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Small-Conductance Calcium-Activated Potassium Current Is Activated During Hypokalemia and Masks Short-Term Cardiac Memory Induced by Ventricular Pacing

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Background—Hypokalemia increases the vulnerability to ventricular fibrillation. We hypothesize that the apamin-sensitive small-conductance calcium-activated potassium current (I$_{KAS}$) is activated during hypokalemia and that I$_{KAS}$ blockade is proarrhythmic.

Methods and Results—Optical mapping was performed in 23 Langendorff-perfused rabbit ventricles with ativoventricular block and either right or left ventricular pacing during normokalemia or hypokalemia. Apamin prolonged the action potential duration (APD) measured to 80% repolarization (APD$_{80}$) by 26 milliseconds (95% confidence interval [CI], 14–37) during normokalemia and by 54 milliseconds (95% CI, 40–68) during hypokalemia (P=0.01) at a 1000-millisecond pacing cycle length. In hypokalemic ventricles, apamin increased the maximal slope of APD restitution, the pacing cycle length threshold of APD alternans, the pacing cycle length for wave-break induction, and the area of spatially discordant APD alternans. Apamin significantly facilitated the induction of sustained ventricular fibrillation (from 3 of 9 hearts to 9 of 9 hearts; P=0.009). Short-term cardiac memory was assessed by the slope of APD$_{80}$ versus activation time. The slope increased from 0.01 (95% CI, −0.09 to 0.12) at baseline to 0.34 (95% CI, 0.23–0.44) after apamin (P<0.001) during right ventricular pacing and from 0.07 (95% CI, −0.05 to 0.20) to 0.54 (95% CI, 0.06–1.03) after apamin infusion (P=0.045) during left ventricular pacing. Patch-clamp studies confirmed increased I$_{KAS}$ in isolated rabbit ventricular myocytes during hypokalemia (P=0.038).

Conclusions—Hypokalemia activates I$_{KAS}$ to shorten APD and maintain repolarization reserve at late activation sites during ventricular pacing. I$_{KAS}$ blockade prominently lengthens the APD at late activation sites and facilitates ventricular fibrillation induction. (Circulation. 2015;132:1377–1386. DOI: 10.1161/CIRCULATIONAHA.114.015125.)

Key Words: arrhythmias, cardiac death, sudden, cardiac ion channels

Hypokalemia is a known risk factor of sudden cardiac death. Hypokalemia promotes ventricular tachyarrhythmias via multiple electrophysiological mechanisms, including prolonged ventricular repolarization, slowed conduction, steepened electric restitution, and abnormal pacemaker activity. Hypokalemia directly suppresses several repolarization K$^+$ currents, including the inward rectifier potassium currents (I$_{K1}$)$_{1,3,4}$ rapid component of the delayed rectifier potassium currents (I$_{Kd}$)$_{3,6}$ and transient outward currents (I$_{to}$)$_{4}$ In addition, hypokalemia induces intracellular Ca$^{2+}$ (Ca$^2+$) overload secondary to inhibition of Na$^+$-K$^+$ pump and suppression of forward mode Na$^+$-Ca$^{2+}$ exchanger. The apamin-sensitive small-conductance calcium-activated K$^+$ current (I$_{KAS}$) is known to influence repolarization of normal atria and in failing or infarcted ventricles. In contrast, it is generally believed that I$_{KAS}$ is not important in ventricular repolarization in normal ventricles during normokalemia. By inducing Ca$^2+$ overload, hypokalemia might augment the conductance and trafficking of small-conductance calcium-activated K$^+$ (SK) channels to upregulate I$_{KAS}$ in normal ventricles. The increased I$_{KAS}$ may

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help maintain repolarization reserve when other K currents are suppressed by hypokalemia. Blocking $I_{KAS}$ during hypokalemia may be proarrhythmic. Because of the importance of hypokalemia in cardiac arrhythmogenesis, we investigated whether $I_{KAS}$ is activated during hypokalemia and, if so, whether $I_{KAS}$ blockade during hypokalemia is proarrhythmic.

**Methods**

Detailed methods can be found in the online-only Data Supplement.

**Optical Mapping Studies**

**Surgical Preparation**

The protocol was approved by the Institutional Animal Care and Use Committee. A total of 23 New Zealand white rabbits were used for optical mapping studies. Among them, 7 were used for normokalemic experiments, 15 for hypokalemic experiments which include 9 with right ventricular (RV) pacing and 4 with left ventricular (LV) pacing, and 3 for hypokalemia experiments during atrial pacing. The hearts were Langendorff perfused with 37°C oxygenated Tyrode solution. The composition of Tyrode solution in normokalemic experiments (n=7) was (in mmol/L) as follows: NaCl 128, KCl 4.7, NaHCO3 24, NaH2PO4 1.8, CaCl2 1.8, MgCl2 1.2, glucose 11.1, and bovine serum albumin 40 mg/L, pH 7.40. In hypokalemic experiments, the KCl was decreased to 2.4 mmol/L once hearts were cannulated. We then performed radiofrequency atrioventricular node ablation to reduce the ventricular rate. Hypokalemia in cardiac arrhythmogenesis, we investigated whether $I_{KAS}$ Densities Determined by Voltage-Clamp Techniques

Additional rabbit hearts were used for patch-clamp studies according to previously described methods. Briefly, isolated ventricular myocytes were used for whole-cell $I_{KAS}$ recording with the voltage-clamp technique in the ruptured-patch configuration. The extracellular solution contained (in mmol/L) the following: N-methylglucamine 140, MgCl2, 1, glucose 5, HEPES 10 (pH 7.4 using HCl), and KCl 4.7 or 2.4. The internal solution consisted of (in mmol/L) the following: potassium gluconate 144, MgCl2, 1.15, EGTA 5, HEPES 10, and CaCl2, yielding a free-Ca2+ concentration of 1 mmol/L. All experiments were carried out at room temperature. Currents were elicited with the use of a voltage ramp from +40 to −100 mV (0.35 mV/s) from a holding potential of −50 mV. Voltage ramps were repeated every 10 seconds. Once the currents had stabilized, the cell was exposed to the same bath solution supplemented with apamin (100 nmol/L). Currents recorded in the presence of apamin were digitally subtracted from those measured in its absence, and the density of the apamin-sensitive current at 0 mV was calculated.

