Small Conductance Calcium-Activated Potassium Current Is Important in Transmural Repolarization of Failing Human Ventricles

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Background—The transmural distribution of apamin-sensitive small conductance Ca^{2+}-activated K^+ (SK) current (I_{KAS}) in failing human ventricles remains unclear.

Methods and Results—We optically mapped left ventricular wedge preparations from 12 failing native hearts and 2 rejected cardiac allografts explanted during transplant surgery. We determined transmural action potential duration (APD) before and after 100 nmol/L apamin administration in all wedges and after sequential administration of apamin, chromanol, and E4031 in 4 wedges. Apamin prolonged APD from 363 ms (95% confidence interval [CI], 341–385) to 409 (95% CI, 385–434; P<0.001) in all hearts, and reduced the transmural conduction velocity from 36 cm/s (95% CI, 30–42) to 32 cm/s (95% CI, 27–37; P=0.001) in 12 native failing hearts at 1000 ms pacing cycle length (PCL). The percent APD prolongation is negatively correlated with baseline APD and positively correlated with PCL. Only 1 wedge had M-cell islands. The percentages of APD prolongation in the last 4 hearts at 2000 ms PCL after apamin, chromanol, and E4031 were 9.1% (95% CI, 3.9–14.2), 17.3% (95% CI, 3.1–31.5), and 35.9% (95% CI, 15.7–56.1), respectively. Immunohistochemical staining of subtype 2 of SK protein showed increased expression in intercalated discs of myocytes.

Conclusions—SK current is important in the transmural repolarization in failing human ventricles. The magnitude of I_{KAS} is positively correlated with the PCL, but negatively correlated with APD when PCL is fixed. There is abundant subtype 2 of SK protein in the intercalated discs of myocytes. (Circ Arrhythm Electrophysiol. 2015;8:667-676. DOI: 10.1161/CIRCEP.114.002296.)

Key Words: action potential ■ calcium ■ heart failure ■ ion channels ■ ventricular remodeling

Heart failure is associated with significant electrophysiological remodeling that includes a downregulation of most potassium currents and upregulation of late sodium and sodium–calcium exchange currents.4,5 These changes reduce the repolarization reserve, prolong the action potential (APD) duration (APD) and facilitate the development of ventricular arrhythmias and sudden cardiac death. Small conductance Ca^{2+}-activated K^+ (SK) current is a repolarization current responsible for afterhyperpolarization of the neurons in the central nervous system.4,5 Apamin, a Western honey bee toxin, is a specific blocker of the SK current in both neurons and cardiac myocytes.4,5 Studies from Chiamvimonvat’s laboratory showed that apamin-sensitive SK current (I_{KAS}) is important in the repolarization of atrial myocytes and plays important roles in automaticity and atrioventricular node conduction.4,9 Although I_{KAS} contributes little in the normal ventricles at normal pacing rates,10 I_{KAS} blockade by apamin can prolong APD in normal ventricles when the pacing rate is slow.11 More importantly, I_{KAS} is significantly upregulated in myocardial infarction and heart failure. The effects of apamin on APD in diseased hearts also increase with increased pacing cycle length (PCL).11-17 In addition, blocking I_{KAS} at long PCL may result in spontaneous afterdepolarization, torsades de pointes ventricular arrhythmia, and ventricular fibrillation in Langendorff-perfused rabbit hearts.11 Although I_{KAS} is upregulated in diseased hearts, the...
WHAT IS KNOWN

• Heart failure is associated with significant electrophysiological remodeling that includes a downregulation of most potassium currents and upregulation of late sodium and sodium–calcium exchange currents, resulting in the reduction of the repolarization reserve, prolongation of the action potential duration, and facilitation of the development of ventricular arrhythmias and sudden cardiac death.

• Small conductance Ca²⁺-activated K⁺ current (I_{KAS}) is significantly upregulated in heart failure.

• Blocking I_{KAS} results in spontaneous afterdepolarization, torsades de pointes ventricular arrhythmia, and ventricular fibrillation in Langendorff-perfused rabbit hearts.

WHAT THE STUDY ADDS

• I_{KAS} is important in the transmural repolarization in failing human ventricles.

• The M cells seem to have less I_{KAS} upregulation than the non-M cells in failing ventricles.

• There is abundant SK2 protein in the intercalated discs; blocking I_{KAS} reduces transmural conduction velocity in native failing ventricles.

• These results suggest that I_{KAS} is important in transmural conduction and repolarization of failing human ventricles.

magnitude of upregulation may vary transmurally. In both failing human and rabbit ventricles, cells isolated from the midmyocardium had lower I_{KAS} density than the cells isolated from the epicardial layer. However, because only a small number of cells were studied, the transmural distribution of I_{KAS} remains unclear. We sought to determine the transmural distribution of I_{KAS} in failing human ventricles by studying the wedge preparation using optical mapping techniques. We also identified the comparative importance of I_{KAS}, rapid delayed rectifier potassium current (I_{K}) and slow delayed rectifier potassium current (I_{Kr}) in human ventricles by sequential application of specific blockers. The results are used to test the hypothesis that I_{KAS} inhibition by apamin has significant effects on transmural repolarization in diseased human ventricles.

