Small-Conductance Calcium-Activated Potassium Channel and Recurrent Ventricular Fibrillation in Failing Rabbit Ventricles


Rationale: Fibrillation/defibrillation episodes in failing ventricles may be followed by action potential duration (APD) shortening and recurrent spontaneous ventricular fibrillation (SVF).

Objective: We hypothesized that activation of apamin-sensitive small-conductance Ca$^{2+}$-activated K$^+$ (SK) channels are responsible for the postshock APD shortening in failing ventricles.

Methods and Results: A rabbit model of tachycardia-induced heart failure was used. Simultaneous optical mapping of intracellular Ca$^{2+}$ and membrane potential ($V_m$) was performed in failing and nonfailing ventricles. Three failing ventricles developed SVF (SVF group); 9 did not (no-SVF group). None of the 10 nonfailing ventricles developed SVF. Increased pacing rate and duration augmented the magnitude of APD shortening. Apamin (1 μmol/L) eliminated recurrent SVF and increased postshock APD$_{50}$ in the SVF group from 126±5 to 153±4 ms ($P<0.05$) and from 147±2 to 162±3 ms ($P<0.05$) in the no-SVF group but did not change APD$_{50}$ in nonfailing group. Whole cell patch-clamp studies at 36°C showed that the apamin-sensitive K$^+$ current ($I_{KAS}$) density was significantly larger in the failing than in the normal ventricular epicardial myocytes, and epicardial $I_{KAS}$ density was significantly higher than midmyocardial and endocardial myocytes. Steady-state Ca$^{2+}$ response of $I_{KAS}$ was leftward-shifted in the failing cells compared with the normal control cells, indicating increased Ca$^{2+}$ sensitivity of $I_{KAS}$ in failing ventricles. The $K_d$ was 232±5 nmol/L for failing myocytes and 553±78 nmol/L for normal myocytes ($P=0.002$).

Conclusions: Heart failure heterogeneously increases the sensitivity of $I_{KAS}$ to intracellular Ca$^{2+}$, leading to upregulation of $I_{KAS}$ postshock APD shortening, and recurrent SVF. (Circ Res. 2011;108:00-00.)

Key Words: arrhythmia ■ intracellular calcium ■ ion channels ■ ventricular fibrillation

Electrical storm describes a clinical condition in which the patients experience recurrent spontaneous ventricular fibrillation (SVF) requiring multiple defibrillation shocks within a short period of time. It occurs frequently in patients with heart failure (HF) and is an important reason for rehospitalization. In addition, recurrent SVF occurs in roughly half of the patients undergoing cardiopulmonary resuscitation and may be associated with significant morbidity and mortality. The mechanisms of electrical storm remain unclear. We recently developed a rabbit model of electrical storm with pacing-induced HF. In that model, acute but reversible postshock action potential duration (APD) shortening induced recurrent SVF by promoting late phase 3 early afterdepolarizations (EADs) and triggered activity. However, the mechanisms underlying acute APD shortening after fibrillation/defibrillation episodes in failing ventricles remain unclear. Because VF is associated with intracellular Ca$^{2+}$ (Ca$_i$) accumulation, especially in failing ventricles, we hypothesized that apamin-sensitive small-conductance Ca$^{2+}$-activated K$^+$ (SK) channels may be responsible, in part, for postshock APD shortening. Apamin is a neurotoxin that selectively blocks SK channels. Several studies show that apamin-sensitive K$^+$ current ($I_{KAS}$) is abundantly present in cardiac atrial cells but not in normal ventricular cells. We were unable to find any studies on $I_{KAS}$ regulation in heart failure (HF) ventricles. Therefore, we
studied normal and HF rabbits to test the hypotheses that IKas is significantly increased in HF rabbit ventricular myocytes and that IKas blockade prevents postshock APD shortening and recurrent SVF in Langendorff-perfused HF ventricles.

**Methods**

An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org.

New Zealand White rabbits (N=39) were used in the study. Among them, 5 died during rapid pacing, 22 were used for optical mapping (including 12 with pacing-induced HF, 3 sham with pacemaker implanted but not paced and 7 normal control) and 12 were used for patch clamp studies (6 HF and 6 normal control).

**Optical Mapping Studies**

We simultaneously mapped intracellular Ca2+ (Ca2) and membrane potential (Vm) using Rhod-2 AM and RH237, respectively, in ventricles of Langendorff-perfused hearts. Rapid ventricular pacing and 3 to 5 ventricular fibrillation (VF)-defibrillation episodes were mapped. Apamin (1 μmol/L) was added to the perfusate for 30 minutes before the same protocols were repeated. We then waited for 30 minutes to determine whether the effects of apamin can be washed out.

**Patch Clamp Studies**

Whole-cell patch-clamp technique was used to record apamin-sensitive K+ currents (IKas) in isolated ventricular cardiomyocytes. All experiments were performed at 36°C. To study Ca2+-dependence of IKas, various combinations of EGTA and CaCl2 were used in the pipette solutions.

**Real-Time PCR**

Ventricular tissues were sampled from 5 normal and 5 HF rabbits before cell isolation for patch-clamp studies. Real-time PCR was performed using iQ SYBR Green Supermix with iCycler (Bio-Rad, Hercules, CA).

