Role of Apamin-Sensitive Calcium-Activated Small-Conductance Potassium Currents on the Mechanisms of Ventricular Fibrillation in Pacing-Induced Failing Rabbit Hearts

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Background—Ventricular fibrillation (VF) during heart failure is characterized by stable reentrant spiral waves (rotors). Apamin-sensitive small-conductance calcium-activated potassium currents (I_{KAS}) are heterogeneously upregulated in failing hearts. We hypothesized that I_{KAS} influences the location and stability of rotors during VF.

Methods and Results—Optical mapping was performed on 9 rabbit hearts with pacing-induced heart failure. The epicardial right ventricular and left ventricular surfaces were simultaneously mapped in a Langendorff preparation. At baseline and after apamin (100 nmol/L) infusion, the action potential duration (APD_{80}) was determined, and VF was induced. Areas with a >50% increase in the maximum action potential duration (ΔAPD) after apamin infusion were considered to have a high I_{KAS} distribution. At baseline, the distribution density of phase singularities during VF in high I_{KAS} distribution areas was higher than at other areas (0.0035±0.0011 versus 0.0014±0.0010 phase singularities/pixel; P=0.004). In addition, high dominant frequencies also colocalized to high I_{KAS} distribution areas (26.0 versus 17.9 Hz; P=0.003). These correlations were eliminated during VF after apamin infusion, as the number of phase singularities (17.2 versus 11.0; P=0.009) and dominant frequencies (22.1 versus 16.2 Hz; P=0.022) were all significantly decreased. In addition, reentrant spiral waves became unstable after apamin infusion, and the duration of VF decreased.

Conclusions—The I_{KAS} current influences the mechanism of VF in failing hearts as phase singularities, high dominant frequencies, and reentrant spiral waves all correlated to areas of high I_{KAS}. Apamin eliminated this relationship and reduced VF vulnerability. (Circ Arrhythm Electrophysiol. 2017;10:e004434. DOI: 10.1161/CIRCEP.116.004434.)

Key Words: apamin • electrophysiology • heart failure • potassium • ventricular fibrillation

Heart failure (HF) is associated with structural and electrophysiological remodeling that leads to tissue heterogeneities that enhance arrhythmogenesis and the propensity of sudden cardiac death.1-3 Mapping studies of ventricular fibrillation (VF) have shown various mechanisms from multiple wavelets of excitation to reentrant spiral waves. The mechanisms of VF within the HF substrate have been shown to be dominated by either stable reentrant spiral waves or focal activity,4 correlating to stable high dominant frequency (DF) areas.5 This is in contrast to structurally normal hearts that have VF characterized by unstable reentrant spiral waves or multiple waves and transient high DF areas.4,5 The underlying mechanisms that support the stability of the reentrant spiral waves within the HF substrate are still unknown.

Small-conductance calcium-activated potassium (SK) channels have been shown to contribute to ventricular repolarization and are mediated by calcium binding.6 With each new study on SK channels, their importance is further demonstrated in arrhythmia vulnerability in both the atria7 and ventricles.8 Apamin is a specific blocker of SK currents, and...
WHAT IS KNOWN

- Ventricular fibrillation (VF) during heart failure is characterized by stable reentrant spiral waves (rotors).
- Apamin-sensitive small-conductance calcium-activated potassium currents (I_{KAS}) are heterogeneously upregulated in failing hearts.

WHAT THE STUDY ADDS

- The I_{KAS} current influences the mechanism of VF in failing hearts.
- Heterogeneous upregulation of I_{KAS} within this substrate provides a milieu for rotor location and stabilization.
- The spatial distribution of phase singularities and dominant frequencies, and the location of stable reentrant spiral waves correlated to greater ΔAPD (action potential duration) after apamin infusion.
- VF after apamin infusion eliminated this relationship as the rotors became unstable.