**Data Analysis**

APD90 was measured at the level of 80% repolarization of the action potential. The mean APD90 was calculated for all available ventricular pixels. The $F/F_0$ ratio was used to estimate the relative concentration of Ca2+ between normokalemia and hypokalemia and between early and late activation sites. Continuous variables are expressed as mean and 95% confidence interval (CI). We used the term delta to indicate the mean on the corresponding difference between 2 measures (ie, post minus pre). Paired Student t tests were used to compare continuous variables measured at baseline and during apamin infusion. An independent-sample t test was used to compare the $I_{KAS}$ current densities with 4.7- and 2.4-mmol/L potassium concentration in the Tyrode solution. Comparison of prevalence of ventricular fibrillation (VF) inducibility between baseline and during apamin infusion was performed with the Fisher exact test. A 2-sided value of P≤0.05 was considered statistically significant.

**Results**

Effects of $I_{KAS}$ Blockade on Rabbit Ventricles During Hypokalemia

The maximum Ca2+ $F/F_0$ at an LV apex during ventricular pacing at a 300-millisecond PCL was 1.12 (95% CI, 1.08–1.17; n=7) when potassium concentration was 4.7 mmol/L and increased to 1.29 (95% CI, 1.23–1.35; n=13) when the potassium concentration decreased to 2.4 mmol/L (P<0.001). The $F/F_0$ was 1.17 (95% CI, 1.14–1.20) at early activation sites and 1.29 (95% CI, 1.22–1.35; delta=0.12 [95% CI, 0.07–0.17]; P=0.001; n=13) at late activation sites, respectively, at baseline and 1.14 (95% CI, 1.11–1.18) and 1.27 (95% CI, 1.21–1.32) for the hypokalemic hearts (delta=0.12 [95% CI, 0.08–0.16]; P=0.001; n=13), respectively, after the addition of apamin. The latter findings were consistent with Ca2+ accumulation at late activation sites during ventricular pacing. Typical examples are shown in the Figure I in the online-only Data Supplement.

We tested the effects of apamin on 9 rabbit ventricles with RV pacing during hypokalemia ([K+]o=2.4 mmol/L). Optical images were captured from the whole ventricle. As shown in Figure 1A, apamin prolonged APD90 at all PCLs during hypokalemia. However, the effects were more apparent at long than short PCLs (Figure 1B and 1C). At the PCL of 1000 and 250 milliseconds, apamin prolonged the APD90 from 215 milliseconds (95% CI, 205–226) to 269 milliseconds (95% CI, 250–289; delta=54 [95% CI, 40–68]; P<0.01) and from 173 milliseconds (95% CI, 165–180) to 189 milliseconds (95% CI, 178–199), respectively (delta=16 [95% CI, 10–22]; P<0.01). The average magnitudes of APD90 prolongation at 1000- and 250-millisecond PCLs were 54 milliseconds (95% CI, 40–68) and 16 milliseconds (95% CI, 10–22), respectively. The percentage of prolongation at PCLs of 1000 and 250 milliseconds was 25.38% (95% CI, 19.24–31.52) and 9.23% (95% CI, 6.09–12.38), respectively. We also tested the effects of apamin in 7 normokalemic ventricles ([K+]o=4.7 mmol/L). Apamin had very little effect on APD90 at the 250- and 300-millisecond PCL but increased the APD90 by 14% at the 1000-millisecond PCL (from 182 milliseconds [95% CI, 170–194] to 208 milliseconds [95% CI, 188–227]; delta=26
[95% CI, 14–37]; *P* = 0.01). The average magnitude of APD<sub>80</sub> prolongation at the 1000-millisecond PCL was 26 milliseconds (95% CI, 14–37), which was significantly less than that during hypokalemia (*P* = 0.01). Figure II in the online-only Data Supplement summarizes the effects of apamin on APD in normokalemic ventricles.

APD heterogeneity has been recognized as an important factor contributing to reentrant ventricular arrhythmia. We used the standard deviation and correlation of variance generated from the optically imaged region to quantify APD heterogeneity. Figure 2A shows APD maps at baseline and after apamin and the ΔAPD maps. Figure 2B shows that apamin significantly increased the standard deviation of APD<sub>80</sub> at all PCLs. Apamin also significantly increased the correlation of variance of APD<sub>80</sub> at 250-, 300-, and 500-millisecond PCLs. The changes in the correlation of variance at 800- and 1000-millisecond PCL were insignificant.