**Statistical Analyses**

Statistical analyses were performed using SPSS PASW Statistics 17 software (IBM, Chicago, IL). We used Student t test for a comparison between 2 groups. One-way ANOVA with post hoc Bonferroni tests were performed for comparisons among 3 or more groups. A P≤0.05 was considered as statistically significant.

**Results**

**Evidence of HF**

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. Echocardiograms of sham-operated rabbits (N=3) at baseline and at second surgery showed no changes of left ventricular (LV) end-diastolic dimension (12.5±0.9 mm versus 13.0±0.6 mm), end-systolic dimension (6.5±1.5 mm versus 6.9±1.7 mm), or fractional shortening (0.49±0.08 versus 0.48±0.11) (P=NS for all comparisons). In contrast, HF rabbits (N=13) showed significant increases in end-diastolic dimension (14.5±0.2 mm versus 19.9±0.5 mm) and in end-systolic dimension (8.4±0.3 mm versus 17.0±0.3 mm) and reduced fractional shortening (0.42±0.01 versus 0.10±0.01) (P<0.05 for all comparisons). The percent LV fibrosis was 5%±1% for normal (N=3) or sham-operated hearts and 21%±6% for failing hearts (N=5) (P<0.05). Action potential duration measured at 80% repolarization (APD80) with pacing cycle length (PCL) of 300 ms were not different between sham-operated and normal rabbits (166±5 ms versus 168±2 ms, P>0.05). Therefore, these data were combined into a single nonfailing group. There were no significant differences in RR intervals during the sinus rhythm (SRm) between nonfailing and failing hearts (464±38 ms versus 509±42 ms, P=NS). Figure 1A shows marked cardiac enlargement (right) typical for all failing hearts. Action potential duration (APD) prolongation was present in all failing ventricles. Figure 1B shows APD80 at 300 ms PCL recorded from the site marked by an asterisk in APD80 maps in Figure 1C. Overall, the failing ventricles (N=7) had a longer APD80 than nonfailing ventricles (N=7) (181±5 ms versus 167±2 ms, P<0.05; Figure 1D). The Ca2+ transient duration at 80% repolarization (Ca2+TD80) was 184±5 ms for failing ventricles and 176±3 ms for nonfailing ventricles (P=NS).

**Effects of Apamin on Acute APD Shortening and Postshock SVF**

After fibrillation/defibrillation episodes, acute but transient shortening of APD occurred in all 12 failing ventricles. Three of them developed SVF in the postshock period (the “SVF-Group”). Figure 2A (left) shows Vm (black line) and Ca2+ (red line) optical signals recorded during one of the SVF episodes. There were 2 postshock beats (1 and 2) before VF termination. Optical maps confirmed complete cessation of wave fronts after beat 2, consistent with type B successful defibrillation.17 However, there was a large Ca2+ transient and short APD (beat 3). The first beat of SVF (beat 4) began during late phase 3 of the preceding sinus beat. In Figure 2A (b), an isochronal map illustrates that beat 4 originated from the basal portion of the RV. The right 2 columns are ratio maps of Vm and Ca2+, respectively, of the same beat. The Ca2+ remained elevated throughout the mapped field, whereas Vm had already repolarized. The focal beat originated from the left upper quadrant during persistent Ca2+ elevation, consistent with the late phase 3 EAD mechanism.5,18 Five SVF episodes were recorded after initial successful defibrillation in this
CaiTD80 and APD80 (Figure 3B). The pseudo-ECG tracings baseline, associated with smaller differences between defibrillation had less APD shortening as compared with 2. After pretreatment with apamin, beats 1 and 2 after defibrillation, whereas Cai remained elevated after repolarization. Acute APD shortening occurred after optical traces of SRm and in the postshock period in the failing hearts. B, Black and red lines indicate optical tracings ofVm and intracellular calcium (Ca2+), respectively. The optical tracings were recorded from the site labeled by an asterisk in APD80 map and Ca2+TD80 map in C. C, APD80 map (upper images) and Ca2+TD80 map (lower images) obtained from normal (left images) and failing ventricles (right images). Averages of APD80 and Ca2+TD80 in the study were measured from all pixels in the map, excluding the atrial signals and the pixels at the edge of ventricles. D. Average of APD80 and Ca2+TD80 at fixed PCL of 300 ms in 7 nonfailing and 7 failing ventricles. Bar graphs represent means±SEM; APD80 and Ca2+TD80 indicate APD and Ca2+ transient duration, respectively, measured at 80% repolarization. HF indicates heart failure; LAD, left anterior descending artery; LV, left ventricle; no-HF, nonfailing heart; RV, right ventricle.