Methods

This research project is approved by the Institutional Review Board of the Indiana University Purdue University Indianapolis. We consented and studied 20 consecutive transplant recipients who underwent orthotopic cardiac transplantation. Among them 6 were excluded because of poor signal quality. The remaining 14 were successfully studied (Table). A detailed Methods section can be found in the Data Supplement.

Human Wedge Preparation

A wedge of the left ventricular free wall was perfused by an isolated left circumflex coronary artery branch (Figure 1A). The wedge was Langendorff perfused with 37°C oxygenated Tyrode’s solution. Two pseudo-ECG electrodes were mounted on the opposite sides of the wedge. A bipolar pacing lead was hooked onto the endocardium.

Imaging System: Single and Dual Mapping

For the first 10 hearts, a single camera was used for mapping the AP. We used blebbistatin to arrest contraction and Pyridinium, 4-(2-(6-(dibutylamino)-2-naphthalenyl)ethenyl)-1-(3-sulfopropyl)-hydroxide, inner salt 90134-00-2 (di-4ANEPPS) for voltage mapping. The last 4 hearts were stained with Rhod-2 AM and RH237 for simultaneous AP and calcium transient mapping (Figure 1B). The activation time, APD, calcium transient duration (CaTD), and the difference between CaTD and APD (CaTD–APD) of the mapped region were examined (Figure 1C).

Experimental Protocol

Protocol I: The Effects of Apamin on APD

To determine APD restitution curve before and after apamin, 10 preparations were sequentially paced (in ms) at 2000, 1500, 1000, 900, 800, 700, 600, and 500 PCL. We then determined the steep portion of the APD restitution curve by reducing the PCL from 400 ms in 10 ms steps until loss of capture. Apamin (100 nmol/L) was then added into the perfusate and the protocol was repeated 30 minutes later.

Protocol II: The Comparative Importance of I_{KAS}, I_{K}, and I_{Kr} in Ventricular Repolarization

The last 4 hearts were first treated with apamin, followed by chromanol (50 µmol/L) and then E4031 (100 nmol/L). Optical mapping was performed at baseline and after each drug was administered.

Data Processing

The subepicardium and subendocardium were defined by 20% of transmural thickness from epicardium and endocardium, respectively. The remaining 60% of tissue was the midmyocardium. To determine the APD, we selected for analysis 4 pixels in each layer and obtained a mean of these 4 pixels to represent the APD of that layer. Transmural APD or CaTD gradients were defined by the difference of APD or CaTD, respectively, between endocardial and epicardial layers. M-cell islands were defined as the regions that had longer APD than neighboring myocardium surrounded by a local APD gradient >15 ms/mm. Transmural conduction time was defined by the difference of activation time between the earliest activation site at endocardium and the corresponding epicardial site. The transmural conduction velocity (TCV; cm/s) was calculated by the ratio between the transmural thickness and the transmural conduction time. The magnitude of drug-induced changes of APD (% APD prolongation) was calculated by the ratio between the ΔAPD (postdrug APD–predrug APD) and the baseline APD.

Immunohistochemical staining was performed using an anti-SK2 rabbit polyclonal antibody ( Sigma-Aldrich, St. Louis, MO).

Statistical Analysis

Results are summarized as mean and 95% confidence interval (CI). Pearson correlation coefficient was used to assess the association between baseline APD and APD prolongation. The paired T tests were used to compare APD, maximal restitution slope and conduction velocity between baseline and postapamin. Bonferroni method was used to adjust for multiple comparisons. Fisher exact test was used to compare the occurrence of electric alternans between baseline and postapamin. The 95% CI of the proportion of the wedges with strong intercalated discs SK2 staining is based on the exact binomial distribution. Linear mixed-effects models were used to estimate the effects of different drugs averaged over PCL by treating patients as the random effect. All statistical analyses were performed in IBM SPSS Statistics V21 and SAS 9.3 (SAS Inc., Cary, NC). A 2-sided P≤0.05 was considered statistically significant.
Clinical Characteristics

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%APD indicates prolongation percentage of APD; A, amiodarone; AP, optical mapping of the action potential; B, β-blocker; CaT, optical mapping of calcium transient; CM, cardiomyopathy; CRT, cardiac resynchronization therapy; F, female; IHC, immunohistochemical staining; LVEF, left ventricular ejection fraction; M, male; TCV, transmural conduction velocity; and VAD, ventricular assist device.

Results

Effects of Apamin on Transmural APD
Apamin significantly altered transmural APD distribution. Figure 2A and 2B shows typical APD maps before and after apamin, respectively, at 2000 ms PCL. Apamin administration prolonged APD throughout the mapped region, as shown by the changing colors between Figure 2A and 2B. Figure 2C shows a significant negative correlation between the magnitudes of APD prolongation and baseline APD in all wedges studied was −0.35 (95% CI, −0.14 to −0.57) by Pearson correlation (r) when PCL was fixed at 2000 ms.