### Effect of \( I_{\text{KAS}} \) Blockade on the Maximal Slope of APDR in Hypokalemic Ventricles

APDR curves were sampled at a basal and apical area over the LV in each heart studied. In a representative ventricle (Figure 3A), APDR slope after \( I_{\text{KAS}} \) inhibition was consistently higher than baseline at both slow (1000 milliseconds) and fast (200 milliseconds) PCLs. \( I_{\text{KAS}} \) blockade also increased the maximal slope of APDR compared with baseline in both the basal and apical regions. For 9 hearts with RV pacing studied, \( I_{\text{KAS}} \) blockade increased the maximal slope of APDR from 0.99 (95% CI, 0.81–1.17) at baseline to 1.26 (95% CI, 1.04–1.47; delta=0.26 [95% CI, 0.14–0.39]; *P* < 0.01; Figure 3B).

### \( I_{\text{KAS}} \) Blockade Facilitated the Development of 2:2 Alternans and Wave Breaks in Hypokalemic Ventricles

Rapid pacing was associated with a heterogeneous distribution of APD and the calcium transient duration during...
hypokalemia, but less so during normokalemia (Figure III in the online-only Data Supplement). In that example, calcium transient duration alternans developed at the 220-millisecond PCL, whereas both calcium transient duration and APD alternans were observed at the 190-millisecond PCL during hypokalemia, but no alternans was observed during normokalemia at 190-millisecond PCL. For all hearts studied, the longest PCL that induced calcium transient duration alternans during hypokalemia was 237 milliseconds (95% CI, 223–250), significantly longer than the longest PCL associated with APD alternans (201 milliseconds; 95% CI, 189–214; delta=36 [95% CI, 20–51]; \( P < 0.001 \)).

Rapid pacing caused conduction delay during hypokalemia, which was exacerbated by apamin. For 9 hypokalemic ventricles with RV pacing, apamin increased the total activation time (AT) from 26 milliseconds (95% CI, 21–31) to 36 milliseconds (95% CI, 28–43) at the 250-millisecond PCL (delta=9 [95% CI, 2–16]; \( P = 0.014 \)). In addition, rapid pacing causes APD alternans, especially at sites remote from the pacing site (Figure 4A). As shown in Figure 4B, the PCL threshold of alternans was significantly prolonged by apamin (250 milliseconds [95% CI, 225–274] versus 201 milliseconds [95% CI, 189–214] at baseline; delta=49 [95% CI, 20–78], \( P < 0.01 \)). Further decreases in PCL caused wave break in 4 ventricles (44.4%) at baseline and in all ventricles (100%) after apamin. The PCL threshold inducing wave break was also longer after the addition of apamin (186 milliseconds; 95% CI, 163–208) than at baseline (160 milliseconds; 95% CI, 147–173; delta=26 [95% CI, 11–40]; \( P < 0.01 \)).

APD alternans was initially concordant but became discordant when PCL was shortened. Figure 5 shows examples of discordant alternans induced by rapid pacing at baseline and after apamin. To quantify the degree of spatially discordant APD alternans in each ventricle, we calculated the total pixel numbers showing discordant alternans before and after apamin infusion at the shortest PCL that allowed 1:1
capture. At baseline, 4 ventricles (44.4%) showed small areas of spatially discordant APD alternans at 140- to 200-millisecond PCLs. Apamin facilitated the formation of spatially discordant APD alternans by lengthening the nodal line and enlarging the area involved in alternans (Figure 5A). For all 9 hypokalemic hearts with RV pacing, apamin significantly increased the area ratio (percent of mapped region) of spatially discordant APD alternans from 7.89% (95% CI, −0.46 to 16.23) to 45.3% (95% CI, 32.86–57.81) at the shortest PCL that allowed 1:1 capture (delta=37.44% [95% CI, 27.21–47.69]; P<0.001; Figure 5B). Further decreasing the PCL resulted in wave break in 4 ventricles at baseline and in all 9 ventricles after apamin.

\( I_{\text{KAS}} \) Blockade and Activation-Repolarization Coupling

Activation-repolarization coupling was assessed by plotting APD\(_{80}\) against the AT during pacing. The PCL used for these analyses was 300 milliseconds. Representative plots of APD\(_{80}\) versus AT in 2 hearts with different RV pacing sites are shown in Figure 6A and 6B. The slope of APD to AT was flat at baseline. Apamin caused more APD prolongation at late activation sites than early activation sites, leading to increased APD-to-AT slope. There was a negative correlation of \( \Delta \)APD\(_{80}\) (APD\(_{80}\) after apamin minus that at baseline) versus baseline APD\(_{80}\). For all 9 hearts with RV pacing during hypokalemia, apamin increased the APD-to-AT slope from 0.01 (95% CI, −0.09 to 0.12) to 0.34 (95% CI, 0.23–0.44; delta=0.32 [95% CI, 0.19–0.45]; P<0.001). We measured the APD-to-AT slope in 4 hypokalemic ventricles studied with recordings available at both 30 and 90 minutes after apamin administration. The APD-to-AT slopes at 30 and 90 minutes were 0.30 (95% CI, 0.25–0.35) and 0.32 (95% CI, 0.19–0.45), respectively (P=0.98), indicating a stable APD-to-AT relationship (Figure 7).

We also tested the effect of activation-repolarization coupling in 4 additional ventricles paced from the LV base. A representative plot of APD\(_{80}\) versus AT in 1 heart with LV base pacing is shown in Figure IV in the online-only Data Supplement. For 4 hearts paced from the LV base, apamin increased the APD-to-AT slope from 0.07 (95% CI, −0.05 to 0.20) at baseline to 0.54 (95% CI, 0.06–1.03; delta=0.47 [95% CI, 0.02–0.93]; P=0.045).

