the restitution hypothesis as follows: The increase in activation rate towards shorter cycle lengths initiates alternans by some, as yet unknown, mechanism that is dependent only on rate of activation. Once alternans are initiated, since the cycle length is constant, alternans of APD are always accompanied with alternans of DI with equal amplitude of alternation. Therefore, when the activation rate then decreases back to below the onset rate of alternans, APD may not stop alternating as the APD would still be affected by the alternation in preceding DI, i.e. by the restitution mechanism. As activation rate decreases further, the slope of restitution relationship becomes shallower, eventually diminishing the amplitude of alternation of DI as well as APD, resulting in a steady state, i.e. 1:1 rhythm.

TABLE I. Summary of Hysteresis Results for stepwise DI protocols

Beats at each Level of DI	DI (msec)		CL (msec)		Average Alternans Amplitude (msec)	
	Onset	*Termination*	*Onset*	*Termination*	*DI Decrease*	*DI Increase*
30 (N=4)	27±6*	47±6*	162±32‡	182±32‡	22±9	26±11
15 (N=3)	33±6†	49±5†	175±37	194±38	13±3	15±4

Onset (termination) DI/CL for alternans: the value of DI/cycle length (Mean±SEM, in milliseconds) when alternans of APD started (terminated). The table shows that in both protocols, APD alternans terminated at longer DIs and CLs than the initiation. */‡ indicate significant differences between the onset and termination DI/CL in the 30 beats protocol; † indicates significant difference between the onset of termination DI in the 15 beats protocol.)

However, in the current study, the DIs were explicitly controlled at a constant value at each step, therefore the contribution of DI dependent restitution mechanism to alternans was eliminated. Therefore, our results indicate that there must exist mechanisms other than restitution that results in the hysteresis. Walker et al. [5] observed hysteresis in both, threshold of alternans of APD and calcium currents, suggesting that cardiac memory, i.e. the dependence of APD on the electrical history in the past several seconds, as an intrinsic property of cardiac myocytes and an important mechanism contributing to the hysteresis effect. At the same DI (or same cycle length in their case), we also observed larger APD alternans when DI was increasing than that when DI was decreasing (figure 3) consistent with their findings. However, as stated before, a critical difference between our study and those reported previously is noteworthy: in our study we used a novel pacing protocol to eliminate beat by beat alterations in DIs preceding each activation, thus allowing us to explore hysteresis in the absence of restitution effects. In the previous studies, because cycle lengths were constant, alternans of APD was always preceded by alternating DIs, therefore, the restitution effects were also always present. Results from our previous studies [9, 10] where we used sinusoidally oscillating DIs also showed that at the same DI, the average APD was smaller when DI was increasing than that when DI was decreasing. These observations suggests an interesting possibility; Because at the same DI, cycle length was longer when DI was decreasing, if a rate of activation threshold is a mechanism of initiation of alternans, then the alternans would be expected to terminate at longer DI than the onset threshold of DI, which is consistent with the results observed in our study. However, according to this hypothesized mechanism by itself, the cycle length at the termination of alternans should be the same as the cycle length at the onset of alternans, which is not consistent with the results of the current study (table I). Therefore some other mechanism(s) must come into play for the termination of alternans at longer cycle lengths. Compared to the 30 beats protocol, the difference between the DIs at the onset and termination of alternans and the loop area were smaller (16 msec vs 20 msec, and 160 vs 197 $msec^2$) in the 15 beats protocol. If cardiac memory were to affect hysteresis in alternans, as hypothesized previously, a possible explanation could be that in the 15 beats protocol, memory is less accumulated, at every level of DI, compared to the 30 beats protocol. Thus, even though APD alternans persisted after the cycle length reached the rate threshold, it would diminish faster as a result of less accumulated memory. The smaller difference between the amplitude of alternans during descending and ascending phases of DI could also be a result of the same phenomenon. The exact role of memory in the above scenario, however, is speculative and needs further exploration. The APD accommodation, due to increased pacing rate as seen in figure 1C is also a possible factor contributing to the hysteresis, but if so, the mechanism is unclear. Nevertheless, the hysteresis phenomenon is the reason why once alternans is initiated, it persists even when activation rates increase and decrease around an operating mean value, a fluctuation that is often observed clinically.

V. CONCLUSION

In conclusion, our results show that even during DI-independent activation, hysteresis in thresholds for alternans onset and termination rate exists. Because our use of novel pacing protocol eliminated the effects of restitution mechanism, the observed hysteresis effect in state transition suggests that this hysteresis exists in mechanisms other than restitution.

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REFERENCES

[1] I. Banville, N. Chattipakorn, and R. A. Gray, "Restitution dynamics during pacing and arrhythmias in isolated pig hearts," *J Cardiovasc Electrophysiol,* vol. 15, pp. 455-63, Apr 2004.

[2] J. J. Fox, J. L. McHarg, and R. F. Gilmour, Jr., "Ionic mechanism of electrical alternans," *Am J Physiol Heart Circ Physiol,* vol. 282, pp. H516-30, Feb 2002.

[3] M. L. Koller, M. L. Riccio, and R. F. Gilmour, Jr., "Dynamic restitution of action potential duration during electrical alternans and ventricular fibrillation," *Am J Physiol,* vol. 275, pp. H1635-42, Nov 1998.

[4] G. M. Hall, S. Bahar, and D. J. Gauthier, "Prevalence of rate-dependent behaviors in cardiac muscle," *Physical Review Letters,* vol. 82, pp. 2995-2998, Apr 5 1999.

[5] M. L. Walker, X. Wan, G. E. Kirsch, and D. S. Rosenbaum, "Hysteresis effect implicates calcium cycling as a mechanism of repolarization alternans," *Circulation,* vol. 108, pp. 2704-9, Nov 25 2003.

[6] A. R. Yehia, D. Jeandupeux, F. Alonso, and M. R. Guevara, "Hysteresis and bistability in the direct transition from 1:1 to 2:1 rhythm in periodically driven single ventricular cells," *Chaos,* vol. 9, pp. 916-931, Dec 1999.

[7] R. Wu and A. Patwardhan, "Mechanism of repolarization alternans has restitution of action potential duration dependent and independent components," *J Cardiovasc Electrophysiol,* vol. 17, pp. 87-93, Jan 2006.

[8] E. J. Pruvot, R. P. Katra, D. S. Rosenbaum, and K. R. Laurita, "Role of calcium cycling versus restitution in the mechanism of repolarization alternans," *Circ Res,* vol. 94, pp. 1083-90, Apr 30 2004.

[9] K. M. Guzman, L. Jing, and A. Patwardhan, "Effects of changes in the L-type calcium current on hysteresis in restitution of action potential duration," *Pacing Clin Electrophysiol,* vol. 33, pp. 451-9, Apr 2010.

[10] L. Jing, S. Chourasia, and A. Patwardhan, "Heterogeneous memory in restitution of action potential duration in pig ventricles," *J Electrocardiol,* vol. 43, pp. 425-432, September - October.