In addition to baseline APD, PCL was also an important factor that determines the magnitudes of APD prolongation after apamin. Figure 3A shows the effects of PCL on APD at 3 different myocardial layers of the wedge preparation, both at baseline and after apamin. Apamin prolonged APD significantly in all 3 layers at all PCL (P value <0.05 for all). Among them, 22 of 27 comparisons remained significant after Bonferroni adjustment. The ΔAPD progressively increased with lengthening of PCL. Figure 3B shows the APD restitution curve before and after apamin. Apamin administration increased the maximal slope of APD restitution curve in the subendocardium from 0.49 (95% CI, 0.41−0.57) to 0.58 (95% CI, 0.48−0.69; P=0.033), but did not significantly change the maximal restitution slope of the subepicardium (from 0.44 [95% CI, 0.35−0.54] to 0.54 [95% CI, 0.40−0.48]; P=0.058) or midmyocardium (from 0.42 [95% CI, 0.35−0.50] to 0.51 [95% CI, 0.41−0.61]; P=0.096). The observations of electric alternans when approaching effective refractory periods (ERP) were 6 of 10 before apamin (ERP=304 ms [95% CI, 262–346]) and 9 of 10 after apamin (ERP=312 ms [95% CI, 262–361]; P=0.4). No arrhythmia was induced with programmed stimulation before and after apamin in the first 2 wedges studied. Figure 3C plots the magnitudes of APD prolongation against the PCL at all 3 layers of the myocardium. There was a positive correlation between the magnitudes of APD prolongation and the PCL at all 3 layers (subendocardium, r=0.423 [95% CI, 0.131−0.521]; midmyocardium, r=0.338 [95% CI, 0.094−0.624]; and subepicardium, r=0.437 [95% CI, 0.243−0.622]). The differences of APD between subepicardium and subendocardium (the transmural APD gradient) at 1000 ms PCL was 33 ms (95% CI, 21–44) at baseline and 42 ms (95% CI, 21–62) after apamin (P=0.244). The transmural APD gradient correlated positively with PCL both at baseline (r=0.816; P=0.007) and after apamin (r=0.870; P=0.002; Figure I in the Data Supplement).

There were large individual variations of APD responses to apamin (Figure 3D). A possible mechanism for differential APD response to apamin is pretransplant treatment with...
amiodarone, a known inhibitor of $I_{KAS}$. However, the magnitudes of APD prolongation in patients treated with amiodarone (11.6% [95% CI, −0.5 to 23.7]; N=7) did not differ significantly from patients not treated with amiodarone (14.9% [95% CI, −8.2 to 38.0]; N=7) at 1000 ms PCL ($P=0.55$). None of the other clinical characteristics was significantly associated with APD prolongation induced by apamin (See Results in the Data Supplement).

M-Cell Islands

We applied the criteria used by Glukhov et al to identify the M-cell islands in the transmural wedge preparation. Only 1 of 14 wedges had any M-cell island according to those criteria. In that wedge, there were 2 M-cell islands at subendocardial and subepicardial regions, respectively, at 2000 ms PCL (Figure 4A, red arrows). The M-cell islands had long APD and were surrounded by steep APD gradients. APD in M-cell islands averaged 457 ms (95% CI, 454–460), which was increased to 496 ms (95% CI, 495–498) after apamin ($P<0.001$). Figure 4B shows a map of ΔAPD. The M-cell islands (red arrows) show less APD prolongation (smaller ΔAPD) than the surrounding tissues. Figure 4C shows the local APD gradients of the mapped area. Red arrows point to the M-cell islands surrounded by large local APD gradients. Figure 4D shows the %APD prolongation as a function of baseline APD. There was a highly heterogeneous prolongation of APD when the baseline APD was <440 ms, with a magnitude of APD prolongation as high as 70%. In contrast, the M-cell islands (data points within the red circle) had APD prolongation of only 0% to 20%, consistent with the observation that the magnitudes of APD prolongation are reversely correlated with the baseline APD. No other wedges had M-cell islands detected. When paced at fast rates approaching the ERP, APD alternans was not observed at baseline (Figure 4E, data from point no. 1 and no. 2 in Figure 4A). However, after apamin administration, APD alternans was observed in M-cell islands (Figure 4F, point no. 2 in Figure 4A) but not in the tissues outside M-cell islands (Figure 4F, point no. 1 in Figure 4A). The maximal slope of APD restitution curve increased from 0.75 to 1.25 after apamin (Figure 4G and 4H).

Effects of Apamin on TCV

There was no difference of TCV between baseline (37.0 cm/s [95% CI, 31.5–42.3]) and after apamin (35.3 cm/s [95% CI, 28.8–41.8]; $P=0.333$) at 1000 ms PCL. Two wedge preparations from rejected cardiac allografts (patient no. 8 and no. 14) showed accelerated TCV after apamin (from 43.5 cm/s at baseline [95% CI, 43.2–43.8] to 55.0 cm/s [95% CI, 54.3–55.8]; statistical analysis was not performed because of limited case number). In the remaining 12 patients, the TCV reduced from 35.9 cm/s (95% CI, 30.1–41.7) to 32.0 cm/s (95% CI,
26.8–37.2; \(P=0.001\). Figure 5A shows the representative activation maps (left panel) and AP tracings (right panel) before and after apamin at 1000 ms PCL. Figure 5B demonstrates the change of TCV at all PCL after the administration of apamin in wedges from either all 14 wedges together (green and blue lines) or from only the 12 wedges from native failing hearts (black and red lines). The reductions of TCV after apamin were significant for the latter group over all PCLs (red line). There was no significant difference between patients with ischemic or nonischemic cardiomyopathy (−4.0 cm/s [95% CI, −6.2 to −1.8], \(n=4\) versus −0.8 cm/s [95% CI, −3.8 to 2.2], \(n=10\); \(P=0.132\)) or in patients with normal (>55%) or abnormal ejection fraction (0.7 cm/s [95% CI, −7.8 to 9.2], \(n=3\) versus −2.4 cm/s [95% CI, −4.6 to −0.1], \(n=11\); \(P=0.553\)).