Apamin-sensitive SK currents (I_{KAS}) have been shown to be heterogeneously upregulated within the HF substrate in both human studies with transplant hearts and animal models of HF. This heterogeneity has been observed both transmurally and on the same surface. Several studies have been performed investigating the role of various potassium channels (I_{KATP}, I_{K1}, I_{Kv}, I_{Ks}, I_{KATP}) and apamin-sensitive small-conductance calcium-activated potassium currents (I_{KAS}) during VF. The spatial distribution of phase singularities (PS) within the HF substrate, which correlates to areas of upregulated SK current, has been observed. At baseline and after apamin infusion, pacing was performed from the apex at 300 ms to determine ΔAPD. The cycle length was then decreased by 10 ms steps until either VF was induced or there was loss of capture. If VF was not induced, burst pacing was used for VF initiation. VF was allowed to continue for at least 5 minutes before defibrillation attempts were made. From the optical VF recordings, voltage, phase, and PS mapping was performed. In addition, an automated PS detection algorithm was applied over a 100-ms window from each VF recording as previously described. The PS number was counted at 5 time points before a defibrillation shock was delivered, with each time point separated by 10 frames (20 ms) of data. Spiral waves were defined as PS with more than one revolution period. PSs associated with reentrant spiral waves were noted, and their locations were determined. The correlation of the change in ΔAPD to the location of the PS was analyzed with custom LabView software. The maximum change in ΔAPD, with apamin infusion set at 100%, and no change in the APD in the absence of apamin, was set at 0. The software then distributed the numbers between 0% and 100% based on their value, and colors were then assigned with blue at 0% and red at 100%. Areas with a >50% change in ΔAPD after apamin infusion were considered to have a high I_{KAS} distribution. Above the 50%, change in ΔAPD was the colors yellow, orange, and red. Below the 50% level were the colors green and blue. The area of the high I_{KAS}-expressing regions was then calculated as a sum of the number of pixels expressing yellow, orange, or red. The area of low I_{KAS}-expressing regions was calculated as the sum of the number of pixels expressing either green or blue. To further strengthen the results of the study, we also analyzed the data using 75% level change in ΔAPD after apamin infusion to define a high I_{KAS} distribution. Frequency domain analysis was also performed to quantify the VF characteristics. The highest peak within the magnitude spectrum was defined as the DF.

Methods

This study protocol was approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine and the Methodist Research Institute and conforms to the Guide for the Care and Use of Laboratory Animals. HF induction was attempted in 12 New Zealand white female rabbits via rapid ventricular pacing. Among them, 9 completed the pacing protocol and developed HF. Five additional rabbits were used as controls for histology.

Pacing-Induced HF and Optical Mapping

For creation of the rabbit HF model, rapid ventricular pacing was performed for 3 to 5 weeks. Using isoflurane for general anesthesia, a left lateral thoracotomy was performed, and an epicardial pacing lead was sutured to the lateral wall of the left ventricle and connected to a modified single-chamber ventricular pacemaker (Kappa or Enpulse Pacemaker, Medtronic, Inc, Minneapolis, MN). After recovery for 1 week, the ventricles were paced at 250 beats per minute for 3 days, 300 beats per minute for 3 days, and 350 beats per minute for 3 to 5 weeks to induce HF. Echocardiography was performed before the pacemaker was implanted and at follow-up. Measurements of left ventricular diameters and fractional shortening were made from M-mode recordings of the parasternal short-axis view at the tips of the papillary muscles using the leading edge technique in 3 to 4 cardiac cycles and averaged. After the echocardiographic recordings were made, optical mapping was performed as previously described. Briefly, hearts were Langendorff perfused with oxygenated Tyrode solution (in mmol/L: NaCl, 125; KCl, 4.5; NaHCO₃, 24; NaH₂PO₄, 1.8; CaCl₂, 1.8; MgCl₂, 0.5; and glucose, 5.5) with a pH of 7.40. The hearts were stained with RH237 (10 μmol/L) for voltage (V_m) mapping. The epicardial right ventricular and left ventricular surfaces were illuminated at a wavelength of 532 nm, and the emitted fluorescence was filtered at 715 nm long pass. Optical recordings were made from a 100x100 pixel area with a spatial resolution of 0.35x0.35 mm² per pixel. The signals were sampled at 2 ms/frame. Apamin, a specific SK channel blocker, has been shown to heterogeneously increase the action potential duration (ΔAPD) within the HF substrate, which correlates to areas of upregulated SK current. The ΔAPD after baseline and after apamin infusion was set at 100%, and no change in the APD was set at 0. The software then distributed the numbers between 0% and 100% based on their value, and colors were then assigned with blue at 0% and red at 100%. Areas with a >50% change in ΔAPD after apamin infusion were considered to have a high I_{KAS} distribution. Above the 50%, change in ΔAPD was the colors yellow, orange, and red. Below the 50% level were the colors green and blue. The area of the high I_{KAS}-expressing regions was then calculated as a sum of the number of pixels expressing yellow, orange, or red. The area of low I_{KAS}-expressing regions was calculated as the sum of the number of pixels expressing either green or blue. To further strengthen the results of the study, we also analyzed the data using 75% level change in ΔAPD after apamin infusion to define a high I_{KAS} distribution. Frequency domain analysis was also performed to quantify the VF characteristics. The highest peak within the magnitude spectrum was defined as the DF.