Effects of Apamin in Atrially Paced Hearts

The APD\(_{80}\) at the 300-millisecond PCL at baseline was 153 milliseconds (95% CI, 128–178). Apamin prolonged the APD\(_{80}\) to 176 milliseconds (95% CI, 143–208; delta=23 [95% CI, 7–39]; P=0.024). The entire epicardium was activated within 13 milliseconds (95% CI, 6–21) at baseline and 17 milliseconds (95% CI, 6–27; delta=3 [95% CI, 0–6]; P=0.038) after apamin. Although apamin increased AT, it did not change the ventricular activation sequence. The APD-to-AT slope in these 3 hearts was 0.98 (95% CI, −2.61 to 4.58) at baseline and −0.22 (95% CI, −1.57 to 1.13) after apamin (delta=−1.20 [95% CI, −4.62 to 2.23]; P=0.271). A representative response to apamin is shown in Figure V in the online-only Data Supplement.

Effects of Hypokalemia on \( I_{\text{KAS}} \) Current Density

We successfully completed patch-clamp studies in isolated rabbit ventricular myocytes with the potassium concentration at UCLA BIOMED LIBRARY SERIALS on October 21, 2015http://circ.ahajournals.org/Downloaded from
at 4.7 mmol/L (n=3) and 2.4 mmol/L (n=3) in the Tyrode solution. The densities of $I_{KAS}$ were 0.04 pA/pF (95% CI, −0.04 to 0.12) and 2.20 pA/pF (95% CI, 0.33–4.06), respectively (delta=2.16 [95% CI, 0.30–4.02]; $P=0.038$). An example of the patch-clamp study is shown in Figure VI in the online-only Data Supplement.

$I_{KAS}$ Blockade Increased Ventricular Vulnerability to Fibrillation in Hypokalemic Ventricles

No spontaneous tachyarrhythmias were observed during the study. Rapid pacing induced VF episodes in 3 of 9 ventricles (33.3%) studied at baseline. VF episodes were successfully induced in all 9 ventricles (100%) after apamin infusion.

Figure 6. Effects of apamin-sensitive small-conductance calcium-activated potassium current ($I_{KAS}$) blockade on the relation of action potential duration (APD) to activation time (AT) on the epicardium determined during right ventricular (RV) pacing at a 300-millisecond pacing cycle length (PCL) in hypokalemic ventricles. A, The AT maps, APD maps, and corresponding plot of the APD-AT relationship at baseline (1) and after apamin infusion (2) in 1 ventricle. The pacing site (asterisk) was located at the RV base. Apamin increased the APD-AT slope from −0.12 to 0.32. (3) The ΔAPD map and correlation between ΔAPD and baseline APD$_{80}$ in the ventricle at a 300-millisecond PCL. (4) The corresponding ΔAPD-AT plot at a PCL of 300 milliseconds. ΔAPD is APD$_{80}$ after apamin minus APD$_{80}$ at baseline.

Figure 7. Relationship of action potential duration (APD) to activation time (AT) over time. The rabbit ventricles were paced from the right ventricle (RV; asterisk) at a 300-millisecond pacing cycle length (PCL). Maps were obtained at different times of the experiment as labeled on the left. The sequence of activation on the left ventricle was stable over time. The APD measured to 80% repolarization (APD$_{80}$) at baseline was stable for 2 hours. After the addition of apamin, the APD$_{80}$ was lengthened, and the APD-to-AT slope increased at 30 minutes. These changes were stable 1 hour later. The pacing site (asterisk) is located at the RV base.
Diac memory is also modulated by longer PCL than at shorter PCL. Blockade of IKAS with atrioventricular block. The effect on APD was greater at I under these conditions was proarrhythmic, suggesting that 1KAS blockade increased the number of 1 phase singularities of VF episodes (P=0.002). Representative recording of optical mapping in 1 heart revealed that apamin increased the slope of APD to AT from −0.04 to 0.49 at a PCL of 1000 milliseconds compared with baseline (Figure VIIA in the online-only Data Supplement). The premature stimulation (230 milliseconds coupling interval) captured the tissue near pacing site. However, because of the prolongation of APD remote from pacing site, the impulse was blocked half-way through the ventricle, leading to reentry and VF (Figure VIIIB in the online-only Data Supplement).

Discussion

We found that hypokalemia activated IKAS in normal ventricles with atrioventricular block. The effect on APD was greater at longer PCL than at shorter PCL. Blockade of IKAS by apamin under these conditions was proarrhythmic, suggesting that IKAS activation plays an important role in protecting ventricular repolarization reserve and in preventing the development of sustained ventricular arrhythmias of the nonfailing ventricles. In addition, apamin unmasked a positive correlation between ΔAPD and AT, indicating that pacing-induced cardiac memory is also modulated by IKAS.

Mechanisms of Cardiac Memory

Cardiac memory is a term coined by Rosenbaum et al to describe a specialized form of remodeling characterized by an altered T wave recorded induced by a preceding period of altered electric activation. In the heart, short-term memory refers to the effects of pacing history on the APD and AT, indicating that pacing-induced cardiac memory can also be induced in rabbit ventricles by 5 minutes of ventricular pacing that returned to control in 5 to 10 minutes. However, losartan, an angiotensin receptor type 1 blocker, did not influence the expression of memory in that study. The native rabbit ventricular Ito (chiefly Ito1 encoded by Kv1.4) has an unusually long time constant of recovery from inactivation. Thus, Ito is almost completely inactivated and makes a negligible contribution to the AP at a PCL of <1 second. IKAS acts like an Ito in that its conductance tracks the Ca2+ transient. It is possible that, in the rabbit ventricles paced at a 300-millisecond PCL, IKAS could form the basis of the memory effect rather than Ito.