Figure 5C shows the average of local conduction velocity in 3 muscle layers at 1000 ms PCL. There were significant differences among the 3 muscle layers (\(P<0.001\) when \(n=14\), \(P=0.004\) when \(n=12\)). The local conduction velocity was the fastest at the subendocardium and the slowest at the midmyocardium. After applying apamin, a significant reduction of local conduction velocity was observed at the subendocardium in native hearts (\(N=12\)): from 66.9 (95% CI, 49.4–84.3) to 51.7 cm/s (95% CI, 34.7–68.8; \(P=0.011\)). No significant change of local conduction velocity was noted in other 2 layers.

**Immunohistochemical Staining**

Immunohistochemical staining of the ventricular myocytes showed strong staining of the SK2 protein in the intercalated discs between ventricular myocytes. Figure 5D and 5E is a typical example of the SK2 staining. Figure 5D shows that both the nerves and the myocardium were positively stained with the SK2 protein in patient no. 4. Figure 5E shows a magnified view of the SK2 proteins in the intercalated discs in patient no. 5. Strong SK2 staining was found in the intercalated discs of all 14 wedges studied (100% [95% CI, 80–100]).

**Importance of \(I_{\text{KAS}}\) Relative to \(I_{\text{Ks}}, I_{\text{Kr}}\)**

The effects of apamin, chromanol, and E4031 on APD prolongation were studied sequentially in wedges from patient no. 11 to no. 14. Figure 6A shows representative AP tracings from patient no. 12 at baseline and after sequential administration of apamin, chromanol, and E4031. In these 4 hearts, APDs were significantly prolonged by chromanol (\(P<0.001\)) and E4031.
The maximal slopes of APD restitution curve of subepicardium, midmyocardium, and subendocardium after chromanol are 0.50 (95% CI, 0.33–0.67), 0.56 (95% CI, 0.40–0.71), and 0.49 (95% CI, 0.37–0.61), respectively ($P=0.181$, $P=0.141$, and $P=0.588$, respectively, compared with post apamin) and after E4031 were 1.01 (95% CI, 0.36–1.67), 0.90 (95% CI, 0.48–1.32), and 0.76 (95% CI, 0.56–0.97), respectively ($P=0.212$, $P=0.139$, and $P=0.049$, respectively, compared with post chromanol).

Figure 7A shows representative calcium transient tracings and CaTD maps at baseline and after apamin, chromanol, and E4031 from patient no. 12. Apamin did not significantly prolong the CaTD ($P=0.502$), but chromanol ($P=0.042$) and E4031 ($P<0.001$) did. The CaTD prolongation was PCL dependent and was different among drugs. As shown in Figure 7B, the effect of apamin could be observed at long PCLs, whereas the effect of chromanol was mainly observed at short PCL. There was no significant prolongation of CaTD by apamin in any of the 3 layers. However, the prolongation of CaTD by chromanol was significant at the subepicardium ($P=0.009$). E4031 significantly prolonged CaTD in all 3 layers of the transmural surface, with larger effects at the subepicardium ($P=0.004$) and midmyocardium ($P=0.013$) compared with the subendocardium. Therefore, the transmural gradient of CaTD (ie, CaTD between subendocardium and subepicardium) was eliminated after E4031 ($P=0.201$; Figure 7C).

$I_{\text{KAS}}$ and the Differences Between APD and CaTD

Figure 8A shows the effects of apamin on the difference between CaTD and APD (CaTD–APD) at the subepicardium and the subendocardium from patient no. 14. Consistent with that reported by Lou et al,21 there was a larger CaTD–APD at the subendocardium than at the subepicardium or midmyocardium ($P<0.001$ for both comparisons). This difference increased with increasing PCL (Figure 8B). Apamin significantly ($P<0.001$) reduced the CaTD–APD on the subendocardium but not in the midmyocardium ($P=0.141$) or the subepicardium ($P=0.0146$; Figure 8C). This differential effect resulted in a reduction, but not elimination, of the differences of CaTD–APD between subendocardium and the other 2 layers ($P=0.078$ compared with midmyocardium; $P<0.001$ compared with subepicardium). CaTD–APD is further reduced by chromanol ($P<0.001$) and E4031 ($P<0.001$), resulting in the elimination of the differential CaTD–APD among the 3 layers after all 3 drugs were given (Figures II and III in the Data Supplement).

