

Role of slow delayed rectifying potassium current in dynamics of repolarization and electrical memory in swine ventricles

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Abstract Dynamics of repolarization, quantified as restitution and electrical memory, impact conduction stability. Relatively less is known about role of slow delayed rectifying potassium current, I_{Ks} , in dynamics of repolarization and memory compared to the rapidly activating current I_{Kr} . Trans-membrane potentials were recorded from right ventricular tissues from pigs during reduction (chromanol 293B) and increases in I_{Ks} (mefenamic acid). A novel pacing protocol was used to explicitly control diastolic intervals to quantify memory. Restitution hysteresis, a consequence of memory, increased after chromanol 293B (loop thickness and area increased 27 and 38 %) and decreased after mefenamic acid (52 and 53 %). Standard and dynamic restitutions showed an increase in average slope after chromanol 293B and a decrease after mefenamic acid. Increase in slope and memory are hypothesized to have opposite effects on electrical stability; therefore, these results suggest that reduction and enhancement of I_{Ks} likely also have offsetting components that affect stability.

Keywords Slow delayed rectifier potassium current · Restitution · Action potential duration · Cardiac memory · Hysteresis · Ventricular arrhythmia

Abbreviations

I_{Ks} Slow delayed rectifier potassium current
AP Action potential
 I_{Kr} Rapid delayed rectifier potassium current
APD Action potential duration
CL Cycle length

DI Diastolic interval
TMP Transmembrane potential
TdP Torsades de Pointes

Introduction

The slow delayed rectifier potassium current, I_{Ks} , plays a critical role in the late repolarization phase of an action potential (AP) by serving as a repolarization reserve when other repolarization currents, such as the rapid delayed rectifier potassium current (I_{Kr}), are reduced during diseased conditions or by the use of drugs such as class III antiarrhythmic drugs [1]. Although prolongation of action potential duration (APD) is proposed to be proarrhythmic in situations such as long QT syndrome [2], there are contrasting results showing that lengthening of APD at short cycle lengths (CLs), i.e. higher heart rate, is an effective way to suppress arrhythmia, and block of I_{Ks} is hypothesized to be more effective in increasing APDs in a frequency-independent way compared to I_{Kr} blockers [3–7]. During inherited or acquired long QT as a result of application of I_{Kr} blocker, activation of I_{Ks} can compensate for the loss of repolarization current, prevent excessive APD prolongation, and reduce proarrhythmic risk [3, 8]. Thus, depending on different pathological conditions, both blockers and activators of I_{Ks} can potentially prove to be beneficial. Although the potential role of modification of I_{Ks} has been considered by several studies, compared to I_{Kr} , less is known about how I_{Ks} affects repolarization dynamics in terms of restitution and electrical memory.

Initiation of ventricular arrhythmias has been reported to be affected by two main factors: restitution of APD, which refers to the relationship between the current APD and its

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preceding diastolic interval (DI), and cardiac memory [9–11], which, in this context, means the dependence of APD on its previous history in the past few seconds. Although the concept of memory is widely hypothesized to impact stability, very few studies have quantified memory. We have previously developed a novel pacing protocol that allows sequential and precise changes in DI, independent of APDs [12, 13], that can be used to quantify memory. Oscillatory changes in DIs result in the trajectories of APD to diverge into the resulting hysteresis in restitution which permits quantification of memory.

Chromanol 293B has been reported to selectively block I_{Ks} [4, 14, 15]. Block of I_{Ks} has been shown to have a frequency-independent effect on APD in guinea pig and in humans [13], but have minimal effect in rabbit and canines [1, 7, 16–19]. L364, 373, a drug which selectively activates I_{Ks} in guinea pigs [20] and rabbits [21], has been recently reported to have no effect in canines while mefenamic acid is a non-selective activator of I_{Ks} which has been shown to shorten APD in canines [8]. Given these previously reported interspecies differences in the effects of this important repolarization current, the main objective of our study was to characterize the effects of manipulation of I_{Ks} on dynamics of repolarization of ventricular AP in the pig, a widely used animal model to study arrhythmia [22–27]. Our results show that reduction (increase) of I_{Ks} increased (decreased) both memory and slope of restitution curves, which suggest a mixed effect on electrical stability. While occurrence of alternans of APD after increase and reduction of I_{Ks} showed results consistent with slope of restitution, there were several trials in the control group where alternans was not seen, yet the slopes of restitution were greater than one. However, at least in terms of generation of alternans, these results suggest a weak pro-arrhythmic effect of I_{Ks} reduction and, likewise, a weak anti-arrhythmic effect of I_{Ks} enhancement.