Histology

For histology, ventricular tissue embedded in paraffin, sectioned, and stained using Masson trichrome stain. The collagen content was quantified by identifying and counting the number of blue-staining pixels as a percentage of the total tissue area using digital photomicrographs in Adobe Photoshop CS6 software.

Statistical Analysis

Data are presented as mean and 95% confidence interval (CI). Wilcoxon signed-rank test was used to compare variables measured at baseline and after apamin infusion. Mann–Whitney–Wilcoxon test was used to compare the correlation between PS density and ΔAPD distribution as measured by ΔAPD. The P values are corrected for multiple comparisons in relevant analyses using Bonferroni adjustment. The generalized estimating equations were used to analyze repeated measures (identify link for VF vulnerability and logit link for dichotomized VF vulnerability) with compound symmetry correlation matrix. A 2-sided P value of ≤0.05 was considered statistically significant.

Results

All rabbits that survived the rapid pacing protocol showed clinical signs of HF as previously described. Table shows the echocardiographic data before and after the development of...
HF. As the results in the table show, significant left ventricular dysfunction developed with ventricular pacing.

Effects of Apamin on APD
In a majority of the mapped ventricular area, apamin significantly prolonged the APD80 in all 9 failing hearts. However, APD80 lengthening was not uniform across the mapped region, with some areas showing no change in APD80 after apamin infusion. An example of the heterogeneous APD80 lengthening with apamin is shown in Figure 1A, with example optical signals recorded during ventricular pacing at a 300 ms cycle length (Figure 1B). Overall, apamin significantly increased the APD80 (183 [95% CI, 172–194] ms versus 215 [95% CI, 205–224] ms, respectively, P=0.009) within the HF substrate as the summary data shows in Figure 1C. The difference in APD80 between baseline and after apamin infusion (ΔAPD80, apamin-treated APD80 minus baseline APD80) was used to characterize the 2-dimensional distribution of I_{KAS}. Areas with a >50% change in the ΔAPD80 after apamin were considered to be areas with I_{KAS} upregulation.

Distribution of PS and Spiral Waves
After VF was induced, optical mapping was used to investigate the VF mechanisms before and after infusion of apamin. PS were used to locate the sources of reentrant VF activation during a 100-ms window from each VF recording. The density of PSs in and out of the areas of I_{KAS} upregulation were calculated by measuring the distribution density of PS versus the ΔAPD80 (Figure 2B). At baseline, PSs correlated to an increase in ΔAPD80; however, this relationship disappeared after apamin infusion. An example of this correlation is demonstrated in Figure 2 (>50% ΔAPD80 versus <50% ΔAPD80; 0.0035±0.0011 versus 0.0014±0.0010 PSs/pixel; P=0.004; Figure 2C). After apamin, there was no significant correlation in the distribution of PS (>50% ΔAPD80 versus <50% ΔAPD80; 0.0016±0.0007 versus 0.0016±0.0010 PS/pixel; P=0.791; Figure 2D). In addition, the number of PSs within the 100-ms window was significantly decreased by apamin (baseline versus apamin infusion, 17.2 versus 11.0 PS; P=0.009; Figure 2E).

