Apamin-Sensitive Potassium Current Modulates Action Potential Duration Restitution and Arrhythmogenesis of Failing Rabbit Ventricles

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Background—Apamin-sensitive K currents (I_{KAS}) are upregulated in heart failure. We hypothesize that apamin can flatten action potential duration restitution (APDR) curve and can reduce ventricular fibrillation duration in failing ventricles.

Methods and Results—We simultaneously mapped membrane potential and intracellular Ca (Ca_i) in 7 rabbit hearts with pacing-induced heart failure and in 7 normal hearts. A dynamic pacing protocol was used to determine APDR at baseline and after apamin (100 nmol/L) infusion. Apamin did not change APD_{80} in normal ventricles, but prolonged APD_{80} in failing ventricles at either long (≥300 ms) or short (≤170 ms) pacing cycle length, but not at intermediate pacing cycle length. The maximal slope of APDR curve was 2.03 (95% confidence interval, 1.73–2.32) in failing ventricles and 1.26 (95% confidence interval, 1.13–1.40) in normal ventricles at baseline (P=0.002). After apamin administration, the maximal slope of APDR in failing ventricles decreased to 1.43 (95% confidence interval, 1.01–1.84; P=0.018), whereas no significant changes were observed in normal ventricles. During ventricular fibrillation in failing ventricles, the number of phase singularities (baseline versus apamin, 4.0 versus 2.5), dominant frequency (13.0 versus 10.0 Hz), and ventricular fibrillation duration (160 versus 80 s) were all significantly (P<0.05) decreased by apamin.

Conclusions—Apamin prolongs APD at long and short, but not at intermediate pacing cycle length in failing ventricles. I_{KAS} upregulation may be antiarrhythmic by preserving the repolarization reserve at slow heart rate, but is proarrhythmic by steepening the slope of APDR curve, which promotes the generation and maintenance of ventricular fibrillation. (Circ Arrhythm Electrophysiol. 2013;6:410-418.)

Key Words: electrophysiology ■ experimental models heart failure ■ optical mapping ■ ventricular fibrillation

Heart failure (HF) is associated with significant electrophysiological remodeling of the repolarization currents, including downregulation of most potassium currents and upregulation of late sodium and sodium–calcium exchange current.1 These changes tend to prolong action potential duration (APD). On the contrary, the APD in patients or animals with structural heart diseases, including HF, shortens more rapidly than normal ventricles during rapid pacing, leading to increased slope of APDR curve.2,3 A steep APDR curve promotes dynamical instability, wavebreaks, and ventricular fibrillation (VF).4,5 The mechanisms by which HF lengthens APD at slow pacing rates but shortens APD and increases the slope of APDR at fast pacing rates remain incompletely understood. One possible explanation is that failing ventricular cells (but not normal ventricular cells) exhibit increased apamin-sensitive small conductance calcium–activated K (SK) current (I_{KAS}).6 The SK channels are first discovered in the brain,7 but it is also known to play an important role in cardiac repolarization.8,9,10 The importance of I_{KAS} in human ventricular repolarization is further documented by studies in the cells isolated from the native hearts of the transplant recipients.11 The latter study showed that blocking I_{KAS} by apamin lengthens the APD for failing human ventricular myocyte by an average of 11.8%. Because multiple other K currents are downregulated in HF,12 upregulation of I_{KAS} plays an important role in ventricular repolarization in failing ventricles. The SK channel is sensitive to intracellular calcium (Ca_i). Because rapid pacing causes Ca_i accumulation, I_{KAS} activation in failing but not normal ventricles could lead to more APD shortening at rapid pacing rate and

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steepened APDR curve. If this is true, then \( I_{\text{KAS}} \) blockade by apamin (a specific \( I_{\text{KAS}} \) blocker)\(^{14}\) should flatten the APDR, reduce wavebreaks, and hinder the maintenance of VF. To determine the importance of \( I_{\text{KAS}} \) on APDR, we simultaneously mapped the membrane potential \((V_m)\) and \( C_a \) in normal and failing ventricles at different pacing cycle lengths (PCLs) before and after apamin administration. We also studied the wavebreaks and the duration of pacing-induced VF in these hearts. The results were used to test the hypotheses that (1) \( I_{\text{KAS}} \) activation promotes steeper APDR in failing ventricles and (2) \( I_{\text{KAS}} \) blockade by apamin flattens APDR curve, prevents wavebreaks, and shortens VF duration in failing ventricles.

**Methods**

This study protocol was approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine and the Methodist Research Institute, and conforms to the Guide for the Care and Use of Laboratory Animals.\(^{15}\) New Zealand white female rabbits (\( N = 27 \)) were used. We attempted to induce HF in 10 rabbits by rapid pacing. Among them, 7 completed the pacing protocol and developed HF. The remaining 3 rabbits died 1.7 (95% confidence interval [CI], 0.2–3.1) days after the onset of tachycardia pacing at 250 beats per minute. We studied 7 normal rabbit hearts as controls. The other 10 hearts were used for Western blot analyses (5 with pacing-induced HF and 5 normal controls). A detailed method section is included in the online-only Data Supplement.