Methods

Data were collected from 7 farm pigs, weighing 18–21 kg. All animal related procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky. Animals were sedated/tranquilized using a combination of telazol (4–8 mg/kg), ketamine (2–4 mg/kg), and xylazine (2–4 mg/kg), and then anesthetized by sodium pentobarbital (30–50 mg/kg, IV). After anesthesia, hearts were quickly excised and placed in cold Tyrode's solution. Two endocardial tissue slices of approximate size 20 mm × 10 mm × 5 mm, one for antagonist and the other for agonist, were collected adjacent to each other from the mid- to apical antero-lateral region of the right ventricle. The tissues were pinned in

plastic tissue chambers, and superfused with warmed ($36 \pm 1^\circ\text{C}$), gassed (95 % O_2 and 5 % CO_2) Tyrode's solution. Composition of the Tyrode's solution was (in mmol/L): 0.5 MgCl_2 , 0.9 NaH_2PO_4 , 2.0 CaCl_2 , 137.0 NaCl , 4.0 KCl and 5.5 Dextrose. NaHCO_3 was added to this solution to obtain a pH of 7.3 ± 0.05 . Tissues were paced at a CL of 500 ms for approximately an hour to allow equilibration before recording was started.

When testing the effect of reduction of I_{Ks} , the superfusate was switched to a buffer of the same composition as given above but containing antagonist chromanol 293B (Sigma-Aldrich, St. Louis, MO, USA). A concentration of 10–50 μM has been used in previous studies to reduce or block I_{Ks} [4, 14, 15]. In the current study, we used a final concentration of 20 μM to substantially reduce I_{Ks} without completely blocking it. To increase I_{Ks} , agonist L364, 373 (dissolved in DMSO; Tocris Bioscience, Bristol, UK) was used at first in one animal with the highest concentration (3 μM) that has been used in a previous study [8]. However, once it became apparent that APDs were not affected at this concentration, mefenamic acid (100 μM , dissolved in 0.1 M NaOH; Sigma-Aldrich) was used as the agonist in all subsequent experiments. The concentration of mefenamic acid was consistent with that used by Magyar et al. [8]. After each drug was added, tissues were paced at 500 ms CL for approximately 40 min for equilibration before data were collected.

Tissues were paced using 3-ms biphasic stimuli delivered via platinum-iridium electrodes. Transmembrane potentials (TMPs) were recorded using machine-pulled glass microelectrodes filled with 3 M KCl. A commercial data acquisition system was used to digitize the TMPs at 50,000 samples/s. The following protocols were used to explore dynamics of repolarization of APs both before and after the drug application. First, standard S1S2 and dynamic protocols similar to that previously described by Gilmour et al. [28] were used to obtain restitution curves. In the standard protocol, S1S1 interval was 300 ms and S1S2 interval decreased from 600 to 300 ms in steps of 50 ms, and then from 300 to 200 ms in steps of 20 ms. For S1S2 < 200 ms, the decrement was 10 ms until block occurred. In the dynamic protocol, CL decreased from 600 ms progressively with the same decrement as used in the standard protocol until block occurred. Second, two feedback-based DI protocols were used to obtain hysteresis, i.e. memory in restitution. The novel feedback-based pacing permitted explicit control of DIs as described previously [12, 29, 30]. Unlike the widely used CL control protocols, which result in a correlation of APD and DI ($\text{DI} = \text{CL} - \text{APD}$), the feedback based protocols allowed precise control of DI, independent of APD. The DIs oscillated in a sinusoidal pattern with a period of 100 beats. Each protocol had two cycles, preceded by 20 beats at its