To strengthen the conclusions of the study, we also analyzed the data using a 75% change in ΔAPD80 after apamin infusion to define the areas with a high I_{KAS} distribution. As shown in Figure 1 in the Data Supplement, there was significantly higher PS density and number of rotors in areas with a >75% change in ΔAPD80 as compared with the remaining portion of the mapped region. In addition to the PS analysis, continuous reentrant spiral waves (lasting longer than 2 rotations) were identified, and their location was correlated to ΔAPD80. As shown in Figure 3, during VF at baseline, the location of reentrant spiral waves correlated with areas of high I_{KAS} distribution. Similar to the PS, this correlation was not significant after apamin infusion.

In addition, the number of reentrant spiral waves significantly decreased after apamin (baseline versus apamin, 3.1 versus 1.3; P=0.008; Figure 3E) along with duration as they became unstable and terminated after 2.3±0.8 rotations at baseline versus 1.4±0.9 rotations after apamin infusion (P=0.015). Figure 4 shows the phase maps of a spiral wave before and after apamin infusion. At baseline, the reentrant spiral wave is spatially stable as the associated PS group together in a single location correlating to an area of high ΔAPD80. After apamin infusion, the reentrant spiral wave meanders around the mapped region, and the associated PS does not remain spatially stable. Movies showing these VF characteristics at baseline and after apamin infusion are available online (Movies I and II in the Data Supplement). The spatial stability of the reentrant spiral wave is quantified by dividing the area occupied by the spiral wave by the total area of the mapped region. The area of the spiral waves doubles.

### Table. Echocardiographic Studies

<table>
<thead>
<tr>
<th></th>
<th>Before Pacing</th>
<th>After Pacing</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD, mm</td>
<td>12.0±1.6</td>
<td>17.2±1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>7.6±1.3</td>
<td>15.1±1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FS, %</td>
<td>36.8±6.1</td>
<td>12.2±6.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EF, %</td>
<td>70.3±7.9</td>
<td>28.4±5.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means±SD. EF indicates ejection fraction; FS, fractional shortening; LVEDD, left ventricular end diastolic diameter; and LVESD, left ventricular end systolic diameter.
Role of \( I_{\text{KAS}} \) in VF

Distribution of Dominant Frequencies

Frequency domain analysis was used to quantify the VF characteristics, and the frequencies were then compared with the \( \Delta \text{APD}_{80} \) after apamin infusion. At baseline, the higher frequencies correlated with a greater change in the APD. This correlation was not significant after apamin infusion because the DFs became more homogeneous. An example is shown in Figure 5 with a \( \Delta \text{APD} \) map and the corresponding static DF maps from the same heart. As the example shows, at baseline, high DF areas were in similar locations as larger \( \Delta \text{APD} \). The DFs during VF with apamin infusion were lower, and the variability did not correlate to the \( \Delta \text{APD} \). These findings are summarized in the data shown in Figure 5D through F.

Effect of Apamin on VF Vulnerability

A total of 45 VF episodes (17 at baseline and 28 after apamin infusion) were induced. At baseline, most of the VF episodes (15 of 17) were shock terminated (>180 seconds in duration). After apamin infusion, most VF episodes (20 of 28) were shock terminated (180 seconds in duration). Figure 2 shows the effects of apamin infusion on phase singularities (PSs) during ventricular fibrillation (VF) in heart failure. A, Consecutive phase maps sampled at 20-ms intervals during VF at baseline (top) and after apamin (bottom). PSs are indicated by black arrowheads. B, Correlation of spiral waves (rotor) to action potential duration (APD). Summary data showing the correlation of the density of PS at baseline (C) and after apamin infusion (D) to \( \Delta \text{APD} \). E, Summary data showing the overall effect of apamin infusion on the number of PS.

Figure 2. Effects of apamin infusion on phase singularities (PSs) during ventricular fibrillation (VF) in heart failure. A, Consecutive phase maps sampled at 20-ms intervals during VF at baseline (top) and after apamin (bottom). PSs are indicated by black arrowheads. B, Correlation of spiral waves (rotor) to action potential duration (APD). Summary data showing the correlation of the density of PS at baseline (C) and after apamin infusion (D) to \( \Delta \text{APD} \). E, Summary data showing the overall effect of apamin infusion on the number of PS.