**Pacing-Induced HF and Optical Mapping**

Rapid ventricular pacing was used to induce HF.\(^{9}\) The hearts were Langendorff perfused at 25 to 35 mL/min with oxygenated Tyrode’s solution (in mmol/L: NaCl 125, KCl 4.5, NaHCO\(_3\) 24, NaH\(_2\)PO\(_4\) 1.8, CaCl\(_2\) 1.8, MgCl\(_2\) 0.5, and glucose 5.5) with a pH of 7.40. The hearts were stained with Rhod-2 AM (1.48 mmol/L) for \( C_a \) and RH237 (10 μmol/L) for \( V_m \). The double-stained hearts were illuminated with a laser at 532 nm wavelength. The fluorescence was filtered and recorded simultaneously 2 ms/frame and 100×100 pixels with a spatial resolution of 0.35×0.35 mm\(^2\) per pixel. Optical signals were processed with both spatial (3×3 pixels Gaussian filter) and temporal (3 frames moving average) filtering. Phase mapping was performed to evaluate the location and evolution of phase singularities.

**Statistical Analysis**

Data are presented as mean and 95% CI. Wilhelm rank-sum test was used to compare the data within and between groups. Categorical parameters between groups were compared by Fisher exact test. Pearson correlation coefficient was used to measure the association of continuous measures. The \( P \) values are corrected for multiple comparison in relevant analyses using Bonferroni adjustment. A 2-sided \( P \) value of ≤0.05 was considered statistically significant.

**Results**

All rabbits that survived the rapid pacing protocol showed clinical signs of HF, including appetite loss, tachypnea, lethargy, pleural effusion, ascites, and visible congestion of lung, liver, and gastrointestinal tract. HF ventricles demonstrated significant increases in left ventricular end-diastolic dimension (12.7 mm [95% CI, 11.0–14.5], 16.7 mm [95% CI, 15.4–17.9], for normal and HF, respectively; \( P = 0.017 \)) and end-systolic dimension (8.2 mm [95% CI, 6.9–9.6] versus 14.8 mm [95% CI, 13.4–16.1]; \( P = 0.012 \)), decreases in fraction of APD 80 prolongation than normal ventricles. The percentage of APD 80 prolongation was PCL dependent (Figure 1). After adding apamin, the HF ventricles have larger percentage of APD 80 prolongation than normal ventricles. The effects were more apparent at very long PCLs (at PCL 350 ms, normal: 2.0% [95% CI, –1.2 to 5.3], HF: 10.7% [95% CI, 4.6–16.9], \( P = 0.04 \)) at PCL 300 ms, normal: 2.3% [95% CI, –1.2 to 5.9], HF: 8.8% [95% CI, 4.9–12.7], \( P = 0.02 \)) at very short PCLs (at PCL 170 ms, normal: 0.4% [95% CI, –3.4 to 4.1], HF: 5.4% [95% CI, 3.5–7.2], \( P = 0.03 \)) at PCL 160 ms, normal: 0.1% [95% CI, –3.9 to 4.2], HF: 6.5% [95% CI, 4.9–8.2], \( P = 0.03 \)). With the intermediate PCLs (280–180 ms), the differences between normal and failing ventricles were not significant (Figure 2).

**Secondary Rise of \( C_a \) in HF Ventricles**

In 4 of the 7 HF ventricles, we observed that the initial \( C_a \) transient was followed by a secondary rise of \( C_a \) when paced at 350 ms PCL (Figure 3A, red arrows at site a). Apamin administration lengthened APD 80 and widened the initial phase of \( C_a \) transient, making the secondary rise of \( C_a \) less apparent (Figure 3A, black arrows at site a). However, apamin could also induce secondary rise of \( C_a \) in areas without secondary rise of \( C_a \) at baseline (Figure 3A, site b). Figure 3B shows the area of the mapped region exhibiting secondary rise of \( C_a \) in these 4 HF ventricles at PCL 350 ms. Before apamin infusion, the area averaged 19% (95% CI, 3–41) of epicardial surface of 4 HF ventricles (Figure 3B, areas encircled by blue line). After apamin, the area with secondary rise of \( C_a \) (Figure 3B, areas encircled by green line) decreased to 9% (95% CI, –1 to 18; \( P = 0.25 \)). Apamin infusion could induce secondary rise of \( C_a \) in 6% (95% CI, –4 to 16) of the mapped areas where no secondary rise of \( C_a \) was observed at baseline. Baseline APD 80 at PCL 350 ms averaged 216 ms (95% CI, 188–243) in areas without \( C_a \) rise area and
245 ms (95% CI, 201–289) in areas with Ca\textsuperscript{i} rise (P=0.12). In 4 HF ventricles with secondary rises of Ca\textsuperscript{i} at PCL 350 ms, the SD of APD\textsubscript{80} was decreased from 17 ms (95% CI, 9–25) to 10 ms (95% CI, 6–13) by apamin (P=0.037). In contrast, in the remaining 3 HF ventricles without secondary rises of Ca\textsuperscript{i} at PCL 350 ms, the SD of APD\textsubscript{80} was not significantly changed by apamin (baseline: 10 ms [95% CI, 1–19], apamin: 7 ms [95% CI, 1–13], P=0.068). The HF ventricles with secondary rise of Ca\textsuperscript{i} (N=4) showed increased thickness of interventricular septum after tachycardia pacing (by 36% [95% CI, −17 to 88]). In comparison, the HF ventricles without secondary rise of Ca\textsuperscript{i} (N=3) showed a reduction of the thickness of interventricular septum (−37% [95% CI, −60 to −14], P=0.034). None of the 7 normal ventricles showed significant APD prolongation at long and short PCLs. P\textsuperscript{*} values in (C) are corrected by Bonferroni method for multiple comparisons. Corrected *P<0.05. LAD indicates left anterior descending coronary artery; LV, left ventricle; and RV, right ventricle.