failing heart. Apamin prevented both the postshock APD shortening and SVF (Figure 2B). We repeated fibrillation/defibrillation episodes four times, but no SVF was observed in the postshock period after apamin. Among the 12 failing ventricles studied, 3 developed a total of 11 episodes of SVF (4 normal and 3 sham-operated ventricles) and 7 failing ventricles. A, Nonfailing and failing hearts. B, Black and red lines indicate optical tracings ofVm and intracellular calcium (Ca2+), respectively. The optical tracings were recorded from the site labeled by an asterisk in APD80 map and Ca2+TD80 map in C. C, APD80 map (upper images) and Ca2+TD80 map (lower images) obtained from normal (left images) and failing ventricles (right images). Averages of APD80 and Ca2+TD80 in the study were measured from all pixels in the map, excluding the atrial signals and the pixels at the edge of ventricles. D, Average of APD80 and Ca2+TD80 at fixed PCL of 300 ms in 7 nonfailing and 7 failing ventricles. Bar graphs represent means±SEM; APD80 and Ca2+TD80 indicate APD and Ca2+ transient duration, respectively, measured at 80% repolarization. HF indicates heart failure; LAD, left anterior descending artery; LV, left ventricle; no-HF, nonfailing heart; RV, right ventricle.

In four of the 12 failing hearts, we also studied different doses of apamin on postshock APD80. The results show that apamin concentration of 0.1, 0.3 and 1.0 μmol/L prolonged postshock APD80 to similar duration, whereas 0.01 μmol/L apamin had little effects on postshock APD80 (Online Figure I).

Effects of Apamin on Baseline APD
Examples of APD80 maps (bottom) and corresponding optical traces of action potentials from a single representative pixel (top) are presented in Figure 4A. Apamin induced more APD80 prolongation in failing than in nonfailing ventricles at PCL of 300 ms. The APD prolongation predominantly occurred in the terminal portion of repolarization. Figure 4B shows the magnitude of APD80 prolongation map obtained from the same nonfailing and failing ventricles presented in Figure 4A. At PCL of 300 ms, the percentage of APD prolongation with apamin was 5.4±0.7% for failing ventricles compared with 1.4±0.6% in nonfailing ventricles (P<0.05) (Figure 4C).

The APD and Ca2+TD were approximately the same, and SVF episodes were completely prevented. PM indicates papillary muscle; S, interventricular septum; RV, right ventricle.
Effects of Apamin on APD After Rapid Pacing

We used rapid pacing to increase $C_{ai}$. After abrupt cessation of rapid ventricular pacing, APD shortening ($\Delta$APD$_{80}$) was greater in failing than in nonfailing ventricles. The dependence of $\Delta$APD$_{80}$ on the PCL (Pacing Protocol I) and pacing duration (Pacing Protocol II) are presented in Figure 5 and Online Figure II, respectively. The shorter PCL and longer pacing duration elicited more APD shortening, and apamin reduced $\Delta$APD$_{80}$ after these rapid pacing protocols in failing ventricles. Figure 5A shows the effects of Pacing protocol I on $\Delta$APD$_{80}$ in a nonfailing ventricle at baseline (control) and after apamin administration. Figure 5B shows that apamin did not significantly change $\Delta$APD$_{80}$ in nonfailing ventricles. Figure 5C shows an example obtained from a failing ventricle with Pacing Protocol I. In this example, apamin reduced $\Delta$APD$_{80}$ from 23 ms to 8 ms. Figure 3D summarizes the effects of apamin in failing ventricles. Apamin significantly reduced $\Delta$APD$_{80}$ in failing but not in nonfailing ventricles.
Similar results were noted for the dependence of \( \Delta \text{APD}_{80} \) on pacing duration (pacing protocol II; Online Figure II).

**Effects of Apamin on Postshock APD in the No-SVF Group**

In 9 of 12 failing ventricles, SVF did not occur after fibrillation/defibrillation episodes (the “no-SVF” Group). Similar to the SVF Group, acute APD shortening was also observed in this group after a fibrillation/defibrillation episode. Figure 6A shows the optical tracing and corresponding APD maps in SRm (left) and after defibrillation (right) in a failing ventricle without SVF. Similarly, apamin prolonged the APD immediately after defibrillation shock in failing ventricles that did not develop SVF (Figure 6A, b). Note the difference between \( \text{Cai}_{TD80} \) and APD maps. In contrast, no acute shortening of APD was observed immediately after a successful defibrillation shock in nonfailing ventricles. Pretreatment with apamin did not result in significant APD changes in nonfailing ventricles (Figure 6B).

**Postshock APD Changes of All Ventricles Studied**

We plotted APD of the first 2 postshock beats against the preceding diastolic interval (DI) for all episodes. Apamin did not change postshock APDs in nonfailing ventricles (Figure 7A), but prolonged postshock APDs significantly in failing ventricles of both the no-SVF Group (Figure 7B) and in the SVF Group (Figure 7C). We performed statistical analyses with repeated-measure ANOVA to compare the \( \Delta \text{APD}_{80} \) before and after apamin. The \( \Delta \text{APD}_{80} \) was 5.8 ms (-2.2 ms to 13.8 ms; \( P < 0.05 \)) for nonfailing ventricles, indicating that apamin did not significantly change the postshock APD. For nonfailing ventricles, there was no significant change in postshock APD. For failing ventricles, the \( \Delta \text{APD}_{80} \) was statistically significant after apamin treatment. The differences in \( \Delta \text{APD}_{80} \) before and after apamin were statistically significant for both the no-SVF and the SVF Groups.