IKAS and Cardiac Memory

The induction of cardiac memory in rabbit ventricles can be detected from the relationship between APD and AT. During sinus rhythm or atrial pacing, ventricular APD varies inversely with respect to AT. During ventricular pacing, however, the negative correlation is replaced by a positive correlation lasting ~60 minutes, after which the negative correlation re-establishes itself. Subsequent studies have suggested that ventricular pacing induces cardiac memory through a mechanoelectric feedback mechanism related to altered sarcomeric Ca2+ handling and cytosolic Ca2+ accumulation at sites remote from the pacing site. That mechanism could potentially explain our findings if the increased Ca at remote sites activates inward currents that prolong APD but are compensated for by activation of IKAS, which attenuates APD prolongation. By inhibiting IKAS, apamin unmasks APD prolongation at remote sites, revealing the underlying positive correlation between APD and AT.

Figure 8. Effects of apamin-sensitive small-conductance calcium-activated potassium current (IKAS) blockade on the wave breaks of ventricular fibrillation (VF) episodes in hypokalemic ventricles. A, Consecutive phase maps sampled at 40 milliseconds during VF at baseline and after apamin infusion in a representative ventricle. Phase singularities (PSs; wave breaks) are indicated by black arrows. The optical signals during VF came from a site marked by an asterisk. B, Effects of apamin on the number of phase singularities in all VF episodes before and after apamin infusion. Note that the phase singularities were significantly increased by apamin infusion. *P=0.001.
and AT as a reflection of short-term cardiac memory. Our data therefore suggest that short-term cardiac memory induced by ventricular pacing is strongly modulated by $I_{\text{KAS}}$.

**Importance of Short-Term Cardiac Memory to Ventricular Arrhythmogenesis**

The safety factor of propagation is determined in part by the spatial patterns of repolarization. Propagation from areas with short APD into areas with long APD is associated with a reduced safety factor of propagation, increasing the propensity for conduction block and reentry. Because pacing-induced short-term cardiac memory is associated with greater APD prolongation remote from the pacing site than close to the pacing site, the safety factor of propagation is reduced particularly during premature depolarization. Consistent with this hypothesis, we have shown that apamin administration makes it easier for a premature stimulation to induce wave breaks.

Another form of cardiac memory is the rate- or diastolic interval–dependent changes in APDR. This type of memory is dependent on the kinetic properties of multiple different ion channels. A previous study and the present study indicated that $I_{\text{KAS}}$ is important in APDR. In computer simulations, adding a potassium memory current to the Luo-Rudy model showed that the accumulation of the memory potassium current played an important role in the progression of the activation patterns of VF over time by progressively shortening the APD. $I_{\text{KAS}}$ activation as Ca overload develops during early VF could act in the same way as a memory K current and thus influences the generation and maintenance of VF.

**$I_{\text{KAS}}$ and the Repolarization Reserve**

Despite significant APD prolongation during moderate hypokalemic ventricles after $I_{\text{KAS}}$ inhibition, spontaneous early afterdepolarizations or torsades de pointes were not observed. However, in case of associated disease conditions such as heart failure and coexistent atrioventricular block and brady-cardia, spontaneous early afterdepolarizations or torsades de pointes may occur after apamin. Another possible explanation is that $I_{\text{KCa}}$ and $I_{\text{Kv}}$ are activated during the phase 3 of the action potential, whereas $I_{\text{KAS}}$ is most important during the phase 2 of the action potential when $I_{\text{CAS}}$ activity, sarcoplasmic Ca$^{2+}$ release, and the Ca$^{2+}$ are high. The effects of $I_{\text{KAS}}$ blockade may be sufficient to prolong the APD. However, without other associated ionic current changes, APD prolongation by itself is insufficient to induce early afterdepolarizations or torsades de pointes.

**$I_{\text{KAS}}$ Blockade Increases Tissue Vulnerability in Hypokalemic Ventricles**

We observed increased APD heterogeneity, steepened maximal slope of APDR, increased PCL threshold for alternans, and increased spatially discordant APD alternans after $I_{\text{KAS}}$ inhibition, factors that increase vulnerability to ventricular arrhythmias. The out-of-phase regions of discordant APD alternans are separated by a nodal line in which the spatial gradients in APD or Ca$^{2+}$ transient amplitude are the steepest, predisposing to localized conduction block. The augmentation of spatially discordant APD alternans by $I_{\text{KAS}}$ blockade in our study can be explained by 2 mechanisms: $I_{\text{KAS}}$ blockade steepened the APDR curve in hypokalemic ventricles, which would facilitate the formation of discordant APD alternans. In addition, because $V_m$ and Ca$^{2+}$ are bidirectionally coupled in myocardial tissue, $I_{\text{KAS}}$ may play an important role to modulate the $V_m$ and Ca$^{2+}$ coupling. Longer Ca$^{2+}$ transient results in greater $I_{\text{KAS}}$ activation to compensate for the longer APD caused by longer Ca$^{2+}$ transient. Therefore, $I_{\text{KAS}}$ inhibition would amplify the effect of Ca$^{2+}$ on APD prolongation and hence prolong the PCL threshold for alternans.

**Study Limitations**

The optical mapping techniques do not allow us to determine the absolute levels of the Ca$^{2+}$. Therefore, we relied on reports by others to support the altered Ca$^{2+}$ handling at late activated regions. We showed that $I_{\text{KAS}}$ blockade facilitated ventricular arrhythmias in hypokalemic normal ventricles, a finding opposite to that in failing ventricles, in which apamin was antifibrillatory. The mechanisms of the opposite observations may be related to additional remodeling changes that are present in failing but not normal ventricles. Apamin has been reported to also block the fetal-type $I_{\text{CaL}}$, implying that it is not a specific ion channel blocker. However,Yu et al recently showed that apamin is a highly specific $I_{\text{KAS}}$ blocker for human type cardiac ion channels and does not block $I_{\text{CaL}}$.