**Effects of Apamin on Spatial Heterogeneity of APD**

Long PCLs are also associated with significant spatial heterogeneity of APD in failing ventricles. We used the SD of APD\textsubscript{80} at all mapped pixels to measure the spatial heterogeneity of

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**Figure 1.** Pacing cycle length (PCL) and the effects of apamin on action potential duration (APD) prolongation. A, B, Heart failure (HF) and normal ventricles, respectively. Note that HF ventricles have longer baseline APD than normal ventricles. Apamin prolonged APD in HF but not in normal ventricles. C, D, APD associated with different PCLs at baseline and after apamin infusion in HF and normal ventricles, respectively. Error bars, SD. The HF ventricles but not normal ventricles showed significant APD prolongation at long and short PCLs. *P\textsuperscript{*} values in (C) are corrected by Bonferroni method for multiple comparisons. Corrected *P<0.05. LAD indicates left anterior descending coronary artery; LV, left ventricle; and RV, right ventricle.

**Figure 2.** Effect of apamin on the percentage of action potential duration (APD) prolongation in normal and heart failure (HF) ventricles. A, APD\textsubscript{80} before (blue line) and after (red line) apamin infusion, and the percentage of APD\textsubscript{80} prolongation at pacing cycle length (PCL) 350, 300, 200, 180, and 160 ms in a normal and a HF ventricle. B, PCL and the percentage of APD\textsubscript{80} prolongation by apamin in all normal and HF ventricles. Note that the differences between HF and normal ventricles were significant only at very long (350–300 ms) and short (170–160 ms) PCLs (asterisks), but not with intermediate (280–180 ms) PCLs.
APD. Figure 4 shows the APD<sub>80</sub> maps before and after apamin infusion in normal (Figure 4A) and HF ventricles (Figure 4B) at 300 ms PCL. At baseline, APD<sub>80</sub> distribution is more heterogeneous in HF than normal ventricles (Figure 4A and 4B). The average SD of APD<sub>80</sub> was consistently higher in HF than normal ventricles (at PCL 350 ms, HF: 14 ms [95% CI, 4–18], P<0.05 vs HF; 6 ms [95% CI, 4–8], normal: 7 ms [95% CI, 4–10], P=0.53; at PCL 260 ms, HF: 5 ms [95% CI, 4–6], normal: 7 ms [95% CI, 4–11], P=0.18). These findings indicate that heterogeneous distribution of I<sub>KAS</sub> is largely responsible for the repolarization heterogeneity in HF ventricles.

**Correlation Between ΔAPD and Baseline APD**

APD difference (ΔAPD, apamin-treated APD<sub>80</sub> minus baseline APD<sub>80</sub>) maps were used to characterize the 2-dimensional distribution of I<sub>KAS</sub> (Figure 5). In normal ventricles, apamin did not significantly prolong the APD<sub>80</sub>. The ΔAPD map showed variable changes of APD<sub>80</sub>, including areas of APD<sub>80</sub> shortening (blue) and prolongation (green; Figure 5A). In HF ventricles, the ΔAPD map showed greater and more heterogeneously distributed ΔAPD than normal ventricles (Figure 5B). Figure

**Figure 3.** Secondary rise of Cai in heart failure (HF) ventricles. A, Intracellular Ca transient duration (CaiTD) map and optical Cai tracings at sites a and b. Red arrows on the tracing marks secondary rise of Cai. Area with secondary rise of Cai at baseline and after apamin infusion were encircled by blue and green lines, respectively, on the CaiTD map. Note that the area with secondary rise of Cai, less apparent (black arrows) probably because of the increased duration of the initial Ca elevation during phase 2 of the action potential. In contrast, apamin infusion can also induce secondary rise of Cai in areas without them at baseline (site b, red arrows). B, The CaiTD map of all 4 hearts with secondary rise in Cai at 350 ms PCL. Note that the distribution of these areas is heterogeneous before and after apamin infusion.