**Figure 5. APD after cessation of rapid pacing.**

In A and C, the first and third rows show action potential at fixed-rate pacing at 300 ms PCL. The second and fourth rows show action potentials during rapid pacing followed by a postpacing beat. The preceding cycle length of the first postpacing beat was controlled by an \( S_2 \) given 300 ms after the last \( S_1 \). The APD of the first postpacing beat was measured to 80% repolarization (APD\(_{80}\)). The same pacing protocol was performed at baseline and after apamin (1 \( \mu \)mol/L). B, Effects of apamin on \( \Delta \text{APD}_{80} \) in all nonfailing ventricles studied. D, Effects of apamin in all failing ventricles studied. Bar graphs represent means±SEM; 1:1 indicates the shortest PCL associated with 1:1 capture. *\( P < 0.05 \) vs control.

**Figure 6. Effects of apamin on postshock APD in no-SVF Group and nonfailing hearts.**

A, Left, Baseline \( V_m \) (black line) and \( \text{Cai} \) (red line) recorded from site marked by an asterisk in APD\(_{80}\) maps in SRm (left) and after defibrillation (right) in a failing ventricle without SVF. Similarly, apamin prolonged the APD immediately after defibrillation shock in failing ventricles that did not develop SVF (Figure 6A, b). Note the difference between \( \text{Cai}_{TD80} \) and APD\(_{80}\) maps. In contrast, no acute shortening of APD was observed immediately after a successful defibrillation shock in nonfailing ventricles. Pretreatment with apamin did not result in significant APD changes in nonfailing ventricles (Figure 6B).

**Figure 7. Postshock APD of all episodes.**

A, no-SVF Group (left) and SVF Group (right). The data are plotted as mean±SEM. B, No-failing hearts. C, Failing hearts. The differences in \( \Delta \text{APD}_{80} \) before and after apamin were statistically significant for both the no-SVF and the SVF Groups.
Effects of Glibenclamide on ∆APD_{80} in Failing Ventricles

To determine whether ATP-sensitive potassium current (I_{KATP}) activation was responsible for the APD shortening in failing ventricles, we examined the effect glibenclamide (10 μmol/L), which mainly inhibit I_{KATP} channel in this range of concentrations, in 3 failing ventricles. As shown in Online Figure III, glibenclamide did not significantly affect the magnitude of post-rapid pacing and postshock APD shortening compared with control.

I_{KAS} in Failing Ventricles

The density and properties of I_{KAS} were examined in cardiomyocytes isolated from normal and failing rabbit left ventricles using the voltage-clamp technique in whole-cell mode. Figure 8A shows representative current traces obtained with a step-pulse protocol (300 ms pulse duration; holding potential, −50 mV; see inset) in the absence and presence of 100 nmol/L apamin in the bath solution. Mean I_{KAS} density (determined as the apamin-sensitive difference current) was significantly larger in failing than in normal ventricular epicardial myocytes (I_{KAS} density at 0 mV with an intrapetite free Ca^{2+} of 863 nmol/L: 8.39 ± 1.00 pA/pF, n = 6 cells from 5 failing rabbits, versus 2.83 ± 0.87 pA/pF, n = 6 cells from 4 normal rabbits, P < 0.01). In contrast, when the intrapetite free-Ca^{2+} was buffered to 100 nmol/L, the apamin-sensitive current was much smaller, with no significant difference between normal and failing ventricular myocytes (Figure 8D). Figure 8B illustrates the I_{KAS}-voltage (I−V) relationships. Transmural distribution of I_{KAS} in failing ventricles was studied using cardiomyocytes isolated from 3 layers (6 epicardial cells, 5 midmyocardial cells, and 7 endocardial cells from 5 animals). Mean I_{KAS} density in epicardial myocytes (8.52 ± 2.47 pA/pF, range 5.21 to 11.46 pA/pF) was significantly larger than midmyocardial cells (2.18 ± 2.14 pA/pF, range 0.00 to 5.70 pA/pF) and endocardial cells (1.87 ± 2.36 pA/pF, range 0.00 to 6.04 pA/pF) (Figure 8C). To further elucidate the mechanisms underlying I_{KAS} upregulation, Ca^{2+}-dependence of I_{KAS} was studied in epicardial cells using pipette solutions containing increasing intracellular free Ca^{2+} concentrations. Figure 8D demonstrates that the steady-state Ca^{2+} response of I_{KAS} was leftward-shifted in the failing cells compared with the normal cells. The data were fitted with the Hill equation, yielding K_d of 232 ± 5 nmol/L for failing and 553 ± 78 nmol/L (P = 0.002) for normal cells, and Hill coefficients of 2.38 ± 0.13 for failing and 1.50 ± 0.30 for normal cells (P = 0.01). These results are compatible with the notion that the K^{+} channels...
carrying $I_{\text{KAS}}$ have increased sensitivity to cytosolic Ca$^{2+}$ in failing ventricles.

Quantitative PCR
The SK2 mRNA levels (normalized to GAPDH) in normal (N=5) and failing (N=5) ventricles were 100±6.4% and 118±8.5%, respectively (P=0.39).