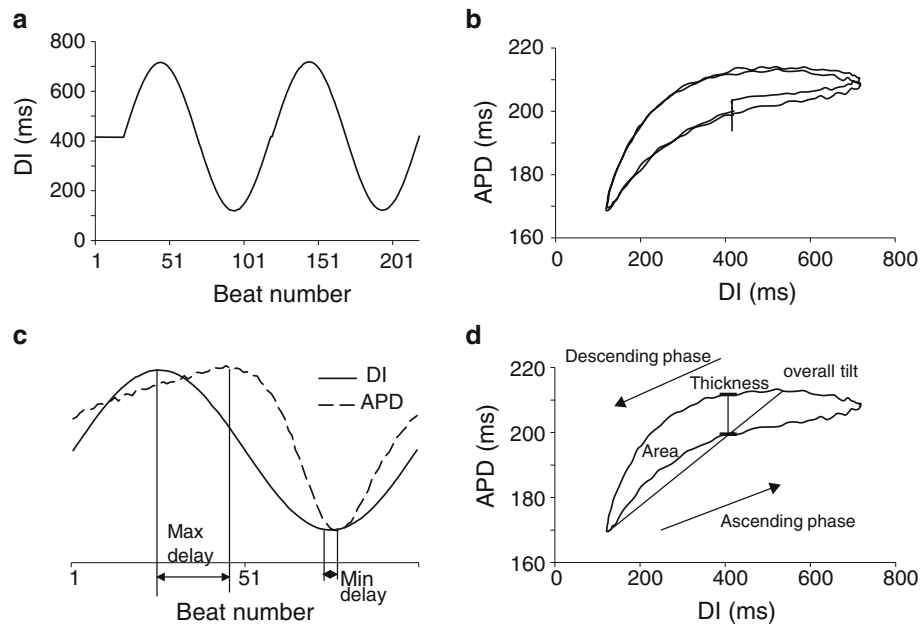


Fig. 1 **a** An example of sinusoidal DI protocol, with 20 beats of DIs at center value of 400 ms followed by 2 cycles of sinusoidal change ranging from 100 to 700 ms. **b** Restitution relationship between APD and DI obtained from the DI change shown in (a), which shows two complete hysteresis loops after 20 beats adaptation at center DI. **c** The second cycle of the DI sequence shown in (a) (solid line) and the

resulting APD trace (dashed line). The APD trace is scaled and offset to clearly illustrate maximum delay and minimum delay, which are measured in beats. **d** Hysteresis loop generated by the second cycle of the DI sequence shown in (a), with illustration of loop thickness, overall tilt and loop area

mean DI value. In one protocol, the mean DI was 400 ms with a variation of ± 300 ms; in the other, the mean value was 150 ms with a variation of ± 140 ms. Figure 1a shows an example of the sinusoidal DI protocol with a mean DI of 400 ms. And, third, to test effects of I_{Ks} changes on arrhythmia, we recorded TMPs when the tissue was paced using constant CL protocols where pacing CLs ≤ 300 ms and constant DI protocols where the DIs ≤ 40 ms were kept constant for all beats in one trial. CLs and DIs were progressively reduced until block occurred. Data were analyzed for the occurrence of alternans of APD, i.e. beat to beat variation in APDs, which is thought to presage and be conducive to ventricular arrhythmia. Each protocol was repeated 2 to 3 times. Between trials, the tissue was paced at 500 ms CL.

A custom developed program written in MATLAB (Mathworks, Natick, MA, USA) was used for offline data analysis. The TMPs were low-pass filtered at 1,000 Hz cutoff frequency, and APD was calculated for each AP as the duration between the start of an AP, i.e. the point where the derivative of TMP becomes positive, and the instance when TMP repolarized to 90 % of its amplitude. Alternans of APD was considered to occur when beat to beat variations in APDs were ≥ 4 ms for 5 successive beats. The threshold of 4 ms is the same as that reported previously by Pruvot et al. [31]. From the hysteresis in restitution between APD and DI resulting from the sinusoidal DI

protocols (Fig. 1b), five parameters were computed to quantify memory: (1) loop thickness, defined as difference in APDs between the ascending and descending phases when DI was at its mean value; (2) loop area, i.e. area contained within the hysteresis loop; (3) overall tilt, defined as the slope of the line composed by connecting the two points where the APD was at its maximum and minimum; (4) maximum delay, which is the number of beats between the peaks of DI and APD; and (5) minimum delay, which is the delay (in number of beats) between the nadirs of DI and APD. In some cases, APD adaptation caused a baseline shift in the first cycle of the sinusoidal DI sequence, which was induced by the transition from 500 ms CL to constant DI pacing; therefore, we only computed the measures from the second cycle. In rare cases when DI control was transiently lost during real-time control for 1 or 2 beats, the missed DI and corresponding APD values were replaced using linear interpolation from their adjacent 2 values. Those trials where DI control was lost for more than 5 beats from the 200 beats sequence were not used in analysis. If there was more than one trial for any protocol that met the conditions stated above, then results from those trials were averaged and the measures were calculated from the averaged loop/restitution curve and used for further analysis. Paired Student's *t* test was conducted to test for statistical significance. Significant difference was accepted at $p \leq 0.05$.