Discussion

The major finding of our study is that $I_{\text{KAS}}$ is important in the repolarization of the transmural wedge preparation in failing human ventricles. The conclusion is strengthened by our recent study that showed apamin is a highly specific SK current blocker. The magnitude of $I_{\text{KAS}}$ was positively correlated with the PCL. At a fixed PCL, the magnitude of $I_{\text{KAS}}$ was negatively correlated with APD. The M cells (which have long APDs) seem to have less $I_{\text{KAS}}$ upregulation than the non-M cells. We also found that there was an abundant expression of SK2 proteins in the intercalated discs, and that apamin may reduce TCV in native failing ventricles but not in the ventricle removed because of rejection. Finally, the presence of
I\text{\textsubscript{KAS}} may be in part responsible for the differences between CaTD and APD in the failing ventricles.

I\text{\textsubscript{KAS}} and Transmural Repolarization in Human Wedge Preparation

We showed that the transmural APD significantly and heterogeneously prolonged when exposed to apamin. However, previous studies from our laboratory found a lower \(I_{\text{KAS}}\) density in cells isolated from the midmyocardial layer than that isolated from subepicardial and subendocardial layers in failing rabbit and human ventricles.\textsuperscript{12,13} In comparison, we found no statistically significant differences of apamin-induced APD changes among the 3 myocardial layers. These findings may be explained by the effects of electric coupling, which may reduce the transmural repolarization heterogeneity in the wedge preparation compared with that in isolated myocytes.\textsuperscript{22,23}

APD and the PCL

At a fixed PCL, there is a clear negative correlation between the magnitude of apamin-induced APD prolongation and the baseline APD. These findings imply that \(I_{\text{KAS}}\) upregulation is important in maintaining transmural repolarization reserve. Failure to upregulate \(I_{\text{KAS}}\) is a characteristic finding in cells with long APDs. If the APD is the only determinant for apamin responsiveness, then conditions that typically lengthen APD (such as long PCL) should be associated with reduced apamin responsiveness. However, lengthening PCL in the human wedge preparation significantly increased the magnitude of APD prolongation induced by apamin. The K channel blockers (class III antiarrhythmic agents) are known to exhibit reverse use-dependence, resulting in greater prolongation of APD at longer PCL.\textsuperscript{24} Apamin may have similar reverse use-dependent properties, resulting in greater effects on \(I_{\text{KAS}}\) at longer PCL. Reverse use-dependence may also underlie proarrhythmic effects of apamin at slow ventricular rates.\textsuperscript{11}

\(I_{\text{KAS}}\) and the Characteristics of M Cells

The M cell is distinguished from other ventricular myocytes based on the ability of its APD to prolong prominently at slower rates.\textsuperscript{25} Distinct M-cell islands are observed frequently in the wedge preparation from normal ventricles but rarely in failing ventricles.\textsuperscript{18,21} Consistent with these previous studies, we found only 2 M-cell islands in one of the 14 wedges studied. In that M-cell island, APD is long and the magnitude of APD prolongation induced by apamin is small. These data suggest that M cells in failing ventricles are characterized by a deficiency in \(I_{\text{KAS}}\) as compared with most of the surrounding myocytes. When the PCL is lengthened, the repolarization reserve of non-M cells is maintained in part by a robust upregulation of the \(I_{\text{KAS}}\). However, because of the relative deficiencies of the \(I_{\text{KAS}}\) in the M-cell island, it was not able to maintain its repolarization reserve thus prolonging its APD more prominently during the slow than the fast rates. This phenomenon
(the ability for APD to prolong prominently at slower rates) fulfills the traditional definition of M cell.25

Although M-cell islands have longer APD than the surrounding tissues, no electric alternans were observed when PCL shortened to near ERP. After apamin administration, the M-cell island and the surrounding tissues had similar APD. However, rapid pacing induced electric alternans only in the M-cell island. Although $I_{KAS}$ is not prominently upregulated in M-cell islands, it may still play an important role in preventing alternans at fast rates. $I_{KAS}$ blockade may have larger effects on the repolarization reserve of the M-cell islands than the surrounding myocardium.

$I_{KAS}$ and Transmural Conduction

We found that apamin prolonged transmural conduction time in 12 wedges isolated from failing native hearts. There is also abundant SK2 protein in the intercalated discs, suggesting that apamin may significantly interfere with the intercalated disc function. Previous studies have shown that SK2 channel knockout in a murine model results in prolongation of the PR interval and that the SK2Δ/Δ mice may develop complete atrioventricular block.9 Studies in mice resistance arteries showed that electric conduction along the endothelium of the arteries is also controlled in part by the SK2.26 Other investigators showed that overexpression of SK3 in murine model

Figure 6. Comparison of action potential duration (APD) prolongation by apamin, chromanol, and E4031. A, Representative action potential tracings from patient no. 12 at baseline, after apamin, chromanol, and E4031 at 2000 ms pacing cycle length (PCL). B, The percentage of APD prolongation by apamin, chromanol, and E4031. †P<0.05 comparing the effect of apamin and chromanol. ‡P<0.05 comparing the effect of chromanol and E4031. C, Transmural APD gradients at different PCL. D, Effects of chromanol on APD in 3 myocardial layers. E, Effects of E4031 on APD in 3 myocardial layers. Bars represent 95% confidence interval. Endo indicates subendocardial layer; Epi, subepicardial layer; and Mid, midmyocardial layer.