Discussion
The primary finding of this study is that heart failure heterogeneously increases the sensitivity of $I_{\text{KAS}}$ to intracellular Ca$^{2+}$, leading to upregulation of $I_{\text{KAS}}$ postshock APD shortening and recurrent SVF.

$I_{\text{KAS}}$ in the Normal and Failing Ventricles
The primary function SK channels in the nervous system is to produce afterhyperpolarization following a neural action potential and to protect the cell from the deleterious effects of continuous tetrac activity. Seminal studies from multiple laboratories show that SK channels are also expressed in cardiac cells, more so in the atria than in the ventricles. Our study confirmed that $I_{\text{KAS}}$ plays little role in APD regulation in normal ventricles. However, the same is not true for failing ventricles in which $I_{\text{KAS}}$ is upregulated in epicardial cells. Previous studies showed that multiple ventricular K$^+$ currents ($I_{\text{f}}, I_{\text{K1}}, I_{\text{Kr}},$ and $I_{\text{Kc}}$) are downregulated in animal models of HF, contributing to the reduced repolarization reserve. In addition, ATP-sensitive K$^+$ currents ($I_{\text{KATP}}$) are also disrupted in HF. The $I_{\text{KATP}}$, which shortens APD and protects the heart from Cai overload, is essential in maintaining the homeostasis during the metabolically demanding adaptive response to stress. In a model of HF induced by transgenic expression of the cytokine tumor necrosis factor-α, structural remodeling in failing ventricles led to disruption of energetic signal-channel communication, resulting in disturbing the $I_{\text{KATP}}$-mediated protection. Another study shows that $I_{\text{KATP}}$ channel activation under metabolic stress was impaired in myocytes from rat hypertrophied ventricle. Our study found that suppression of $I_{\text{KATP}}$ did not reduce postpacing or postshock APD shortening in failing ventricles, but apamin significantly reduced the magnitude of postpacing or postshock APD shortening. Taken together, these findings suggest that because of $I_{\text{KATP}}$ channel malfunction and downregulation of multiple other K$^+$ currents, the failing ventricles may rely on $I_{\text{KAS}}$ to shorten the APD in situations of Cai overload.

Late Phase 3 EAD and Ventricular Arrhythmogenesis
When heart rate increases, APD shortens physiologically to preserve a sufficient diastolic interval for ventricular filling and coronary flow. However, excessive APD shortening might be arrhythmogenic by promoting late phase 3 EAD18 or by inducing transmural heterogeneity of repolarization. Because of SERCA downregulation and decreased driving force of Na$^+$/Ca$^{2+}$ exchange current, myocytes in failing hearts have higher diastolic Cai concentration, slower Cai transient decay, and more Cai accumulation during burst pacing than those in normal hearts. Therefore, during rapid pacing or fibrillation in failing ventricles, the rapid depolarization may increase Cai which in turn activates $I_{\text{KAS}}$ and shortens APD in a spatially heterogeneous fashion. Marked APD shortening after fibrillation/defibrillation episodes with persistent Cai elevation during the late phase 3 of action potential results in the development of late phase 3 early afterdepolarization and recurrent SVF in failing hearts.

Heterogeneous Remodeling of $I_{\text{KAS}}$
The upregulation of $I_{\text{KAS}}$ during HF is highly heterogeneous. Although epicardial cells in average gained much more $I_{\text{KAS}}$
density than midmyocardial and endocardial cells during HF. Large variations of $I_{Kas}$ density are present within the same layer of cells. Previous studies showed that strong intercellular coupling can reduce the differences of APD between different layers of cells, making M cells “invisible” in intact human ventricles. Similarly, the highly heterogeneous distribution of $I_{Kas}$ density and the tight intercellular coupling of intact ventricles allowed cells with high $I_{Kas}$ density and shortened APD to influence the APD of the neighboring cells with low $I_{Kas}$ density. Therefore, optical mapping studies showed that postshock APD shortening occurs both in the epicardium and in the endocardium, and that apamin prevented postshock APD shortening in both layers of the ventricles.

Clinical Implications
Recurrent SVF (electrical storm) is a frequent complication in patients with advanced HF. A recent genome-wide association study showed that variants at the 1q21 locus cluster at KCNN3 (SK3) is associated with atrial fibrillation. The knowledge that $I_{Kas}$ is upregulated in HF should direct broader studies into the role of such currents in determining heart disease risk and as possible targets for preventive therapy in both atrial and ventricular arrhythmias.

Study Limitations
Apamin is a neural toxin and may induce significant neurological side effects when used in live animals and in humans. Therefore, it is necessary to perform nontoxic blockers of $I_{Kas}$ before we can test the effects of $I_{Kas}$ blockade on VF storm in human patients. Another limitation is that apamin is a potent inhibitor of the L-type Ca current ($I_{Ca,L}$). Although $I_{Ca,L}$ blockade cannot be used to explain APD lengthening after fibrillation/defibrillation episodes, this pharmacological effect could help prevent VF. Therefore, whether or not apamin prevented SVF by $I_{Kas}$ blockade or by a combined effects of $I_{Kas}$ and $I_{Ca,L}$ blockade remain unclear.