Results

Effect of I_{Ks} change on baseline APDs

Because L364, 373 had been reported as a selective I_{Ks} agonist in previous studies of guinea pigs and rabbits, we started with L364, 373 in the first animal. However, APDs at 500 ms CL showed no difference between control and post-drug (227 vs. 222 ms). This observation is consistent with a previous study in dogs [8]. As the main objective of the current study was to explore the effect of changes in I_{Ks} on dynamics of repolarization of AP, and not to explore whether L364, 373 works as an agonist in this species, for the rest of the experiments we used mefenamic acid to activate I_{Ks} .

Figure 2 shows an example of TMPs recorded at 500 ms CL before (solid line) and after application of chromanol 293B (dashed line), and mefenamic acid (dotted line). All

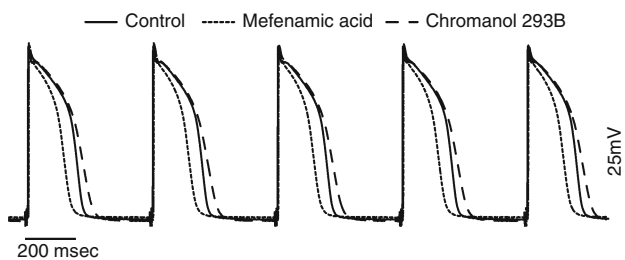
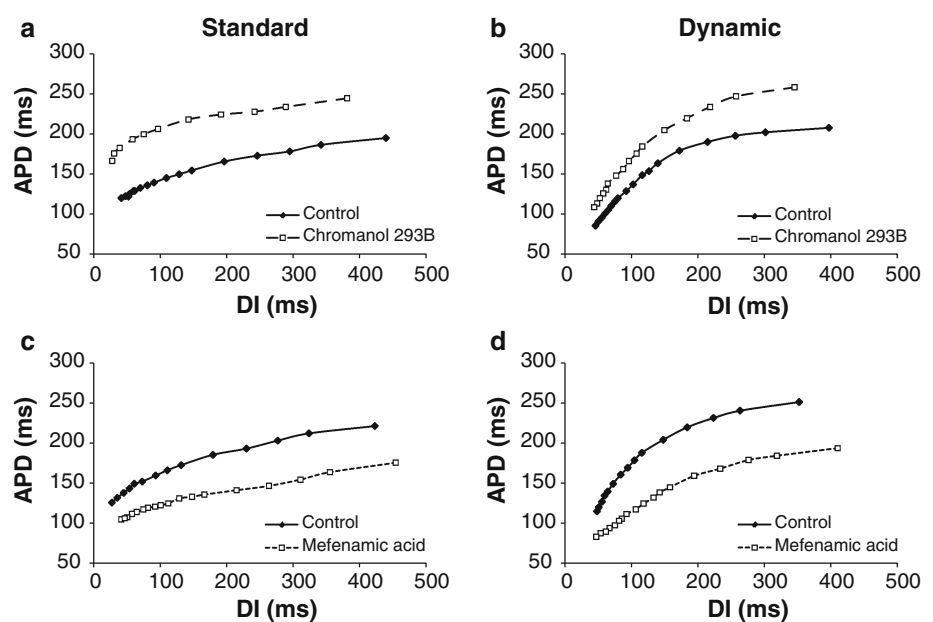


Fig. 2 An example of transmembrane potentials recorded in one pig at CL of 500 ms which shows the APD differences among control (solid line), chromanol 293B (dashed line), and mefenamic acid (dotted line). APD increased after chromanol 293B and decreased after mefenamic acid

Fig. 3 Standard (a, c) and dynamic (b, d) restitution curves recorded from one trial show the change in slope of restitution after chromanol 293B (a, b) and mefenamic acid (c, d). Slopes in this trial are comparable to the average slopes ($n = 6$). Slopes of standard and dynamic restitution both increased after chromanol 293B and decreased after mefenamic acid, with more prominent changes in dynamic restitution



the traces were collected from one animal and the upstroke of APs were aligned to better show the difference among APDs. The average APD ($n = 6$) was lengthened by 14 % after chromanol 293B (205 ± 6 vs. 234 ± 7 ms) and shortened by 20 % after mefenamic acid (218 ± 4 to 173 ± 6 ms), both of which were significant.