**Figure 4.** Effect of apamin on spatial heterogeneity of action potential duration (APD) in normal and heart failure (HF) ventricles. A, B, APD<sub>80</sub> maps of all normal and HF ventricles, respectively, at baseline and after apamin infusion at 300 ms pacing cycle length (PCL). C, D, SD of APD<sub>80</sub> in normal and HF ventricles, respectively, before and after apamin infusion during 3 different PCLs. Apamin significantly reduced the SD of APD<sub>80</sub> to the level observed in normal ventricles. Error bars, SD. Small squares in the data box indicate the mean value.
5C shows the correlation between ΔAPD and baseline APD₀ in a HF and a normal ventricle. HF ventricles showed a steeper negative correlation than normal ventricles at longer PCL (at PCL 350 ms, HF: \( r = -0.74 \) [95% CI, −0.86 to −0.62], normal: \( r = -0.44 \) [95% CI, −0.70 to −0.17], \( P = 0.02 \)); at PCL 300 ms, HF: \( r = -0.77 \) [95% CI, −0.86 to −0.67], normal: \( r = -0.43 \) [95% CI, −0.70 to −0.15], \( P = 0.01 \)) but not at intermediate PCL (260 ms; Figure 5D). The negative correlation indicates that apamin induces more APD₈₀ prolongation in areas with short APD₀ than with long APD₀ at baseline.

**Effect of Apamin on the Maximal Slope of APDR Curves**

APDR curves were sampled at a basal (site a), a middle (site b), and an apical (site c) area in each heart studied (Figure 6A and 6B). In a representative HF ventricle (Figure 6A), APDR curve at these 3 sites (Figure 6A, top) consistently showed that apamin prolonged APD₀ at both very long (350 and 300 ms) and short (160 ms) PCLs, leading to a steeper APDR at long PCL and a flattened APDR at short PCL. In a representative normal ventricle (Figure 6B), APDR was not significantly changed by apamin. At baseline, HF ventricles have higher maximal slopes of APDR than normal ventricles (HF: \( r = -0.44 \) [95% CI, −0.70 to −0.17], \( r = 0.02 \); at PCL 300 ms, \( r < 0.01 \)) but not at intermediate PCL (at PCL 300 ms, \( r = -0.70 \) to −0.15), \( P = 0.01 \)) but not at intermediate PCL (260 ms; Figure 6D). The negative correlation than normal ventricles at longer PCL (at PCL 300 ms, \( r = -0.405 \) [95% CI, −0.70 to −0.17], \( P < 0.001 \)) but not at intermediate PCL (260 ms; Figure 6D). The negative correlation than normal ventricles at longer PCL (at PCL 300 ms, \( r = -0.405 \) [95% CI, −0.70 to −0.17], \( P < 0.001 \)) but not at intermediate PCL (260 ms; Figure 6D).

**Effect of Apamin on VF Dynamics in Failing Ventrices**

At baseline, VF was inducible in all 7 HF ventricles, but in only 1 of the 7 normal ventricles (\( P < 0.01 \)). A total of 36 VF episodes (9 at baseline, 27 after apamin) were induced in HF ventricles. Figure 7A shows the p-ECG recordings of VF at baseline and after apamin infusion in a HF ventricle. The dominant frequency of VF was decreased from 13.0 Hz (95% CI, 8.2–17.8) at baseline to 10.0 Hz (95% CI, 6.8–13.2) after apamin infusion (\( P = 0.028 \); Figure 7B). Consecutive phase maps sampled at 20-ms intervals during VF were analyzed for phase singularities (wavebreaks). Figure 7C shows consecutive phase maps with phase singularities (black arrows) at baseline and after apamin infusion in a failing ventricle. Apamin decreased the number of phase singularities (\( P = 0.028 \); Figure 7D). Figure 8 shows the outcomes of VF episodes in HF ventricles. At baseline, most (8 of 9) VF were shock-terminated (>180 s in duration) episodes (Figure 8A, top and Figure 8B). After apamin infusion, most (15 of 27) VF became self-terminated (<180 s in duration) episodes (Figure 8A, bottom and Figure 8B; \( P = 0.026 \)). We defined 180 s as the duration of VF if VF did not terminate at that time. The VF duration was decreased from 160 s (95% CI, 112–209) at baseline to 80 s (95% CI, 8–151) after apamin infusion in HF ventricles (\( P = 0.043 \); Figure 8C).

Furthermore, there is a positive correlation between SD of APD₀ at all mapped pixels and the duration of VF in failing ventricles (at PCL 300 ms, \( r = 0.57, P = 0.03 \); at PCL 260 ms, \( r = 0.61, P = 0.02 \)).