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Disclosures
None.

References


### Novelty and Significance

**What Is Known?**

- Electrical storm describes a clinical condition in which the patients experience recurrent spontaneous ventricular fibrillation (SVF) requiring multiple defibrillation shocks within a short period of time. It occurs frequently in patients with heart failure (HF).
- We developed a model of electrical storm in failing rabbit ventricles that develops acute but reversible postshock action potential duration (APD) shortening, leading to late phase 3 early afterdepolarizations (EADs), triggered activity and recurrent SVF.
- Small-conductance Ca2+-activated K+ (SK) channels are present in both the atria and ventricles. Although these channels are active in the normal atria, they conduct little or no current in normal ventricles.
- Apamin is a neurotoxin that selectively blocks SK channels.

**What New Information Does This Article Contribute?**

- We found that HF heterogeneously increases the sensitivity of the apamin-sensitive K+ current (I_{KAS}) to intracellular Ca2+, leading to upregulation of I_{KAS}, postshock APD shortening, late phase 3 EAD, triggered activity, and recurrent SVF.
- These new findings suggest that I_{KAS} is a possible new target for preventive therapy in both atrial and ventricular arrhythmias.
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SUPPLEMENTAL MATERIAL
Small-Conductance Calcium-Activated Potassium Channel and Recurrent Ventricular Fibrillation in Failing Rabbit Ventricles

Expanded Material and Methods

Material

The research protocol was approved by the Institutional Animal Care and Use Committees. New Zealand white rabbits (N=39) were used in the study. Among them, 5 died during rapid pacing, 22 were used for optical mapping (including 12 with pacing-induced HF, 3 sham with pacemaker implanted but not paced and 7 normal control) and 12 were used for patch clamp studies (6 HF and 6 normal control).

Pacing-Induced Heart Failure

HF was induced by rapid pacing. Surgery was performed with isoflurane general anesthesia. After left lateral thoracotomy, an epicardial pacing lead was placed in the lateral wall of the 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.

Optical Mapping Studies

Surgical preparation

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 the ascending aorta was retrogradely perfused at 35 to 45 mL/min in Langendorff-mode with 37°C oxygenated Tyrode’s solution equilibrated with 95% O₂ and 5% CO₂ to maintain a pH of 7.40±0.05. The composition of the Tyrode’s solution was as follows (in mmol/L): NaCl 125, KCl 4.5, NaHPO₄ 1.8, NaHCO₃ 24, CaCl₂ 1.8, MgCl₂ 0.5, glucose 5.5 and albumin 50 mg/L in deionized water.

Dual optical mapping

We simultaneously mapped intracellular calcium (Ca) and membrane potential (V_m) as described previously. Briefly, the hearts were stained with 1.48 μmol/L Rhod-2 Am for Ca and with 10 μmol/L RH237 for V_m. The hearts were illuminated with a laser (Verdi, Coherent Inc.) at a wavelength of 532 nm. The fluorescence was filtered and acquired simultaneously with two CMOS cameras (BrainVision, Tokyo, Japan) at 2 ms per frame and 100 x 100 pixels with spatial resolution of 0.35 x 0.35 mm² per pixel. We performed optical mapping of either epicardial (N=14, 7 normal and 7 HF rabbits) or endocardial (N=6, 3 normal and 3 HF rabbits) surface. For epicardial mapping, optical signals were gathered from the anterior surface of the heart including right ventricle (RV) and left ventricle (LV). We made the RV flap preparation for the endocardial mapping. To expose the endocardium, the RV flap was then created by cutting the posterior end of RV free wall from the base to the apex along the posterior descending artery. We ligated major arterial leaks to ensure continuous perfusion of the RV free wall. During optical mapping, blebbistatin (10 μmol/L to 20 μmol/L) were used to inhibit motion.

Construction and Interpretation of Two-Dimensional Maps
The average fluorescence level \( (F) \) of an individual pixel was first calculated for the duration of recording. The ratio on each pixel is then calculated as \( (F-F^{'})/F \). The image data were spatiotemporally filtered with 3 x 3 x 3 averaging. Shades of red were assigned to represent above-average fluorescence (depolarization) and shades of blue to represent below-average fluorescence (repolarization) to generate the ratio maps.

**Experimental Protocol**

Pseudo-ECGs were measured with widely spaced bipolar electrodes on right atrium and RV, and on RV and LV. A quadripolar catheter was inserted into the RV apex for pacing. VF was induced by burst pacing. The hearts were then defibrillated with transvenous electrodes placed on RV and LV with biphasic truncated exponential waveform shocks (100 to 300 V, 6-ms total duration) using a Ventritex defibrillator HVS-02 (Sunnyvale, CA, USA).