**Summary and Clinical Significance**

A large study involving 58,167 hospital inpatients showed that 5.2% had serum potassium <3.0 mmol/L, including 73 patients with potassium <2.0 mmol/L and 472 patients with potassium between 2.0 and 2.4 mmol/L. Hypokalemia significantly increases mortality in hospitalized patients. We showed that $I_{\text{KAS}}$ blockade increased the vulnerability to ventricular tachyarrhythmias in hypokalemic ventricles. $I_{\text{KAS}}$ also plays an important role in modulating cardiac memory. These findings indicate that $I_{\text{KAS}}$ is important in ventricular arrhythmogenesis during hypokalemia. This may be relevant to drug safety because a number of commonly used clinical drugs such as anesthetic agents, quinine, $d$-tubocurarine, and amiodarone are known inhibitors of $I_{\text{KAS}}$. It is possible that further investigations will discover the $I_{\text{KAS}}$-blocking action of other drugs used commonly in clinical practice. The $I_{\text{KAS}}$-blocking action may contribute to their proarrhythmic mechanism. Better understanding the drug effects on $I_{\text{KAS}}$ may be important in the prevention of sudden death and promote drug safety in patients with severe hypokalemia.

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Disclosures
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**CLINICAL PERSPECTIVE**

Hypokalemia is a known risk factor for sudden cardiac death. Proarrhythmic effects of hypokalemia have been related to reduced repolarization reserve as a result of direct suppression of multiple K+ currents and inhibition of the Na-K pump, causing increases in intracellular Ca2+ (Ca2+). We hypothesized that Ca2+ overload in this setting may activate apamin-sensitive small-conductance calcium-activated K+ current ($I_{\text{Kas}}$), attenuating the reduction in repolarization reserve and protecting against arrhythmias. In the present study, we documented that $I_{\text{Kas}}$ was indeed upregulated during hypokalemia, especially at sites of late activation during ventricular pacing, which exacerbated Ca overload. Apamin, a specific $I_{\text{Kas}}$ blocker, increased ventricular vulnerability to fibrillation. These findings support the notion that $I_{\text{Kas}}$ may act as a rescue current to counteract excessive reduction in repolarization reserve during hypokalemia, reducing the risk of arrhythmias. In addition to hypokalemia, other conditions associated with reduced repolarization reserve and abnormal Ca handling such as heart failure and myocardial infarction have also been shown to increase $I_{\text{Kas}}$. Blocking $I_{\text{Kas}}$ by drugs or foods might remove this rescue mechanism and make the patients more vulnerable to ventricular arrhythmias and sudden death, especially in the setting of chronic right ventricular pacing or frequent premature ventricular contractions. We conclude that $I_{\text{Kas}}$ may be an important rescue current that helps to maintain repolarization reserve in diseased conditions and hypokalemia. These findings may be important for understanding drug safety and the detrimental effects of ventricular pacing and frequent premature ventricular contractions.
SUPPLEMENTAL MATERIAL

Methods

Optical mapping studies

Surgical preparation
The study protocol was approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine and the Methodist Research Institute, Indianapolis, Indiana. A total of 23 New Zealand white rabbits with body weight 3.5 to 5.0 Kg were used for optical mapping studies. Among them, 7 were used for normokalemic experiments, 13 for hypokalemic experiments that include 9 with right ventricular (RV) pacing and 4 with left ventricular (LV) pacing. In addition, we studied 3 hypokalemic hearts during atrial pacing. Under isoflurane general anesthesia, the hearts were harvested and Langendorff perfused with 37°C oxygenated Tyrode's solution. The composition of Tyrode's solution in normokalemic experiments (N=7) was (in mmol/L): NaCl 128, KCl 4.7, NaHCO₃ 24, NaH₂PO₄ 1.8, CaCl₂ 1.8, MgCl₂ 1.2, glucose 11.1 and bovine serum albumin 40 mg/L with a pH of 7.40. In hypokalemic experiments (N=13), the KCl in the Tyrode’s solution was decreased to 2.4 mmol/L once hearts were cannulated. We then performed radiofrequency atrioventricular (AV) node ablation to reduce the ventricular rate to < 60 bpm (ventricular escape cycle length > 1000 ms). The slow rates allowed us to study the electrophysiological effects of apamin at both fast and slow pacing rates. The pacing sites were either in the right ventricle (RV, N=16) or in the left ventricle (LV) base (N=4). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Optical Mapping
We performed simultaneous optical mapping of the membrane potential (Vₘ) and Caᵢ using techniques similar to that reported elsewhere.¹ The hearts were stained with Rhod-2 AM (1.2 μmol/L, 0.18 μmol in 150 mL Tyrode's solution, from Invitrogen, Grand Island, NY) for Caᵢ mapping and then RH237 (10 μmol/L, 0.4 μmol in 40 ml, from Invitrogen, Grand Island, NY) for Vₘ mapping. Blebbistatin (15~20 μmol/L, from Tocris Bioscience, Minneapolis, MN) was used to inhibit contraction. The hearts were excited with a laser (Verdi G5, Coherent Inc., Santa Clara, CA) at a wavelength 532 nm. The fluorescence was collected through a lens and dichroic mirror with a 650 nm cut-off wavelength. The signals were further filtered (715 mm for Vₘ and 580 nm for Caᵢ) and recorded simultaneously with two MiCAM Ultima cameras (BrainVision, Tokyo, Japan) at 2 ms/frame temporal resolution and 100 x 100 pixels with spatial resolution of 0.35 x 0.35 mm² per pixel. The average fluorescence level (F) of an individual pixel was first calculated for the duration of recording. The ratio of fluorescence...
\((F-F_0)/F_0\) of the individual pixel was further filtered with 3 x 3 x 3 averaging to generate the maps.