**Discussion**

We found that \( I_{KAS} \) activation is important in preserving repolarization reserve of failing ventricles at slow heart rates. However, \( I_{KAS} \) activation is also a major factor that underlies the repolarization heterogeneity. At very short PCLs, Ca²⁺ accumulation activates \( I_{KAS} \) and steepens the APDR curve, which facilitates the generation and maintenance of VF. Apamin flattens the APDR at short PCL, decreases wavebreaks during VF activation, reduces the dominant frequency of VF and shortens the duration of VF. These findings suggest that \( I_{KAS} \) blockade can potentially be antiarrhythmic by flattening APDR, but proarrhythmic by prolonging APD during bradycardia in failing ventricles.
Secondary Rise of \( \text{Ca}_i \) at Long PCLs

Secondary rise of \( \text{Ca}_i \) during the late action potential plateau is commonly observed in HF but not normal ventricular cells.16 The mechanism of secondary rise of \( \text{Ca}_i \) is attributed to reduced sarcoplasmic reticulum \( \text{Ca}_i \) release, resulting in less \( \text{Ca}_i \)-induced inactivation of L-type \( \text{Ca} \) current (\( I_{\text{CaL}} \)) at the phases 2 and 3 of the action potential.17 As repolarization continues, the driving force for \( \text{Ca} \) entry increases and promotes greater \( \text{Ca} \) entry and additional sarcoplasmic reticulum \( \text{Ca} \) release during the latter phase of the plateau. Under these conditions, the combination of the increased late \( I_{\text{CaL}} \), together with increased Na–\( \text{Ca} \) exchange current (\( I_{\text{exCa}} \)), causes further APD prolongation. In the present study, we showed that nearly 20% of the epicardial cells demonstrated secondary rises of \( \text{Ca}_i \). These same areas also had longer baseline APD\(_{\text{80}}\) which was less responsive to apamin, compared with areas without secondary \( \text{Ca}_i \) rises, although the differences were not statistically significant. A possible explanation is that these areas exhibited less upregulation of \( I_{\text{KAS}} \) than areas without secondary \( \text{Ca}_i \) rises (ie, in the areas without secondary \( \text{Ca}_i \) rises, the upregulation of \( I_{\text{KAS}} \) was sufficient to compensate for the increased inward currents, preventing the secondary \( \text{Ca}_i \) rises and APD prolongation). This notion is supported by the observation that \( I_{\text{KAS}} \) blocker can induce secondary rise of \( \text{Ca}_i \) in nearly 6% of the mapped areas where no secondary rise of \( \text{Ca}_i \) was observed at baseline in failing ventricle. Electrophysiological heterogeneity is commonly observed in failing ventricles, and contributes to the mechanisms of arrhythmogenesis.1,18,19 These heterogeneities are related in part to the differential remodeling of various K channels transmurally. In this study, we found that significant heterogeneity of APD is also present in different regions of the epicardium in failing ventricles at long PCL, which was decreased by apamin. These findings suggest that heterogeneous \( I_{\text{KAS}} \) upregulation contributes to the repolarization heterogeneity in failing ventricles.

\( I_{\text{KAS}} \) at Short and Long PCLs

The ventricular APD and the diastolic interval both shorten during rapid pacing. The relationship between APD and the previous diastolic interval can be used to construct an APDR curve.20 The APDR curve can be measured experimentally by delivering a single extrastimulus after a train of stimuli (extrastimulus technique) or by dynamic pacing protocol with progressively shortened PCL (dynamic pacing technique).9 As compared with the APDR curve determined by extrastimulus techniques, the dynamic protocol generates steeper slopes of the APDR curve that better mimic APD dynamics during rapid ventricular arhythmias, such as VF.6 In the present study using dynamic pacing protocol, we found that APD shortening at very short PCL could be prevented by \( I_{\text{KAS}} \) inhibition,
consistent with the hypothesis that short PCL causes Ca accumulation and activates I_{KAS}, leading to a steeper APDR. If Ca accumulation is important for APD response to rapid pacing, then epicardial cells of the failing ventricles should exhibit greater shortening of APD in failing ventricles because those cells have the highest expression of I_{KAS}. The latter

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**Table 1**

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**Figure 7.** Effects of apamin on the dominant frequency (DF) and wavebreaks of ventricular fibrillation (VF) in heart failure (HF) ventricles. A, p-ECG recordings of pacing-induced sustained VF episodes at baseline and after apamin infusion in a HF ventricle. Right, The DF distribution of VF at baseline and after apamin infusion. B, Effects of apamin on DF of VF in 6 HF ventricles. Note that the DF was decreased by apamin. C, Consecutive phase maps sampled at 20-ms interval during VF at baseline and after apamin infusion. Phase singularities (wavebreaks) are indicated by black arrowheads. Bottom, Corresponding optical recording of VF at asterisk site. D, Effects of apamin on the number of phase singularities before and after apamin infusion in HF ventricles. Note that the numbers of phase singularity are decreased by apamin. Small squares in the data box indicate the mean value. PS indicates phase singularity.