We used 2 pacing protocols to characterize post-rapid pacing action potential duration (APD) shortening. Three to five fibrillation-defibrillation episodes were induced for each ventricle. The 1st and 2nd postshock beats were mapped to measure the postshock APD. Apamin (1 µmol/L), a small conductance Ca\(^{2+}\) activated K\(^+\) (SK) channel blocker,\(^3,4\) was added to the perfusate for 30 min. The dose was chosen because Xu et al\(^5\) reported that the maximum SK channel blocking effects in atria and ventricles is achieved with 1 µmol/L apamin. In four of these failing ventricles, we tested the effects of 0.01, 0.1 and 0.3 µmol/L of apamin before 1.0 µmol/L of apamin to determine if there is dose-dependent effect of apamin on postshock APD\(_{80}\). The same rapid pacing protocols and repeated fibrillation-defibrillation episodes were then performed. In 3 failing hearts, we used glibenclamide (10 µmol/L) prior to apamin administration to determine if \( I_{KATP} \) blockade has any effects on postshock APD\(_{80}\). Apamin was then given after glibenclamide washout. At the end of the study, the hearts were harvested, formalin fixed, and sectioned for Masson’s trichrome.

**Data Analysis**

Optical APD and Ca\(_{\text{r}}\)TD were measured at 80% repolarization and recovery, respectively. Two-dimensional (2D) APD\(_{80}\) maps were constructed to study the spatial distribution of APDs on epicardial or endocardial surfaces of the heart. The APD\(_{80}\) and Ca\(_{\text{r}}\)TD\(_{80}\) were measured by computerized methods using all available pixels on the ventricles, excluding the atrial signals and the pixels at the edge of the ventricle. We performed statistical analyses with repeated-measure ANOVA to compare the differences (delta) of postshock APD before and after apamin. The percentage of fibrosis (blue-stained tissues) was determined in multiple slides per rabbit using computerized morphometry.

**Patch Clamp Studies**

**Cell isolation**

The heart was excised and mounted on a Langendorff perfusion apparatus and perfused for 5 min with Tyrode’s solution followed by perfusion with a buffer containing (in mM): NaCl, 125; MgSO\(_4\), 1.18; KCl, 4.75; KH\(_2\)PO\(_4\), 1.2; HEPES, 10; bovine serum albumin (BSA), 1 g/L; glucose, 10; taurine, 58.5; creatine, 24.9; EGTA, 0.02 (pH 7.2 with NaOH). This was followed by 15 min perfusion with the same buffer containing 200 U/ml collagenase type II (Worthington, Lakewood, NJ). The heart was removed from the perfusion apparatus. The left ventricle was then cut into small pieces and dissected mechanically to obtain cardiomyocytes.

**Electrophysiology**
Apamin-sensitive K⁺ currents (I\textsubscript{KAS}) was studied using whole-cell mode of patch-clamp techniques as described elsewhere. Briefly, whole-cell configuration was made in Tyrode’s solution, and capacitance currents were monitored with a repetitive 5 mV step pulse at least for five minutes. After compensation of whole-cell capacitance, Tyrode’s solution was replaced with a bath solution containing N-methylglucamine (NMG). Stability of total K⁺ currents (I\textsubscript{K}) was monitored with a repetitive ramp pulse protocol (holding potential, -70 mV; test potential from +20 mV to -120 mV for 400 ms; every 10 seconds; Supplemental Figure S-IV). Once the I\textsubscript{K} became stable (usually for five minutes), baseline recording was performed with a step pulse protocol as shown in Figure 8. If the baseline I\textsubscript{K} was stable, apamin was applied and a step pulse protocol was performed when the I\textsubscript{K} reached to a steady state. The stability of I\textsubscript{K} was continuously monitored with a ramp pulse protocol throughout the recordings. All experiments were performed at 36°C. Chamber temperature was precisely controlled with PH-1 heating platform, SH-27B solution heater, and TC-344B temperature controller (Warner Instruments, Hamden, CT). Voltage-pulse protocols were generated with Axopatch 200B amplifier using pCLAMP-9 software (Molecular Device/Axon, Sunnyvale, CA). The data were filtered with a built-in four-pole Bessel low-pass filter (cut-off frequency: 2 kHz), and then digitized at 5 kHz. Extracellular solution contains (in mM): NMG, 140; KCl, 4; MgCl\textsubscript{2}, 1; glucose, 5; and HEPES, 10 (pH 7.4 with HCl). Pipette solution contained (in mM): potassium gluconate, 144; MgCl\textsubscript{2}, 1.15; EGTA, 5; HEPES, 10; and CaCl\textsubscript{2}; 4.2 (pH 7.25 with KOH). This composition yields 863 nM free Ca\textsuperscript{2+} at 36°C based upon the calculation method by Bers et al.\textsuperscript{6} To study Ca\textsuperscript{2+}-dependency, various combinations of EGTA and CaCl\textsubscript{2} were used in the pipette solutions. Current traces were analyzed with Clampfit (Molecular Device/Axon, Sunnyvale, CA), and Igor Pro 6 (WaveMetrics, Lake Oswego, OR).

Supplemental Figure S-IV shows typical time course of K⁺ currents obtained with a ramp pulse protocol. These traces were recorded without interruption by step pulse protocols. As indicated in Panel A, total K⁺ currents with a pipette free Ca\textsuperscript{2+} concentration of 863 nM were significantly and rapidly attenuated after 100 nM apamin application in a cell from a failing heart. Both at baseline (between times a and b) and during steady state in the presence of apamin (time c), K⁺ currents measured at 0 mV did not show apparent spontaneous time-dependent changes (Panel A, right panel). Panel B shows that total K⁺ currents with a pipette free Ca\textsuperscript{2+} concentration of 250 nM were stable over time in the absence of apamin. There was no spontaneous time-dependent current reduction. We observed similar results in all cells studied, and K⁺ currents were always measured at steady state.

