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Effect of Rapid Delayed Rectifier Current on Hysteresis in Restitution of Action Potential Duration in Swine *

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Abstract*—Electrical stability in the heart depends on two important factors; restitution of action potential duration (APD) and memory. Repolarization currents play an important role in determining APD and also affect memory. We determined the effects of blocking the rapid component of the delayed rectifier (I_{Kr}) on a quantifiable measure of memory, i.e. hysteresis in restitution of APD, in swine. Transmembrane potentials were recorded from right ventricular endocardial tissues. Two pacing protocols with explicit control of diastolic interval (DI) were used to change DIs in a sequential and sinusoidal pattern to quantify hysteresis in restitution of APD. E-4031 (5 μM/L) was used to block I_{Kr}. Measures of memory and restitution were quantified by calculating hysteresis loop thickness, area, overall tilt, and maximum and minimum delays between DIs and APDs. Blocking I_{Kr} with E-4031 increased the baseline APD, loop thickness, area, and tilt ($p<0.05$). However, loop thickness did not increase beyond what could be predicted by the increase in baseline APD after block of I_{Kr}. The substantial change in APD after blocking I_{Kr} suggests that this current plays a major role in repolarization in the swine. Loop thickness is a measure of memory, an increase in which is predicted by theory to reduce instability in activation. In our study, the substantial increase in loop thickness could be accounted for by an equally substantial increase in APD and therefore does not necessarily indicate increased memory after blocking I_{Kr}. Our results also suggest that factors based on restitution and memory need to be considered in the context of operating point, i.e. baseline APD, when they are used to explore mechanisms that affect electrical stability in the heart.

I. Introduction

Restitution of action potential duration (APD) and memory are known to play a critical role in stability of electrical activation and predisposition to arrhythmia. Restitution here refers to dependence of an APD on its preceding diastolic interval (DI) and memory refers to dependence of an APD on previous APDs occurring over several seconds. Restitution in APD shows hysteresis when the DI changes in a sequential oscillatory pattern [1]. The parameters of hysteresis provide a quantifiable measure of memory, which theoretically has been shown to dampen activation instability [2]. Drug induced reduction or block of repolarization currents, manifested as QT interval prolongation of the electrocardiogram, increases the risk of ventricular tachyarrhythmia which can lead to sudden cardiac death [3]. Drug related prolongation of QT interval is also known as *acquired long QT syndrome* (LQTS). The Food and Drug Administration (FDA) places great emphasis on pre-clinical testing of many new drugs for potential proarrhythmic effects. Their criteria mainly include in vitro *Human ether-a-go-go-related* gene product (hERG) testing [4]. The hERG channel mediates I_{Kr} and is mostly the cause of drug induced acquired LQTS [4]. In this study, therefore, we explored the effects of this key repolarization current, I_{Kr}, on hysteresis in restitution of APD in swine, which is a widely used animal model to study the link between restitution and arrhythmia [2, 5, 6].

Our results showed that blocking I_{Kr} increased the loop thickness but this increase could be accounted by an equally large increase in baseline APD. We have previously shown that a change in loop thickness can be used as a measure of memory [2]. Therefore, our results also suggest that indexes that predict stability dynamics, such as the loop thickness, need to be considered in the context of baseline APD, if substantial changes in baseline APD are observed such as that seen after blocking I_{Kr}.

II. Methods

Experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky. Narrow strips of tissues, obtained from the right ventricle of swine, were placed in a plastic chamber and superfused with modified Tyrode's solution bubbled with 95% O_2 and 5% CO_2 gas mixture [2]. The temperature and pH of the perfusate were maintained at 36 ± 1°C and 7.3 ± 0.05. Transmembrane potentials (TMP) from the endocardial side of tissue were recorded using glass microelectrodes filled with 3M KCl solution. A stand-alone computer with a commercial data acquisition system was used to digitize and record the TMP at a sampling rate of 10,000 samples / sec. A custom made program in LABVIEW was used to explicitly control the DIs using a feedback-based pacing protocol [1]. To determine hysteresis in restitution, the tissues were paced using two protocols. Both protocols consisted of DI control lasting 220 beats. The first 20 beats consisted of constant DI followed by 2 cycles of a sinusoidal pattern of DI with a period of 100 beats. In both protocols, the constant DI values were equal to the mean values of the sinusoidal oscillation. In the first protocol, the DI sequence had 400 ms as central