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**Figure 8.** Effects of apamin on the maintenance of ventricular fibrillation (VF) in heart failure (HF) ventricles. A, p-ECG recordings of pacing-induced VF episodes at baseline and after apamin infusion in a HF ventricle. Before apamin infusion, the pacing-induced VF episode is shock-terminated (>180 s in duration, top). After apamin infusion, the pacing-induced VF episode became self-terminated (<180 s in duration, bottom). B, C, Effects of apamin on the types (B) and duration (C) of VF episodes. Note that apamin increases self-terminated VF episodes and shortens VF duration. Small squares in the data box indicate the mean value.
prediction was confirmed by Harada et al.,21 who reported that epicardium of the failing rabbit ventricles undergoes significant APD shortening at short PCL, and that shortened APD is important to the generation of ventricular arrhythmias in that model.

A steep slope of APDR curve is associated with increased propensity for cardiac arrhythmias.5,20,22 In the present study, I_{KAS} inhibition flattened the APDRs, which in turn decreased wavebreaks, shortened VF durations, and hindered the maintenance of VF. Taken together, these data suggest that upregulation of I_{KAS} in HF ventricles plays important roles of APD shortening during rapid pacing, which in turn steepens the slope of APDR curve and promotes the initiation and maintenance of VF.

We also found that apamin significantly lengthens APD and steepens the APDR curve at long PCL, suggesting I_{KAS} is also important for ventricular repolarization at slow heart rates. A possible explanation is that failing hearts have increased intracellular Na concentration. At slow pacing rates, the reverse mode of NCX current may promote C_{a} transport and sarcoplasmic reticulum Ca loading with enhanced sarcoplasmic reticulum Ca release.23,24 The increased C_{a} results in more I_{KAS} activation. Apamin, therefore, has greater effects at very long PCL than at the intermediate PCL.

**Apamin as a Selective Blocker of SK Currents**

Apamin is a highly selective blocker of SK currents.14,25,26 Even among the SK channels, apamin only selectively blocks SK2 and SK3. It does not block SK1 at 100 nmol/L,26 the concentration used in the present study. Due to its subtype selectivity, we have used the term I_{KAS} rather than I_{K(Ca)} to describe the K current that is blocked by apamin. The only other current blocked by apamin is the fetal L-type Ca^{2+} current.27 Blocking that inward current should not prolong the APD as observed in the present study. However, it might explain the APD shortening observed in some areas of normal ventricles after apamin administration. Therefore, we propose that the changes of APD and arrhythmia inducibility after apamin administration are due to the blockade of the currents conducted by SK channels.

**Clinical Implications**

K currents are vital for cardiac repolarization. Downregulation of the K currents in HF is thought to contribute significantly to reduced repolarization reserve that promotes afterdepolarizations, ventricular arrhythmias, and sudden death.1,28 Upregulation of I_{KAS} during bradycardia might increase repolarization reserve and prevent afterdepolarizations. On the contrary, I_{KAS} upregulation at tachycardia (short CL) might shorten APD and steepen APDR, promoting ventricular arrhythmia. Therefore, similar to other K channel blockers, our data suggest that I_{KAS} blockers can be both proarrhythmic and antiarrhythmic, depending on the clinical situations associated with arrhythmogenesis. I_{KAS} blockade may prevent transition from ventricular tachycardia to VF and also prevent the spontaneous reinitiation of VF and electrical storm.9 On the contrary, if the arrhythmia is bradycardia dependent, such as those induced by early afterdepolarizations, I_{KAS} blockers may promote triggered activity and ventricular arrhythmia.

**Study Limitation**

A limitation of the study is that the mapping was performed only on the epicardial surface. These findings may not be applicable to midmyocardial or endocardial layers of the cells. A second limitation is that, due to a lack of specific antibody against SK2 channels in rabbits, we were not able to obtain reliable data on SK protein levels in failing rabbit ventricles. We have attempted to use a commercial antibody (Abcam, ab83733) for Western blot analyses of the SK2 protein levels in HF and normal controls rabbit ventricles. Due to low signal:noise ratio, the results showed a statistically insignificant increase of SK2 protein in failing ventricles (see online-only Data Supplement). However, reliable anti-SK2 antibody is available for failing human ventricles. Chang et al.13 showed that the native hearts of transplant recipients have both increased I_{KAS} and SK2 protein concentrations. We did not measure the SK current in this study with patch clamp techniques. However, 2 other studies from our laboratory9,13 have documented the presence of apamin-sensitive K currents in failing ventricles using patch clamp techniques.