Pseudoelectrocardiogram (pECG) was monitored using 2 electrodes placed in the left atrium and the RV, respectively. A bipolar electrode was used to pace the RV with an output at 2.5 times the diastolic pacing threshold in 9 hypokalemic and 7 normokalemic hearts. A dynamic pacing protocol was performed to determine the action potential duration restitution (APDR) curve and the optical signals were mapped at different pacing cycle length (PCL). The PCLs were progressively shortened from 1000 ms until VF was induced or the loss of 1:1 capture of the ventricles. We started to acquire optical mapping signal after at least 30 paced beats at the same PCL. A S1/S2/S3 (short/long/short) pacing protocol (S1 30 beats with S1-S1 300 ms, a long S1-S2 of 1000 ms and a S2-S3 starting from 300 ms and gradually shortened to the ventricular effective refractory period (ERP)) and long/short coupled pacing protocol (a long PCL with 1000 ms coupled with a short PCL starting from 300 ms and gradually shortened to the ventricular ERP) were used to simulate the ECG characteristics that initiates the early afterdepolarizations (EADs) and torsades de pointes (TdP) ventricular tachycardia in humans. Apamin (100 nmol/L) was then added to the perfusate and the ventricles were continuously paced at 300 ms PCL for an hour. The protocols were then repeated. An addition 4 hearts were studied during hypokalemia with LV pacing. The output was set at 2.5 times the diastolic pacing threshold and the PCL was fixed at 300 ms both at baseline and after apamin infusion. An additional 3 hearts were paced from the right atrial appendage at 300 ms PCL. Optical mapping was performed during hypokalemia at baseline. Apamin (100 nmol/L) was then added to the perfusate and the optical mapping was repeated 1 hour later.

\(I_{KAS}\) densities determined by voltage-clamp techniques
Additional rabbit hearts were used for patch clamp studies according to previously described methods. Briefly, the rabbits were anesthetized with pentobarbital (50 mg/Kg) and the hearts were rapidly excised. The ascending aorta was cannulated, and the heart was retrogradely perfused with oxygenated Tyrode’s solution at 37°C. After rinsing off remaining blood, the heart was perfused for 15 min with nominally Ca\(^{2+}\)-free Tyrode’s solution containing collagenase II (200 U/ml; Worthington Biochemicals). Ventricles were then removed and minced into small pieces followed by dispersion with a large bore pipette. The dispersed tissue was filtered through a 100-µm Nylon mesh, the cells were resuspended in Tyrode’s solution containing 100 nmol/L CaCl\(_2\). The cell suspension was centrifuged at 500 rpm for 5 min at room temperature. The supernatant was removed and the cells were resuspended in a buffer containing 200 nmol/L CaCl\(_2\). Centrifugation was repeated one more time and the cells were resuspended in Tyrode’s solution containing 500 nmol/L CaCl\(_2\) until use.

Whole-cell \(I_{KAS}\) was recorded using the voltage-clamp technique in the ruptured-patch
configuration. The extracellular solution contained (in mmol/L): N-methylglucamine (NMG) 140, MgCl₂ 1, glucose 5, HEPES 10 (pH 7.4 using HCl), and KCl 4.7 or 2.4. The internal solution consisted of (in mmol/L): potassium gluconate 144, MgCl₂ 1.15, EGTA 5, HEPES 10, and CaCl₂ yielding a free Ca²⁺-concentration of 1 µmol/L [Ca-EGTA Calculator v1.3 (http://maxchelator.stanford.edu/CaEGTA-TS.htm)]. All experiments were carried out at room temperature. Pipette resistances ranged from 1-1.5 MΩ when filled with the pipette solution. After achieving a gigaseal, the test-pulse current was nulled by adjusting the pipette capacitance compensation. After break-in, the whole-cell charging transient was nulled by adjusting whole-cell capacitance and series resistance. Series resistance was compensated by 70 - 80%. After obtaining whole-cell access, the cell was dialyzed for 15 min before the start of current recordings. Currents were elicited using a voltage-ramp from +40 to -100 mV (0.35 mV/s) from a holding potential of -50 mV. Voltage-ramps were repeated every 10 s. Once the currents had stabilized, the cell was exposed to the same bath solution supplemented with apamin (100 nmol/L). Currents recorded in the presence of apamin were digitally subtracted from those measured in its absence and the density of the apamin-sensitive current at 0 mV was calculated.