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value of DI with ±300 ms oscillation around the central value. The second protocol had 150 ms as central value of DI with a ±140 ms oscillation around the center. An example of one of the DI protocol is shown in Figure 1. We waited about 5 minutes between protocols; the tissue was paced at constant cycle lengths during this period. After obtaining the control recordings, we used 5 μM/L of E-4031 (TOCRIS bioscience) to block the rapidly activating delayed rectifier channel, I_{Kr}. Analyses of data were performed offline using Matlab (MathWorks, Natick, MA, USA). Before analyses, all data were digitally filtered by using a low-pass filter with a cutoff frequency of 500 Hz. The APDs and DIs were calculated from the recorded TMP, using a constant threshold defined at 90% repolarization of action potential.

Figure 1 shows an example of the sinusoidal DI protocol used and the obtained hysteresis in restitution of APD. From the APD versus DI curve, we quantified the following parameters of hysteresis: thickness of loop, overall tilt, maximum delay, minimum delay and area under loop. As defined previously [2], loop thickness was measured as the difference between APDs at mean values of oscillatory DI during increasing and decreasing DI trajectories. Tilt was measured as the ratio of differences of maximum and minimum APD and their corresponding DI values. Maximum delay was measured as the delay in beats, between the maximum values of APD and DI during the sinusoidal change. Minimum delay was measured as the delay in beats, between the minimum values of APD and DI during the sinusoidal change. Area under loop was defined as the area

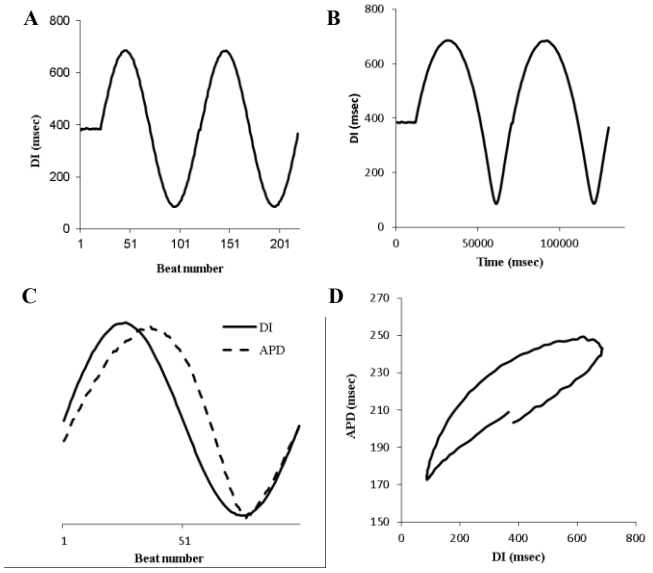

Fig. 1 (A) Example of a sinusoidal DI protocol of 220 beats with central DI value of 400 ms and a ±300 ms oscillation around the central value. (B) Variation of DI with time. (C) The second cycle of the sinusoidal DI protocol (solid line) shown in (A) with the corresponding APDs (dashed line). The APDs are scaled and offset vertically to clearly show the measures of max delay and min delay. (D) The restitution relationship obtained from DI sequence in (A), shown for the second cycle of the sinusoidal DI protocol.

under the hysteresis curves. We used the second cycle of the DI protocol to calculate the hysteresis parameters to minimize the effects of APD adaptation, caused due to a switch to constant DI pacing from cycle length pacing. However in some cases, where the control of DI during the second cycle was not as effective as that during the first cycle, the data from first cycle were used to calculate the hysteresis parameters. Data obtained from multiple trials of the same protocol were first averaged within each animal before averaging across animals. To test the statistical significance between the control and the drug groups, we used the student's paired t-test. Differences were considered significant at $p \leq 0.05$.

Hysteresis parameters were normalized to baseline APD based on results obtained from simulations using the Iyer-Mazhari-Winslow (IMW) model developed for the human ventricular myocyte [7].