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**Disclosures**

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**References**


**CLINICAL PERSPECTIVE**

Heart failure (HF) is associated with prolonged action potential duration (APD) and increased incidence of sudden cardiac death. The mechanisms remain poorly understood. Our recent studies in failing rabbit and human ventricles showed that apamin-sensitive small conductance calcium-activated K current (I_{KAS}) is upregulated. I_{KAS} upregulation may shorten the APD and facilitate spontaneous recurrence of ventricular fibrillation after successful defibrillation. However, because multiple other K currents are downregulated in HF, upregulation of I_{KAS} may also play an important role in maintaining ventricular repolarization reserve, which is antiarrhythmic. In the present study, we found that I_{KAS} activation is a major factor that underlies the repolarization heterogeneity in failing ventricles during slow heart rate. Apamin, which specifically blocks I_{KAS} results in lengthening of the APD and steepens the APD restitution (APDR) curve at slow pacing rates. On the contrary, blocking I_{KAS} by apamin flattens the APD restitution at the fast pacing rates. It also decreases wavebreaks, reduces the dominant frequency, and shortens the duration of ventricular fibrillation. These findings suggest that I_{KAS} upregulation in HF may be antiarrhythmic by preserving the repolarization reserve during slow heart rates but proarrhythmic during fast heart rates or immediately after successful ventricular defibrillation, when there is persistent elevation of intracellular calcium. Thus I_{KAS} blockade may be both antiarrhythmic and proarrhythmic in HF. Understanding these effects may lead to improved safety and efficacy of drugs used in HF.
Apamin-Sensitive Potassium Current Modulates Action Potential Duration Restitution and Arrhythmogenesis of Failing Rabbit Ventricles
Yu-Cheng Hsieh, Po-Cheng Chang, Chia-Hsiang Hsueh, Young Soo Lee, Changyu Shen, James N. Weiss, Zhenhui Chen, Tomohiko Ai, Shien-Fong Lin and Peng-Sheng Chen

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SUPPLEMENTAL MATERIAL

Methods
This study protocol was approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine and the Methodist Research Institute, and conforms to the Guide for the Care and Use of Laboratory Animals. New Zealand white female rabbits (N=27) were used. We attempted to induce HF in 10 rabbits by rapid pacing. Among them, 7 completed the pacing protocol and developed HF. The remaining 3 rabbits died 1.7 [95% CI, 0.2 to 3.1] days after the onset of tachycardia pacing at 250 bpm. We studied 7 normal rabbit hearts as controls. The other 10 hearts were used for western blot analyses (5 with pacing-induced HF and 5 normal controls).

Pacing-induced Heart Failure
Rapid ventricular pacing was used to induce HF. Surgery was performed with isoflurane general anesthesia. After left lateral thoracotomy, an epicardial pacing lead was sutured at the lateral wall of left ventricle and connected to a modified single chamber ventricular pacemaker (Kappa or Enpulse pacemaker, Medtronic, Inc., Minneapolis, MN, USA) for tachycardia pacing. After 1 week of convalescence, the ventricles were paced at 250 bpm for 3 days, 300 bpm for 3 days, and 350 bpm for 3-5 weeks to induce HF. Left ventricular dimension and systolic function were assessed by echocardiography before surgery and after 3-5 weeks of ventricular pacing.

Optical Mapping
The rabbits were intravenously injected with 1,000 units of heparin and anesthetized with sodium pentobarbital (35 mg/kg). After a median sternotomy, the hearts were rapidly excised and Langendorff perfused at 25 to 35 mL/min with oxygenated Tyrode’s solution (in mmol/L: NaCl 125, KCl 4.5, NaHCO₃ 24, NaH₂PO₄ 1.8, CaCl₂ 1.8, MgCl₂ 0.5, and glucose 5.5) with a pH of 7.40. The hearts were stained with Rhod-2 AM (1.48 μmol/L) for Ca and RH237 (10 μmol/L) for Vm mapping. The double-stained hearts were illuminated with a laser at 532 nm
wavelength. The fluorescence was filtered and recorded simultaneously with dual CMOS cameras (Brain Vision, Tokyo, Japan) at 2ms/frame and 100x100 pixels with a spatial resolution of 0.35x0.35 mm² per pixel. The fluorescence obtained through a common lens was separated with a dichroic mirror (650 nm cutoff wavelength), and directed to the respective camera with additional filtering (715 nm long pass for Vm and 580±20 nm for Ca). Optical signals were processed with both spatial (3X3 pixels Gaussian filter) and temporal (3 frames moving average) filtering. Phase mapping was performed to evaluate the location and evolution of phase singularities (PSs). Blebbistatin (10-20 µmol/L, Tocris, Ellisville, MO) was used to inhibit motion artifact during optical mapping.