Effect of I_{Ks} change on restitution

To quantify changes in restitution, standard and dynamic restitution were obtained from all animals. Figure 3 shows examples of standard and dynamic restitution during control and post-drug in one animal. For each restitution curve, overall slope was obtained by computing the average value of all slopes over the entire range of DIs. Average overall slopes ($n = 6$) are summarized in Table 1. Compared to control, both standard (Fig. 3a) and dynamic restitution (Fig. 3b) had steeper slopes after chromanol 293B, especially at short CL. However, only the slopes of dynamic restitution were significantly different (0.75 control vs. 0.97 chromanol 293B). In contrast, slopes of both standard and dynamic restitution flattened after mefenamic acid (Fig. 3c, d); both were significantly smaller than the control (0.26 vs. 0.45, and 0.59 vs. 0.91). In both drugs, changes in slope of dynamic restitution were more prominent than those of standard restitution. Average slope for each restitution curve was also computed for all points where CL < 300 ms, since alternans is mostly seen at higher activation rates. These results are shown in Table 1: all slopes were larger at shorter CL compared to overall slopes, and all dynamic slopes were close to or greater than 1, a condition believed to be requisite for occurrence of alternans and decrease in electrical stability.

Table 1 Average overall slopes for standard and dynamic restitution ($n = 6$)

	Control	Chromanol 293B	Control	Mefenamic acid
Standard				
Overall	0.30 ± 0.05	0.44 ± 0.09	0.45 ± 0.08	0.26 ± 0.03*
CL ≤ 300 ms	0.45 ± 0.06	0.67 ± 0.15	0.63 ± 0.13	0.34 ± 0.04*
Dynamic				
Overall	0.75 ± 0.12	0.97 ± 0.15*	0.91 ± 0.11	0.59 ± 0.08*
CL ≤ 300 ms	1.08 ± 0.14	1.29 ± 0.23	1.19 ± 0.15	0.81 ± 0.09*

Slopes at all values of DIs in each restitution curve were averaged to obtain an overall slope

* $p < 0.05$

Effect of I_{Ks} change on memory

Hysteresis in restitution, i.e. memory, was observed in both sinusoidal DI protocols. Figure 4 shows the averaged hysteresis loops for the sinusoidal protocol with a center DI of 400 ms ($n = 5$; due to technical difficulties with DI control, we could not obtain data in one animal). In the 5 measures of hysteresis, the most prominent changes were observed in loop thickness and area: after chromanol 293B (Fig. 4a), loop thickness increased from 13 ± 2 to 17 ± 2 ms and area enlarged from $8,084 \pm 1,303$ to $11,191 \pm 1,875 \text{ ms}^2$ with a percentage change of 27 and 28 %, respectively; oppositely, after mefenamic acid (Fig. 4b), loop thickness decreased from 16 ± 2 to 8 ± 1 ms, and area shrunk from $8,960 \pm 664$ to $4,215 \pm 810 \text{ ms}^2$, with a percentage change of 52 and 53 %, respectively (all changes significant). Overall tilt showed inconsistent changes during both drugs across animals, where both increases and decreases were observed, resulting in minimal change between control and post-drug. Changes in maximum and minimum delay were also minimal and no significant differences were present. Table 2 includes a summary of these measures (mean ± SEM) for the 400 ms DI protocol.

Figure 5 shows the averaged hysteresis loops ($n = 6$) of control and post-drug data obtained from the sinusoidal DI protocol with a 150 ms center DI. The results are similar to that during the 400 ms DI protocol; loop thickness and area were significantly different while the changes in overall tilt were mixed. Loop thickness increased 52 % after chromanol 293B, decreased 29 % after mefenamic acid, and accordingly loop area increased 31 % and decreased 35 %, respectively. A summary of these measures is in Table 3.

Effects of I_{Ks} change on APD alternans

During reduction of I_{Ks} , alternans occurred only in 2 animals during control, with an average amplitude of 8 ms, and was observed after chromanol 293B in 2 animals (not