III. Results

After administrating E-4031, the average APDs (n=5) changed from 213 ms to 522 ms, a 145% increase. Figure 2 shows examples of action potentials recorded during constant cycle length pacing, pre and post-block I_{Kr}. The two traces in figure 2 are aligned to better show the differences between them. Figure 3 shows the average restitution curves obtained from pre and post-block I_{Kr} for oscillatory DI trials with central DI value at 400 ms (n=5, 3A), and 150 ms (n=3, 3B). Due to difficulties in obtaining 1:1 control for the smaller values of DI in the 150 ms central DI protocols, especially post block, we were able to obtain data from only three animals for that protocol. In both cases, the restitution curves obtained post-block were shifted vertically to facilitate comparison with the pre-block curves. The shift was equal to the difference in the APD (produced by the block of I_{Kr}) at the central value of DI for each oscillatory protocol. Table 1 lists the average hysteresis parameters, and percentage changes before and after the administration of E-4031.

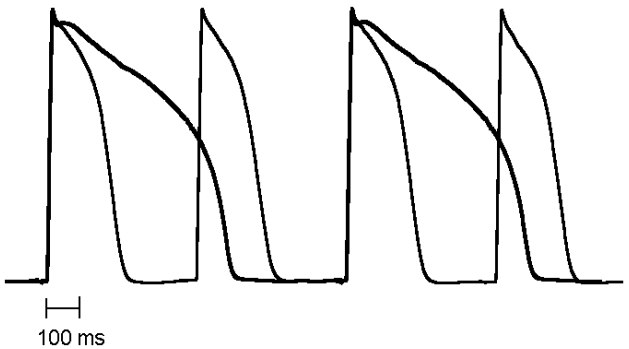

Fig. 2 Example of action potentials recorded, pre-block (thin line) and post-block (thick line) of I_{Kr}. The traces are aligned to better show the differences between them.

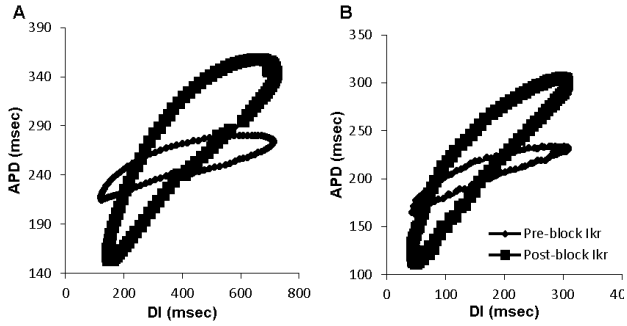

Fig. 3 Effects of blocking I_{Kr}. Shown are average restitution curves obtained pre and post block of I_{Kr} for central DI values equal to (A) 400 ms (n=5) and (B) 150 ms (n=3). The restitution curves post block of I_{Kr} were shifted vertically to facilitate comparison between the two curves.

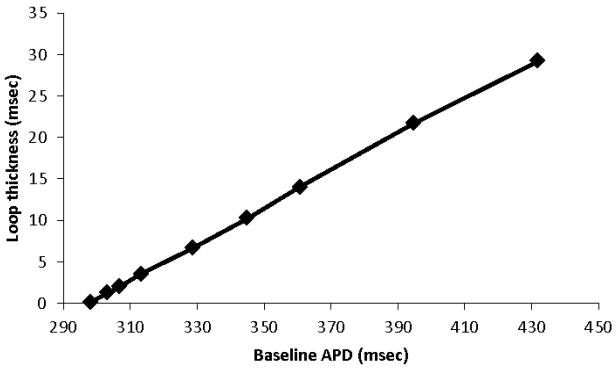

Fig 4. Simulation results from the IMW model based on human ventricular myocyte [7]. Shown is a graph of loop thickness versus baseline APD, obtained at center DI values, from several sinusoidal DI protocols with increasing center DI values and ranges. Note that the first three points, at lowest values of DI, are extrapolated from those at the right.

The table shows that except maximum delay, all other parameters increased after blocking I_{Kr}. In both protocols, the most pronounced and statistically significant changes were seen in overall tilt, loop thickness, and the area under the loop.