Figure 7. Effects of apamin, chromanol, and E4031 on calcium transient duration (CaTD). A, Representative calcium transient tracings and CaTD maps from patient no. 12 at 2000 ms pacing cycle length (PCL) at baseline and after sequential administration of apamin, chromanol, and E4031. B, CaTD at different PCL. †P<0.05 between baseline CaTD and that after apamin. ‡P<0.05 between after apamin and after chromanol. ††P<0.05 between after chromanol and after E4031. C, Transmural gradient of CaTD at different PCLs. Bars represent 95% confidence interval.
is associated with reduced ventricular conduction velocity, bradyarrhythmias, heart block, and sudden death. These findings imply a possible role of SK channels in cell–cell signal transduction and conduction.

Relative Importance of $I_{KAS}$, $I_{Ks}$, and $I_{Kr}$

The magnitude of APD prolongation after apamin varies greatly from site to site. In some locations, the APD could prolong ≤70% after apamin administration. Previous reports about the $I_{Ks}$ and $I_{Kr}$ in transmural preparations were mostly performed in canine models using pharmacological interventions. Because E4031 and chromanol 293 also block the SK current,20 APD prolongation in those studies may be in part because of the inhibition of the SK currents. In our study, we gave apamin first, followed by chromanol 293 and E4031. Our results did not show different magnitudes of APD prolongation by potassium channel blockers in different myocardial layers.

$I_{KAS}$ and CaTD–APD

We found an increased CaTD–APD in the transmural wedge. The magnitudes of CaTD–APD are the largest at the subendocardium, a finding consistent with earlier studies in failing human hearts.21 The CaTD–APD was reduced by apamin, suggesting that $I_{KAS}$ upregulation in failing ventricles is in part responsible for differences between CaTD and APD.

SK Subtypes

The SK channels have 3 different subtypes: SK1, SK2, and SK3.28 Among them, SK2 is most sensitive to apamin, followed by SK3 and SK1, respectively.29 SK1 sensitivity to apamin is species-specific and that SK1 in humans is apamin sensitive.29 Because we used high dose (100 nmol/L) of apamin in this study, all 3 subtypes of SK currents should have been blocked during the drug administration. However, it is not possible to determine the relative importance of these 3 subtypes of SK currents in the conduction and repolarization in the human transmural wedge preparations. Our earlier study had detected only a small amount of SK3 protein in the normal and failing human ventricles.13 In contrast, the SK2 protein is abundantly present in failing human ventricles and is most sensitive to apamin. Therefore, we have focused our study on SK2.

Limitations

There are several limitations in this study. First, the pathogenesis and duration of disease varied greatly among the subjects of the study. Nine of them had received ventricular assist device before receiving transplantation. Four of them received cardiac resynchronization therapy before transplantation. Both forms of therapy may result in ventricular remodeling. Second, we attempted to map 20 hearts between January 2012 and December 2013, but only 14 hearts were successfully studied and included in this analysis. Various technical issues might have been involved in failed mapping studies, but it remains possible that the hearts not successfully mapped may have different SK currents than the hearts successfully mapped. Third, the number of patients in the second part of the experiment is small, although the results among these 4 ventricles consistently showed that all 3 major K channels played important roles in ventricular repolarization. One of the 4 hearts was from a patient with cardiac allograft rejection. The number of patients is too small to determine whether that heart had a different drug response than the other 3 hearts. Fourth, we only studied left ventricular free wall, perfused by a branch of left circumflex artery. Therefore, the finding in this study may not apply to other regions of the ventricles. Finally, the photosensitive dyes and the electromechanical uncoupler may have electrophysiological effects that affect the results of the study.
Conclusions
We conclude that $I_{KAS}$ is important in the transmural repolarization in diseased human ventricles.

The magnitude of $I_{KAS}$ is positively correlated with the PCL, but negatively correlated with APD when PCL is fixed.

These results suggest that SK current may be an important new target for antiarrhythmic drug therapy.

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Disclosures
None.

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Small Conductance Calcium-Activated Potassium Current Is Important in Transmural Repolarization of Failing Human Ventricles

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SUPPLEMENTAL MATERIAL

Methods

The human Heart Failure Tissue Collection Program is approved by the Institutional Review Board of the Indiana University Purdue University Indianapolis. We consented and studied 20 consecutive transplant recipients who underwent orthotopic cardiac transplantation. Among them, 6 were excluded due to poor signal quality (signal to noise ratio < 20). The remaining 14 were successfully studied (Table 1).

Human Wedge Preparation

The explanted hearts were harvested and immediately transferred to the research laboratory in ice cold cardioplegic solution (in mM, NaCl 128.3, KCl 15, NaHCO$_3$ 20.15, NaH$_2$PO$_4$ 0.41, MgSO$_4$ 1.18, CaCl$_2$ 1.8, glucose 11.1, adjusted to pH 7.4 with NaOH). A wedge of the left ventricular free wall was perfused by an isolated left circumflex coronary artery branch (Figure 1A). All leaky vessels were ligated at the edge. The wedge was then connected to a Langendorff perfusion system and perfused with 37°C oxygenated Tyrode’s solution, containing (in mM) NaCl 128.3, KCl 4.7, NaHCO$_3$ 20.15, NaH$_2$PO$_4$ 0.41, MgSO$_4$ 1.18, CaCl$_2$ 1.8, glucose 11.1 and 1.5% bovine serum albumin, adjusted to pH 7.4 with NaOH. The pressure was maintained at 60-70 mmHg throughout the experiment. The wedge was placed in a transparent glass chamber filled with the same 37°C Tyrode’s solution. Two pseudo-ECG electrodes were mounted on the opposite sides of the wedge. An additional reference electrode was positioned in the bath chamber away from the two pseudo-ECG leads. A bipolar pacing lead was hooked onto the center of the endocardium.