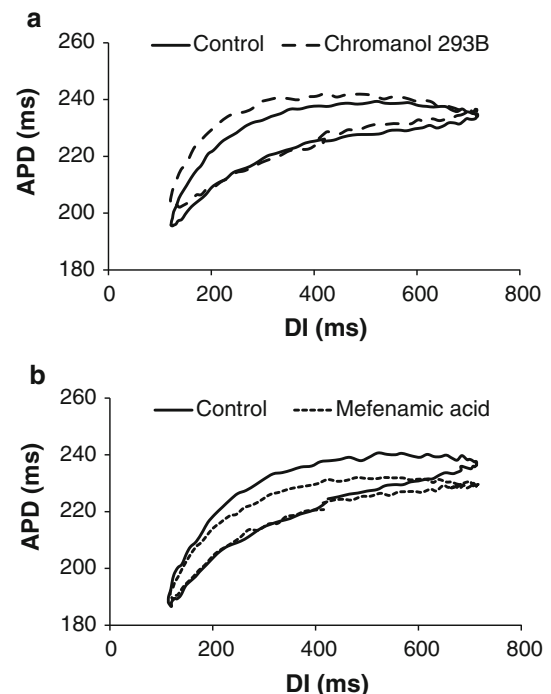


Fig. 4 Averaged hysteresis loops ($n = 5$) during sinusoidal DI protocol with mean DI = 400 ms for control and post-drug. **a** Control (solid line) versus chromanol 293B (dashed line). **b** Control (solid line) versus mefenamic acid (dotted line). Curves were shifted vertically (to adjust for differences in APD) in both panels to facilitate comparison between the hysteresis loops

the same animals) with an amplitude of 5 ms. The average CL when alternans started to occur was longer after chromanol 293B compared with control (200 vs. 130 ms). In all trials, activation blocked at longer CL after chromanol 293B was added, (average CL 118 vs. 150 ms, $p < 0.05$).

During enhancement of I_{Ks} , alternans was present during the control study in 4 animals, with an average amplitude of 12 ms. No alternans was seen after adding mefenamic acid. This result is consistent with what would be predicted by changes in the average slope of dynamic restitution, i.e. 1.19 in control vs. 0.81 post-drug. The average CLs where activation block occurred were 115 and 106 ms for control

Table 2 A summary of mean (\pm SEM) values of measures of hysteresis for 400 ms DI protocol for both drugs (control and post-drug), $n = 5$

	Control	Chromanol 293B	Control	Mefenamic acid
Thickness (ms)	13 ± 2	$17 \pm 2^*$	16 ± 2	$8 \pm 1^*$
Overall tilt	0.065 ± 0.017	0.070 ± 0.023	0.080 ± 0.017	0.063 ± 0.015
Area (ms^2)	$8,084 \pm 1,303$	$11,191 \pm 1,875^*$	$8,960 \pm 664$	$4,215 \pm 810^*$
Max delay (beats)	20 ± 3	23 ± 2	18 ± 3	17 ± 1
Min delay (beats)	1 ± 0	3 ± 1	2 ± 1	3 ± 1

* $p < 0.05$

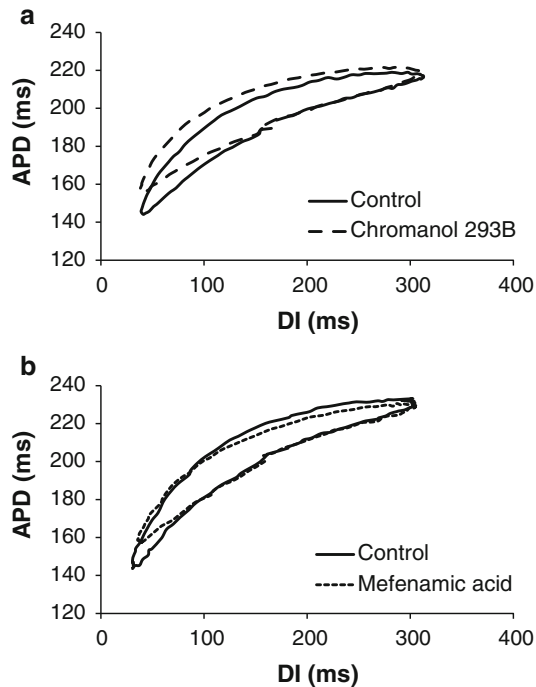


Fig. 5 Averaged hysteresis loops ($n = 6$) during sinusoidal DI protocol with mean DI of 150 ms for control and post-drug data. As in Fig. 4, the curves were vertically shifted to facilitate comparison between the hysteresis loops

and post-drug, respectively, with no significant difference between the two.

Discussion

The focus of the current study was to characterize the effects of changes in I_{Ks} on restitution and memory in swine ventricular tissue. The main observations of our study are: first, chromanol 293B-induced reduction of I_{Ks} in swine ventricles results in APD prolongation, increased measures of hysteresis in restitution, i.e. in memory, but also produces steeper restitution curves. Alternans of APD was present in limited samples during both control and post-drug but occurred at longer CL post-drug; and, second, enhancement of I_{Ks} , which was achieved by mefenamic acid, shortened APD, decreased memory as well as restitution slopes, and minimized occurrence of alternans.