**Experiment Protocol**

A pair of hook bipolar electrodes was inserted into the posterior wall of right ventricle for pacing. A pseudo-ECG was obtained with widely spaced bipolar electrodes to determine ventricular rhythm. S1 dynamic pacing protocol (2X diastolic threshold) was used to determine the APDR at baseline and after 30-min apamin infusion (100 nM). The ventricles were initially paced at a constant PCL of 350 ms. The PCLs were progressively shortened (350, 300, 280, 260, 240, 220, 200, 190, 180, 170, 160, 150 ms) until VF was induced or the loss of 1:1 capture of the ventricles. Optical recording was performed after 30 beats of stable pacing at each PCL. If VF was not induced by the dynamic pacing protocol, 2-3 attempts of burst pacing (PCL 50-100 ms, pacing duration 5-10 s) was used to test whether or not VF was inducible. The same protocol was used to test VF inducibility before and after apamin infusion in both normal and HF ventricles. Optical recordings were then performed during VF. We allowed VF to continue for at least 180 s before defibrillation.

**Western Blotting**

Myocardial blocks from left ventricles (5 HF and 5 normal controls) were excised and chopped immediately after harvesting. 100 mg tissues were homogenized by POLY TRON in 1 ml RIPA buffer with protease inhibitor (50 mM Tris pH 8.4, 150 mM NaCl, 1% NP40, 0.5%
sodium deoxycholate 1 mM PMSF, 2 µg/ml leupeptin, 1 µg/ml pepstatin A, and 5 µg/ml aprotinin). Homogenates were incubated on ice for 30 min and then centrifuged at 14,000 rpm for 15 min. 20 µg of supernatants were subjected to electrophoresis using Bio-Rad mini gel system. The separated proteins were transferred to PVDF (Millipore). The membrane was bathed in TBS with 5% milk for one hour, and probed with either anti-KCNN2 antibody (for detecting SK2 channel, Abcam, ab83733, 1:2500) or anti-GAPDH antibody (PIERCE, MA1-22670, 1:2500) overnight. After the interaction with primary antibody, the membrane was incubated with HRP-conjugated anti-rabbit or anti-mouse secondary antibodies (sigma, 1:5000) for 30 min. Finally, Luminata Crescendo HRP substrate (Millipore, WBLUR100) was added onto the membrane according to manufacturer’s instruction.

Data Analysis

Construction of APDR Curves

Optical APD\textsubscript{80} was measured at 80% repolarization. The APD\textsubscript{80} was measured by computerized methods using all available pixels on the ventricles, excluding the atria and the pixels at the edge of the ventricles. APDR curve was constructed by plotting APD\textsubscript{80} against the preceding diastolic interval (DI), defined by the interval between 80% repolarization and the onset of the next action potential. APD alternans was defined as the difference in APD\textsubscript{80} of 2 consecutive beats of $\geq$4 ms during dynamic pacing. Both long and short APDs observed during alternans are included in plotting APDR curve. The slopes of APDR were calculated by first-order exponential fitting with ORIGIN software (Microcal). Two-dimensional (2D) APD\textsubscript{80} maps were constructed to study the spatial distribution of APDs on the epicardial surfaces of the hearts. We also analyzed the correlation between baseline APD\textsubscript{80} and delta APD (apamin-treated APD\textsubscript{80} minus baseline APD\textsubscript{80}) at PCL of 350, 300, and 260 ms.

Fast Fourier Transforms (FFTs) Analysis and Epicardial Wavebreaks during VF

FFTs of pseudo-ECGs (4 s in duration) were used to determine the dominant frequency (DF) of VF at baseline and after apamin infusion.\textsuperscript{3, 4} For each optical recording, optical data were
acquired continuously for 4.096 s (2048 frames). A PS shown on the phase maps was defined as a site with an ambiguous phase surrounded by pixels exhibiting a continuous phase progression from $-\pi$ to $+\pi$. Previous studies suggest that PSs are a robust alternate representation of wavebreaks, which serve as the source of VF. To quantify wavebreaks during VF, the numbers of PSs in the phase map were counted manually every 10 frames for 1,000 frames in each episode of VF.

**Statistical Analysis**

Data are presented as mean and 95% confidence interval (CI). Paired and unpaired Student's t-tests were used to compare the data within and between groups. Categorical parameters between groups were compared by Fisher's exact test. A two-sided p-value of $\leq 0.05$ was considered significant.

**Results**

**SK2 Protein**

Western blotting was performed in a separate group of 5 normal control and 5 failing hearts which were not used for optical mapping. Immunoblot in Figure A shows antibody weakly identified expression of SK2 channels in these samples. Figure B shows the SK2/GAPDH ratio of all hearts studied. The difference between normal and failing ventricles was not statistically significant (for at least 3 measurements, $p=0.078$).

**References**


**Online Supplement Figure 1.** SK2 protein analysis. A. An example of immunoblot shows that the anti-SK2 antibody weakly identified expression of SK2 channels in these samples. B. Plot shows a compilation of the SK2/GAPDH ratio of all ventricles studied (at least 3 measurements). Note that there was statistically insignificant increase of the SK2/GAPDH ratio in HF ventricles as compared with normal ventricles. SK2, small conductance Ca-activated K channel subtype 2.