Figure 3. Spatial distribution of reentrant spiral waves (rotors). A and B, Correlation of the location of rotors to action potential duration (\( \Delta \text{APD}_{80} \)). At baseline, most of the rotors occur in high apamin-sensitive small-conductance calcium-activated potassium current (\( I_{\text{KAS}} \)) areas. This correlation is not seen with apamin infusion. C, Consecutive phase maps sampled at 20-ms intervals during ventricular fibrillation (VF) at baseline (top) and after apamin (bottom). Arrows indicate the direction of rotation. The rotor observed at baseline remains spatially stable, whereas the rotor with apamin meanders around the field of view. D, Summary data showing the correlation of the location of rotors to \( \Delta \text{APD} \). E, Summary data showing the overall effect of apamin on the number of rotors.
Role of $I_{KAS}$ in VF

The main finding of this study is that significant heterogeneity of $I_{KAS}$ is present in different regions of the epicardium in failing ventricles as indicated by the change in APD$_{80}$ after apamin. Detailed quantitative analysis and cumulative display of PS, DFs, and spiral waves showed a close colocalization to the underlying $I_{KAS}$ distribution within the HF substrate. The correlation was eliminated after apamin infusion. This study further shows that the reentrant spiral waves were spatially stable at baseline but showed meandering and wavebreak after apamin infusion. These data indicate that $I_{KAS}$ channels may play a role in the stability of reentrant spiral waves in the HF model.

Mechanisms of VF Within the HF Substrate

The structural and electric remodeling that occur within the HF substrate can lead to an increase in arrhythmogenicity and VF. Previous studies have shown that this substrate can have a significant effect on the resulting VF characteristics. Huang et al showed that with epicardial plaque mapping, VF in HF was more organized with a slower activation rate, increased incidence of block, and less reentrant wave fronts compared with structurally normal hearts. Optical mapping of the epicardial surface in sheep hearts with HF showed a decrease in DFs and number of rotors as compared with control. Recently, optical mapping of the epicardial, endocardial, and transmural surfaces in a canine HF model showed that stable, high DF areas that correlated with spiral waves or focal activation were observed on the transmural surface at the site of the papillary muscle. The control group showed...
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significantly fewer stable, high DF areas. Noncontact mapping also showed stable reentrant rotors or focal activation in the HF model, whereas unstable rotors characterized the VF in structurally normal hearts. The current study also demonstrated that VF within the HF substrate was characterized by stable reentrant wave fronts. When apamin was introduced, the number of reentrant rotors decreased, and when they did occur, they would not stabilize to a specific location but would meander around the field of view.

Phase mapping is another approach that is used to further quantify VF characteristics. PSs (or singularity points) are used to determine the location of wave breaks and rotor formation. It has been previously shown that VF in a HF model has less singularity points than control. In a separate study,
PSs were shown to be anatomically based in structurally normal hearts. In the current study, the location of PSs were shown to correlate to areas of $I_{KAS}$ upregulation in the HF substrate. Infusion of the $I_{KAS}$ channel blocker apamin resulted in a reduced number of PS and the disappearance of an $I_{KAS}$ correlation. These results suggest that the $I_{KAS}$ may play a role in the stabilization of rotors during VF in the HF model. $I_{KAS}$ blockade by apamin shortens VF duration and promotes its spontaneous termination.12

**SK Expression in the HF Substrate**

Recent studies have shown that SK currents are upregulated in failing ventricular cardiomyocytes, along with increased SK channel protein expression and enhanced sensitivity to intracellular Ca$^{2+}$. Chua et al11 previously showed that $I_{KAS}$ was heterogeneously upregulated in a rabbit HF model. This heterogeneity was observed both transmurally and within the same surface. Transmural heterogeneity of $I_{KAS}$ expression has also been shown in cardiomyocytes from HF transplant recipients.7 Bonilla et al12 and Ni et al20 confirmed these observations by showing that apamin significantly prolonged APD in failing human and canine ventricular cardiomyocytes, along with the increased expression of SK channel protein in failing ventricles. In the current study, the APD was heterogeneously lengthened with apamin infusion, indicating a heterogeneous upregulation of $I_{KAS}$ and supporting the previous studies. In addition, this study shows a correlation between $I_{KAS}$ expression and the spatial distribution of PS, DFs, and spiral waves. Stable reentrant spiral waves occurred in areas with a greater APD and were subsequently unstable and transient after apamin infusion. This further suggests that $I_{KAS}$ may play an important role in stabilizing reentrant spiral waves during VF within the HF substrate.