Effect of I_{Ks} manipulation on APDs

I_{Kr} blocking drugs are most commonly class III antiarrhythmic drugs used to prolong repolarization and prevent arrhythmia; however, due to their reverse frequency response, they have limited therapeutic effect on arrhythmia suppression and, moreover, have increased risk of Torsades de Pointes (TdP) [32–34]. In the current study, our results show that chromanol 293B produced frequency independent changes in APDs during fast CL pacing (Fig. 3), presumably by reducing I_{Ks} at all levels of CL. If prolonging repolarization prevents re-entrant arrhythmia and ventricular fibrillation as suggested by previous studies [5, 6], these results suggest that blockade of I_{Ks} could provide antiarrhythmic benefit. Heterogeneity in I_{Ks} expression in different species and in its kinetic properties has been widely reported. Presence of I_{Ks} channels in pigs and its sensitivity to chromanol 293B has also been shown in previous studies [31, 35]. Our results show that I_{Ks} is more functional in repolarization in pigs compared with rabbits and dogs [7, 16, 19, 36], another two species widely used in arrhythmia research. Considering the consistent observations in our study with those reported in humans [14], in regards to effects on APD, one would predict similar changes in restitution properties with manipulation of I_{Ks} in human ventricles to those observed in pigs in the current study.

Electrical stability indicated by changes in dynamics of repolarization

Restitution of APD has been believed to be the dominant mechanism underlying initiation of APD alternans, which is hypothesized to presage and be conducive to re-entry and ventricular arrhythmias [28, 37, 38]. The restitution hypothesis states that an increase in restitution slope indicates pro-arrhythmic effect and electrical instability. In our results, we observed an increase in average restitution slope after chromanol 293B, i.e. after decrease of I_{Ks} , and a decrease in slope after mefenamic acid, i.e. after an increase of I_{Ks} (Fig. 3; Table 1). These observations are consistent with results from our previous simulation study using the Luo Rudy model, a model of guinea pig ventricular cell [13]. Therefore, based on the restitution theory,

Table 3 A summary of mean (\pm SEM) values of measures of hysteresis for 150 ms DI protocol, $n = 6$

	Control	Chromanol 293B	Control	Mefenamic acid
Thickness (ms)	15 \pm 2	23 \pm 2*	19 \pm 1	14 \pm 2*
Overall tilt	0.257 \pm 0.035	0.234 \pm 0.028	0.311 \pm 0.039	0.259 \pm 0.045
Area (ms ²)	4,283 \pm 636	5,629 \pm 954*	5,267 \pm 745	3,409 \pm 535*
Max delay (beats)	10 \pm 2	10 \pm 1	8 \pm 1	6 \pm 1
Min delay (beats)	2 \pm 1	2 \pm 1	3 \pm 1	3 \pm 1

* $p < 0.05$

our results would suggest that electrical stability in tissues with decreased I_{Ks} is compromised and it would be more susceptible to arrhythmia induction, while enhancement of I_{Ks} could stabilize activation and provide antiarrhythmic protection. Our results of APD alternans are partly consistent with this conclusion, as alternans was only present when the dynamic curves were steeper than 1, during both controls and after chromanol 293B, but was not present after mefenamic acid. However, studies [9–11] related to cardiac memory have shown that restitution alone is not adequate to predict initiation of arrhythmia, rather, memory should be taken into account to provide a more comprehensive prediction. The fact that, in the current study, although the slopes were consistently >1 for all chromanol 293B trials, yet alternans was only observed in 2 out of 6 animals supports this conclusion. Increased memory is proposed to be indicative of increase in electrical stability [9, 39]. In the current study, 2 out of 5 measures of hysteresis were significantly larger (smaller) after chromanol 293B (mefenamic acid) suggesting an increase (decrease) in memory. Therefore, in the context of the hypothesized effects of memory, our results suggest that reduction of I_{Ks} would decrease electrical stability while enhancement of I_{Ks} would have a stabilizing effect. This conclusion is opposite to that predicted by results of restitution slopes. Therefore, the effect of I_{Ks} manipulation, i.e. reduction and enhancement, using the contemporary hypothesized mechanisms affecting stability of activation, is mixed. With the presence of two offsetting components that affect stability, the ultimate effect of I_{Ks} manipulation on stability would depend on which of the two mechanisms plays a dominant role in generation of certain type of arrhythmias. Unfortunately, at this stage, without further investigations and experimental or clinical evidence, it is not clear which of the two, i.e. restitution or memory, is the predominant contributor.