Imaging system: single and dual mapping

For the first 10 hearts (Table 1), a single camera was used for mapping the action potential (AP). An excitation-contraction uncoupler blebbistatin (20 μM, from Tocris Bioscience, Minneapolis, MN) was used during optical mapping. The stock aliquots of blebbistatin were prepared as 38
mM in dimethyl sulfoxide (DMSO) and stored at -20°C. After the hearts stopped contracting, 100 μl of voltage-sensitive dye di-4ANEPPS (from Biotium, Hayward, CA) stock aliquots were injected into the perfusion system. Additional 50~100 μl di-4ANEPPS stock solution was added if the signal-to-noise ratio was deemed to be low. The last 4 hearts were stained with a calcium indicator Rhod-2 AM (from Molecular Probes, Eugene, OR) and a voltage-sensitive dye RH237 (from Molecular Probes, Eugene, OR) for simultaneous AP and calcium transient (CaT) mapping (Figure 1B). The stock aliquots of Rhod-2 AM were prepared as 2 mM in DMSO and stored at -20°C. Before adding into recirculated Tyrode’s solution, 75 μl of stock aliquots were mixed well with 75 μl of Pluronic F-127 (20% w/v) and perfused into the hearts for 10 min. This was followed by dye-free Tyrode’s solution for another 10 min for de-esterification of Rhod-2 AM. For AP mapping, 150 μl of RH237 stock aliquots were injected in 50 ml Tyrode’s solution and followed by dye-free Tyrode’s solution for another 10 min. The stock aliquots of RH237 were prepared as 2 mM in DMSO and stored at -20°C. Blebbistatin was recirculated afterwards as described above.

The stained hearts were excited with laser light at 532 nm (Verdi, Coherent Inc., Santa Clara, CA). The emitted fluorescence was collected using either one or two CMOS cameras (MiCAM Ultima, BrainVision, Tokyo, Japan) at 2 ms/frame and 100 x 100 pixels with spatial resolution of 0.35 x 0.35 mm² per pixel. The fluorescence induced by the laser illumination was obtained through a common lens, separated by a dichroic mirror (650 nm cutoff wavelength), and directed to the respective camera with additional filtering (715 nm longpass for AP and 580 ± 20 nm for CaT). The image acquisition was controlled by a custom-designed program based on LabView and the IMAQ Vision toolset (National Instruments, Austin, TX). The activation time, APD, calcium transient duration (CaTD) and the difference between CaTD and APD (CaTD-APD) of the mapped region were color coded and displayed in 2 dimensional maps for examination (Figure 1C).
Experimental Protocol

We performed programmed stimulation with up to 3 premature stimuli in two wedges (#1 and #2) to test the inducibility. No arrhythmias were induced. In subsequent studies, we eliminated the programmed stimulation to shorten the duration of the study.

*Protocol I: the effects of apamin on APD*

The first 10 hearts were tested using this protocol. The wedge was paced at 1000 ms PCL during tissue stabilization and staining. To determine APD restitution curve before and after apamin, the preparation was sequentially paced (in ms) at 2000, 1500, 1000, 900, 800, 700, 600 and 500 PCL. We then determined the steep portion of the APD restitution curve by reducing the PCL from 400 ms in 10 ms steps until loss of capture. Each cycle length was paced for at least 30 beats or 30 s before optical imaging. Apamin (100 nM, Tocris Bioscience, Minneapolis, MN) was then added into the perfusate and the protocol was repeated 30 min later while the tissues were paced at 1000 ms PCL.

*Protocol II: The comparative importance of $I_{KAS}$, $I_{Ks}$ and $I_{Kr}$ in ventricular repolarization*

The last 4 hearts were first treated with apamin (100 nM), followed by Chromanol (50 µM) and then E4031 (100 nM, both from Tocris Bioscience, Minneapolis, MN) to determine the magnitude of APD prolongation after each drug. The same stimulation protocol with PCL sequentially shortened from 2000 to 500 ms was repeated 15 min after each drug was added into the perfusate.