Effects of apamin concentration
We studied three cells from failing ventricles with both 100 nM and 1 µM of apamin at 36°C. I\textsubscript{KAS} density was 9.87 ± 4.57 pA/pF in 100 nM apamin, and was 11.17 ± 4.63 pA/pF in 1 µM (12.66 ± 7.27% difference). These findings are consistent with that reported in Figure 2 of Xu et al.\textsuperscript{5}

Real-time PCR
The tissue was cut into small pieces in RNA later (Applied Biosystems/Amibion, Austin, TX) and immersed at 4°C over night, then stored at -80°C. Total RNA was extracted from the tissues using random primers with RNAqueous 4PCR kit (Applied Biosystems/Amibion, Austin, TX). cDNA was synthesized from 100 ng total RNA for each sample using iScript Select cDNA Synthesis Kit (BioRad, Hercules, CA). Real-time PCR was performed using qSYBR Green Supermix with iCycler (BioRad, Hercules, CA). Specific primers for PCR were: for SK2, sense 5'-CCACCGTCATCCTGCTGGTC-3' and antisense 5'-AAATGCGCTCGTAGGTCATCGC-3'; for GAPDH, sense 5'- CCATGGAGAAGCCGGGG-3' and antisense 5'-
CGAAGTGAGTGAGTAGAC-3'. The general protocol for amplification was 95°C for 20 s, 58°C for 20 s, and 72°C for 20 s, for 40 cycles. PCR products were analyzed in agarose gels, and the amplicons were gel-purified with QIAquick Gel Extraction Kit (QIAGEN Sciences, Valencia, CA), and sequence was confirmed by a core sequencing laboratory at Indiana University. The sequentially diluted SK2 and GAPDH amplicons were used for standard curves. The quantitative PCR analyses were performed using MyiQ Software (BioRad, Hercules, CA).

**Statistical analyses**

Statistical analyses were performed using SPSS PASW Statistics 17 software (IBM, Chicago, IL). We used Student’s t-test for a comparison between two groups. One-way ANOVA with post hoc Bonferroni tests were performed for comparisons among three or more groups. A $p \leq 0.05$ was considered as statistically significant.

**References**

Online Figure I. The effect of apamin on postshock APD$_{80}$ in failing ventricles (N=4). Apamin concentration of 0.1, 0.3 and 1.0 µmol/L had similar effects on postshock APD$_{80}$ while 0.01 µmol/L had no significant effects. Bar graphs represent means ± SEM; * p<0.05 vs control.
Online Figure II. Examples of optical $V_m$ traces in figure A and C were obtained from normal and failing ventricles, respectively. In each ventricle, the “control” indicates baseline (before apamin) while “apamin” indicates the data obtained > 30 min after apamin administration. A, An example from pacing protocol II, with application of $S_1$ at PCL of 200 ms for 400 beats, an extrastimulus ($S_2$) was given 300 ms after the last $S_1$ to control the diastolic interval. A shows that apamin have little effect on $\Delta APD_{80}$ in normal ventricles. B, The effect of apamin on pacing protocol II in all normal ventricles studied. C shows an example in failing ventricles. Apamin decreased the $\Delta APD_{80}$ from 18 ms to 9 ms. D, The effect of apamin in pacing protocol II in all failing ventricles studied. These data show that the magnitude of post-pacing APD shortening increased with the number of paced beats, and that apamin reduced $\Delta APD_{80}$ in HF but not in normal control ventricles. Bar graphs represent means ± SEM; * p<0.05 vs control.
Online Figure III. Effects of glibenclamide on post-rapid pacing and postshock APD shortening in failing ventricles. A and B, represent the effect of glibenclamide on pacing protocols I and II, respectively. Glibenclamide does not affect the magnitude of $\Delta APD_{80}$ in failing ventricles. C, Postshock $APD_{80}$ versus diastolic interval in failing ventricles. The postshock $APD_{80}$ with glibenclamide (filled circles) are similar to control (unfilled circles) in failing ventricles. Bar graph represents means ± SEM.
Supplemental Figure IV. Stability of current measurements. (A) Left panel shows typical K⁺ current traces recorded with ramp pulses in a failing cardiomyocyte with a pipette free Ca²⁺ concentration of 863 nM: (a) indicates baseline; (b), immediately before the application of 100 nM apamin and (c), steady state in the presence of 100 nM apamin. The graph on the right side of Panel A shows time course of total K⁺ currents measured at 0 mV. The arrows correspond to the traces in the left panel. (B) Left panel shows typical K⁺ traces recorded with ramp pulses in a failing cardiomyocyte with a pipette free Ca²⁺ concentration of 250 nM. Right panel shows time course of total K⁺ currents. Note that in the absence of apamin, the K⁺ currents did not change significantly over time.