We have previously reported that loop thickness is larger when baseline APDs are longer [2]. Table 2 shows control, i.e. pre block, values of the average APDs at center DI values of 400 and 150 ms and the corresponding loop thickness. The increase in loop thickness of 8.29 and in APD of 43 in going from center DI 150 ms to center DI 400 ms, gives a gain of 0.19 (8.29 / 43) in units of loop thickness per unit increase in APD. Post-block of I_{Kr}, APDs increased from 213 ms to 522 ms, a 309 ms increase. Using linear extrapolation, the expected increase in loop thickness, post-block I_{Kr}, is about 59 ms (309 * 0.19). Results in Table 1 show that the increase in loop thickness post-block I_{Kr}, for both 150 ms center DI and 400 ms DI protocol was approximately 49 ms. Thus the loop thickness that was observed was less than that predicted by the increased APD. The assumption of a linear dependence of loop thickness on APD was supported by simulation results shown in figure 4, obtained using the IMW model [7]. We used this model because of a lack of adequate swine model. Figure 4 shows a graph of loop thickness versus baseline APD, obtained at the center DI values, from several sinusoidal DI protocols with increasing center DI values and ranges. The first three data points in the graph were extrapolated backwards because the human model, as compared to swine, has higher steady state APD values and does not exhibit hysteresis at lower ranges of sinusoidally varying DI. The graph in figure 4 shows a linear increase in loop thickness with increasing APD values.

IV. Discussion

The main objective of this study was to determine the effects of blocking repolarization current I_{Kr} on hysteresis in restitution of APD and thus on memory in electrical restitution in swine. E-4031 was used to block I_{Kr} in swine and the block of this current caused a marked increase in the baseline APD, and parameters of hysteresis such as tilt and loop thickness. However, the increase in loop thickness was less than that predicted by increase in baseline APD resulting from block of I_{Kr}. While the large change in baseline APDs make interpretation of the results difficult since the operating points are dramatically different, the less than predicted increase in loop thickness suggest that blocking the rapidly activating delayed rectifier channel I_{Kr} in swine does not affect memory substantially or may decrease it slightly.

A. Effect of I_{kr} manipulation on APD and measures of memory

Ionic manipulations, for example, blocking an ion channel current, often have an impact on APD. It is important to consider the effects of change in baseline APD (post manipulation) on measures of memory, such as loop thickness, because these measures are used to iterate a disturbance around an operating point and large changes in APD indicate large changes in operating point. In our study the increase in baseline APD, after adding E-4031, was substantial (about 145%) and thus the loop thickness was normalized with the change in baseline APD.

V. Limitations

We obtained data only from the endocardium of right ventricle in swine. Given the heterogeneity that exists within a heart, based on these results it is difficult to predict whether other regions in the heart would also behave similarly in response to E-4031. The concentration of E4031 that we used was based on previously reported studies in canines by Hua et al. [8] and Fish et al. [9] in which the change in the baseline APD, post E-4031, was less than 30%. However, in swine, this same concentration of I_{Kr} blocker resulted in increase in APD much larger than that reported by the studies in canines. Thus, our study also points out critical differences in expression of this current between these two widely used species. As stated above, the substantial increase in APD made interpretation of changes in loop thickness, in terms of changes in memory, difficult. Studies using lower concentrations of E4031 where the changes in APD are modest are required to better determine the effects of the block of this repolarization current on measures of memory.

Table 1. Changes in maximum delay, minimum delay, tilt, loop thickness and area under loop of hysteresis in restitution, pre and post block of I_{Kr}. Measures for both, the 400 ms central DI value protocol and the 150 ms central DI value protocol are shown.

I_{kr}	Central DI 400 ms			Central DI 150 ms		
	Pre block	Post block	% change	Pre block	Post block	% change
Parameters	**Mean±SEM**	**Mean±SEM**		**Mean±SEM**	**Mean±SEM**	
Max delay (beats)	12.93 ±0.91	11.66±0.53	-9.8	10.33±2.19	6.5±0.29	-37.1
Min delay (beats)	2.83±0.4	3.6±0.24	27.2	3.5±2.36	4.67±1.01	33.4
Tilt	0.137**±0.005	0 .414**±0.034	202.2	0.314*±0.032	0.786*±0.029	150.3
Loop thickness (msec)	31.87*±3.48	81.07*±8.11	154.4	23.58**±7.37	72.67**±6.78	208.2
Area under loop ($msec^2$)	14865.43**±1172.71	39058.73**±3567.35	162.7	4519.2**±1351.92	13199.33**±624.83	192.1

*p<0.05, **p<0.01

Table 2. Average values of APDs and loop thickness at center DI values of 150 ms and 400 ms during control condition (i.e. pre block of I_{Kr}).

	Value at Center DI 150 ms	Value at Center DI 400 ms	Difference
Average APD (ms)	196.6	239.7	43.1
Loop thickness	23.58	31.87	8.29

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