Data processing

Optical APD and CaTD were measured at the level of 80% repolarization and recovery, respectively. Two-dimensional maps were constructed to study the spatial distribution of APD and CaTD. The wedge preparation was divided into three layers: subepicardium, midmyocardium and subendocardium. The subepicardium and subendocardium were defined by 20% of transmural thickness from epicardium and endocardium, respectively. The remaining
60% of tissue was the midmyocardium. To determine the APD, we selected for analysis four pixels in each layer and obtained a mean of these four pixels to represent the APD of that layer. These pixels were chosen at 4 evenly spaced locations in each layer. All chosen pixels had a stable baseline and a signal to noise ratio > 20. Transmural APD or CaTD gradients were defined by the difference of APD or CaTD, respectively, between endocardial and epicardial layers.\(^{19}\) The local APD gradient at a pixel with coordinate \((m, n)\) was calculated as \([(\text{APD}_{m+1} - \text{APD}_m)^2 + (\text{APD}_{n+1} - \text{APD}_n)^2]^{0.5}.\(^{20}\) M cell islands were defined as the regions that had longer APD than neighboring myocardium surrounded by a local APD gradient > 15 ms/mm.\(^{19}\) Transmural conduction time was defined by the difference of activation time between the earliest activation site at endocardium and the corresponding epicardial site. The transmural conduction time was subdivided into 3 components of local activation times: subendocardial, midmyocardial and subepicardial. The time of activation on the AP was determined by the time when phase 0 of the action potential reached 50% of maximal depolarization. The transmural conduction velocity (TCV) (cm/s) was calculated by the ratio between the transmural thickness and the transmural conduction time. The magnitude of drug-induced changes of APD (% APD prolongation) was calculated by the ratio between the \(\Delta\text{APD}\) (post-drug APD – pre-drug APD) and the baseline APD.

**Immunohistochemistry studies**

Sections of the myocardium were harvested and fixed in 4% formalin for 45 min, followed by storage in 70% alcohol.\(^{21}\) The tissues were processed routinely, paraffin embedded and cut into 5-µm thick sections. Immunohistochemical staining was performed using an anti-SK2 rabbit polyclonal antibody (Sigma-Aldrich, St. Louis, MO).\(^{22}\)

**Western blot analysis**

Human heart membrane microsomes were obtained by homogenization and centrifugation. Sixty µg of microsomes were subjected to a SDS-polyacrylamide gel
electrophoresis and transferred to a nitrocellulose membrane. The blot was probed with a rabbit anti-SK2 polyclonal antibody (Sigma, 1:1000). Antibody-bound protein bands were visualized with 125I-protein A followed by autoradiography.

**Statistical analysis**

Results are summarized as mean and 95% confidence interval (CI). Pearson correlation coefficient was used to assess the association between baseline APD and APD prolongation. The paired T tests were used to compare APD, maximal restitution slope and conduction velocity between baseline and post-apamin. Bonferroni method was used to adjust for multiple comparisons. Fisher's exact test was used to compare the occurrence of electrical alternans between baseline and post-apamin. The CI of the proportion of the wedges with strong intercalated discs SK2 staining is based on the exact binomial distribution. Linear mixed-effects models were used to estimate the effects of different drugs averaged over PCL by treating patients as the random effect. All statistical analyses were performed in IBM SPSS Statistics V21 and SAS 9.3 (SAS Inc., Cary, NC). A two sided p ≤ 0.05 was considered statistically significant.

**Results**

**APD prolongation is not associated with clinical characteristics**

Further analysis of the patients’ clinical characteristics showed that APD prolongation did not correlate with patients’ age (r=0.465, p=0.094) or left ventricular ejection fraction (r=-0.141, p=0.315). APD prolongation was not different between men and women (14.0% [CI, 7.3% to 20.7%], N=12 vs. 9.0%, N=2, p=0.531); between ischemic and non-ischemic cardiomyopathy (10.6% [CI, 3.5% to 17.7%], N=4 vs.14.3% [CI, 6.1% to 22.5%], N=10, p=0.550); between native and rejection hearts (14.4% [CI, 8.8% to 19.9%], N=12 vs. 6.7% [CI, -26.0% to 33.3%], N=2, p=0.214); between patients treated with or without beta blocker (16.6% [CI, -0.15% to
34.7\%, N=5 vs. 11.4\% [CI, 6.3\% to 16.4\%], N=9, p=0.366); with or without ventricular assist device (14.8\% [CI, 6.7\% to 22.9\%], N=9 vs 10.5\% [CI, -0.7\% to 21.7\%], N=5, p=0.460); or with or without cardiac resynchronization therapy (17.4\% [CI, -7.3\% to 42.0\%], N=4 vs. 11.6\% [CI, 6.5\% to 16.7\%], N=10, p=0.342).
Supplement Figure 1. Transmural APD gradient at baseline and after apamin. Bars represent 2 times of the standard error.
Supplement Figure 2. The differences between calcium transient duration and action potential duration (CaTD-APD) at baseline and after sequential administration of apamin, chromanol and E4031. * represents p<0.05 when comparing the CaTD-APD at baseline with that after apamin. \( \gamma \) represents p<0.05 when comparing CaTD-APD after apamin and that after chromanol. ‡ represents p<0.05 comparing the CaTD-APD after chromanol and that after E4031. Bars represent 2 times of the standard error.
Supplement Figure 3. CaTD-APD of all three myocardial layers after chromanol and E4031.

Bars represent 2 times of the standard error.
Supplement Figure 4. SK2 protein expression determined by Western blot analyses. Immunoblot was probed with a rabbit anti-SK2 polyclonal antibody (Sigma, 1:1000). Con: membranes from a normal human heart. +: Positive control using human embryonic kidney cells transfected with KCNN2.