**Data Analysis**

APD₈₀ was measured at the level of 80% repolarization of the action potential. The mean APD₈₀ was calculated for all available ventricular pixels. The F/F₀ ratio was used to estimate the relative concentration of Ca⁴⁺ between normokalemia and hypokalemia and between early and late activation sites. Continuous variables are expressed as mean and 95% confidence interval (CI). Paired Student's t-tests were used to compare continuous variables measured at baseline and during apamin infusion. Non-parametric Mann-Whitney-Wilcoxon test was used to compare the I_KAS current densities with 4.7 mmol/L and 2.4 mmol/L potassium concentration in the Tyrode solution. Comparison of prevalence of VF inducibility between baseline and during apamin infusion was performed using Fisher's exact test. A 2-sided $P \leq 0.05$ was considered statistically significant.
Figure 1. Effect of extracellular [K⁺]₀ concentration and activation sequence on Ca²⁺ transient. Panel A shows representative Ca²⁺ transient in ventricles at two different K⁺ concentrations. The F/F₀ was much higher with 2.4 mmol/L [K⁺]₀ than with 4.7 mmol/L [K⁺]₀ in the Tyrode solution. Panel B shows representative tracings of Ca²⁺ transient at two different sites on one ventricle perfused with low (2.4 mmol/L) [K⁺]₀ Tyrode solution with PCL of 300 ms. The F/F₀ in the late activation site (remote site) was much higher than that at the early activation site (near pacing site). F = fluorescence of calcium transient; F₀ = fluorescence of calcium transient during late diastole. PCL = pacing cycle length.
Figure 2. Effects of \( I_{\text{KAS}} \) blockade on APD at different PCLs in normokalemic (4.7 mmol/L) ventricles. A, Representative \( V_m \) traces and APD\(_{80}\) maps at baseline and in the presence of apamin (100 nmol/L). The magnitude of APD prolongation was more prominent at long PCLs than at short PCLs. The pacing site (asterisk) was located at RV apex. B, Apamin significantly prolonged APD\(_{80}\) at 500, 800, and 1000 ms PCLs, and the prolongation was more prominent at longer PCLs. *\( P < 0.05 \) vs baseline \( N = 7 \). C, A plot of \( \Delta \text{APD}_{80} \) ratio \([\text{APD}_{80} \text{ after apamin} - \text{APD}_{80} \text{ at baseline}] / \text{APD}_{80} \text{ at baseline}\) vs PCL shows that apamin prolonged APD\(_{80}\) by approximately 14% at a PCL of 1000 ms but only by 3% at a PCL of 250 ms. APD = action potential duration; \( I_{\text{KAS}} \) = apamin sensitive small conductance calcium activated potassium current; PCL = pacing cycle length.
**Figure 3.** Effects of PCL on CaTD alternans. A shows no alternans when PCL shortened from 300 ms to 190 ms when the extracellular [K\(^+\)]\(_o\) concentration was 4.7 mmol/L. B shows the development of CaTD alternans at 220 ms PCL at [K\(^+\)]\(_o\) concentration of 2.4 mmol/L. The APD alternans was observed only when PCL was shortened to 190 ms. The area of APD alternans was much smaller than the area of CaTD alternans. The pacing site on the RV was marked by an asterisk. The optical signal was recorded from the site marked by a plus sign, which is remote from the site of the pacing. The red and black tracings indicated the calcium and voltage signals, respectively. APD = action potential duration; CaTD = calcium transient duration; L = long; PCL = pacing cycle length; S = short.
**Figure 4.** Effects of $I_{\text{KAS}}$ blockade on epicardial action APD-to-AT relationship determined during LV basal pacing with PCL 300 ms in hypokalemic (4.7 mmol/L) ventricles. The top two rows show the AT maps, APD maps and corresponding plot of APD-AT relationship at baseline (1) and after apamin (2) infusion in one ventricle during 300 ms PCL. The pacing site (asterisk) is located at LV base. Apamin increased the slope of APD-AT from 0.06 to 0.95. (3) The $\Delta$APD map and correlation between $\Delta$APD and baseline APD$_{80}$ in the ventricle at PCL 300 ms. (4) The corresponding plot of $\Delta$APD-AT at PCL 300 ms. APD = action potential duration; AT = activation time; $I_{\text{KAS}}$ = apamin sensitive small conductance calcium activated potassium current; LV = left ventricle; PCL = pacing cycle length; $\Delta$APD = APD$_{80}$ after apamin - APD$_{80}$ at baseline.
Figure 5. Effects of apamin (100 nmol/L) on atrially paced hearts. The experiment was done at 300 ms PCL and 2.4 mmol/L [K⁺]₀. Apamin increased the ventricular APD in this and two additional hearts studied. The sequence of activation on the epicardium remained stable. APD = action potential duration; AT = activation time; PCL = pacing cycle length
Figure 6. Densities of $I_{KAS}$ at low and normal extracellular K$^+$ concentration in isolated ventricular myocytes. A shows the effects of apamin on K$^+$ currents at 2.4 mmol/L [K$^+$]o. The differences between Control tracing and Apamin tracing is the apamin-sensitive K$^+$ current ($I_{KAS}$). B shows an absence of $I_{KAS}$ when the [K$^+$]o concentration was 4.7 mmol/L.
Figure 7. Induction of reentry and VF by long/short (L/S) pacing protocol during hypokalemia, after $I_{KAS}$ blockade. A, APD maps and the corresponding APD-to-AT relationship from the representative ventricle at baseline and after apamin infusion. Map were generated at the PCL of 1000 ms. B, Representative sustained ventricular arrhythmia (VF) induced by L/S (1000/230 ms) coupled pacing protocol. The pacing site is located at RV base (asterisk). The premature contraction (S) led to ventricular activation near pacing site (blue) and local conduction block leading to delayed activation remote from pacing (red). The voltage ($V_m$) map at the bottom shows conduction block in the center and activation circling the periphery of the blocked region (arrows). The phase map shows the development of phase singularities at the site of block, causing reentry that initiates VF. APD = action potential duration; AT = activation time; $I_{KAS}$ = apamin sensitive small conductance calcium activated potassium current. L = long; S = short.
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