**Ionic Currents on Rotor Dynamics**

Several studies have been performed in both animal models and computer simulation investigating the role various ionic currents have on reentrant wave fronts (ie, rotors). These studies have focused on rotor frequency, breakup, and stabilization versus meandering and have shown that the potassium currents ($I_{KACH}$, $I_{K1}$, $I_{Ks}$, and $I_{Kf}$) all play a role in rotor dynamics. Studies are still conflicting on the role Ca$^{2+}$ plays. Increased intracellular Ca$^{2+}$ has been shown to play a role in arrhythmia triggers such as delayed afterdepolarizations and early afterdepolarizations; however, its influence on rotor dynamics is still unclear. Intracellular Ca$^{2+}$ is also tied to $I_{KAS}$ current. Recent studies have confirmed the importance of the $I_{KAS}$ current on the repolarization reserve in failing hearts. Blocking $I_{KAS}$ with apamin infusion was shown to decrease PSs and DFs during VF in a rabbit HF model.12 It has previously been shown that VF within the canine HF substrate is characterized by stable reentrant spiral waves correlating to discrete high DF areas.5 This study has shown that the heterogeneous upregulation of $I_{KAS}$ plays a role in the location and stability of the PS and reentrant spiral waves. Apamin infusion reduced the number of PS and reduced the stability of any rotors within the field of view.

**Limitations**

We did not measure the actual $I_{KAS}$ current in this study with patch-clamp techniques or determine the distribution of $I_{KAS}$ channel proteins with immunohistochemistry. However, 2 other studies11,12 have documented the heterogeneous upregulation of $I_{KAS}$ in failing ventricles. In addition, the limitations of determining the $I_{KAS}$ protein levels in the rabbit ventricles have been discussed at length elsewhere. Another limitation of the study is that the mapping was performed only on the epicardial surface. These findings may not be applicable to the midmyocardial or endocardial layers of the myocardium.

**Conclusions**

VF within the HF substrate is characterized by spatially stable rotors. Heterogeneous upregulation of $I_{KAS}$ within this substructure provides a milieu for rotor location and stabilization. PS and DF spatial distribution and the location of stable reentrant spiral waves correlated to greater APD changes after apamin infusion. VF after apamin infusion eliminated this relationship as the rotors became unstable.

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**Disclosures**

None.
References


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Supplemental Material

Data Supplement Figure 1: Effects of apamin on phase singularities and rotors during VF in heart failure. The figures show the results from data using a 75% change in ΔAPD_{80} after apamin infusion to define the areas with a high I_{KAS} distribution. Similar to the data using a 50% change in ΔAPD_{80}, PSs (panel B) correlated to an increase in ΔAPD_{80} at baseline, however, this relationship disappeared after apamin infusion. Summary data showing the correlation of the location of rotors to ΔAPD is shown in Panel C. The figure demonstrates a significantly higher number of rotors in areas with a >75% change in ΔAPD_{80}. 
Data Supplement Figure 2. SK2 protein expression determined by Western blot analysis.

In four failing hearts, crude membrane vesicles were prepared from the high and low ΔAPD$_{80}$ areas determined with optical mapping after apamin infusion. Western blot analysis was then performed to determine the expression of SK2 proteins. SK2 expression in each heart is shown in panel A. Summary data is shown in panel B. R:rabbit. +: Positive control using human embryonic kidney cells transfected with KCNN2.
**Movie 1 – Baseline** – Continuous phase map during VF at baseline in a rabbit heart failure model. A spatially stable phase singularity is observed correlating to a stable reentrant spiral wave.

**Movie 2 – Apamin** – Continuous phase map during VF after apamin infusion in a rabbit heart failure model. Transient phase singularities are observed as no spatially stable reentrant spiral waves were seen with apamin.