Clinical indications of arrhythmogenic effect of I_{Ks} blocker/activator

The divergent effects on restitution and memory are consistent with divergent effects of changes in I_{Ks} on electrical stability that have been reported previously. A previous study [40] has shown that I_{Ks} blockade reduced dispersion of

repolarization, which is a critical mechanism underlying discordant alternans and ventricular fibrillation. However, other studies reported that suppression of I_{Ks} decreased the repolarization reserve and increased the risk of TdP generation [41, 42] as a result of excessive prolongation. In the current study, we did not observe early after-depolarization after I_{Ks} reduction. However, observations that reduction of I_{Ks} leading to alternans and conduction block at longer CL suggest, albeit weakly, a proarrhythmic effect of I_{Ks} blocker. The experiments were conducted in tissue samples that were small in size; therefore, induction of ventricular tachycardia or fibrillation was not possible. Future studies using optical mapping of isolated heart would be needed to investigate the effect of chromanol on initiation of arrhythmia.

The agonist that we used, mefenamic acid, is somewhat non-selective and is known as a blocker of Cl^- current. However, it has also been shown to increase I_{Ks} in several studies (which is also shown by Magyar et al. in their fig. 1) [8, 43, 44]. Results show that increase in I_{Ks} by mefenamic acid decreases slope of restitution and measures of memory (Figs. 3, 4), suggesting the existence of both stabilizing and destabilizing effects. Further, our results of alternans suggest that this drug might have a suppressive effect on alternans generation. As stated above, induction of ventricular tachycardia is not possible in small-sized tissues; therefore, the exact role of mefenamic acid on arrhythmia generation remains to be determined. Therapeutic usefulness of enhancement of I_{Ks} is uncertain, but it is hypothesized to be able to prevent excessive APD prolongation by increasing the repolarization reserve, which could compensate the adverse effect caused by application of I_{Kr} blocking drugs [18, 33, 42]. Nevertheless, our results suggest that the effect of I_{Ks} activator on restitution and memory should be taken into account when considering the therapeutic benefit of I_{Kr} blocking drugs.

Limitations

As stated above, we used a non-selective I_{Ks} agonist to test the effect of increase in I_{Ks} . Currently, L364, 373 is the only drug that selectively activates I_{Ks} , but it has been proven only in rodents and rabbits. Consistent with a previous study on canines, our results also showed that it did

not have an effect on APD at 500 ms CL in pigs. Previously, several studies have used mefenamic acid to activate I_{Ks} [8, 43, 44]. However, these previous studies also showed that, in addition to I_{Ks} enhancement, mefenamic acid could inhibit Cl^- current and Ca^{2+} current. Block of Cl^- would prolong APD, while block of Ca^{2+} would shorten APD, i.e. the effect on these two currents on APD would be opposite and potentially offsetting each other. Therefore, the net shortening of APD observed in this study after mefenamic acid was likely due to I_{Ks} activation.

We chose to use one concentration of each drug without testing the dose effect on I_{Ks} , as the focus of the current study was to investigate the effect of changing I_{Ks} on dynamics of repolarization, but not on the way these change I_{Ks} per se. Therefore, we chose one concentration from what has been reported in the literature to meet our objective of manipulating the current by both attenuating and accentuating it.

All our recordings were made from the endocardial side of the right ventricle. Whether similar results would be obtained from other cell types in other areas of the heart is unknown. Heterogeneity of I_{Ks} expression has been reported by a number of studies in different species. For example, Bryant et al. reported a smaller I_{Ks} density in endocardium compared to epicardium [45]; in rabbits, I_{Ks} expression was more abundant in the base than in the apex [46]; I_{Ks} density was reported to be similar between endo- and epicardial myocytes in canine ventricles [47]; and, in minipigs, the expression of mRNA for I_{Ks} was similar among endo-, mid- and epicardial myocytes in the left ventricle as well as the right ventricle [35]. However, the regional differences in I_{Ks} expression in pig ventricles are unknown. Considering the heterogeneity of I_{Ks} expression in other species, it is likely that heterogeneity exists in pig ventricles, and changes in this current may also alter electrical substrate heterogeneously. Future studies will be required to further investigate the role of heterogeneity in dynamics of repolarization.

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