Apamin does not inhibit human cardiac Na+ current, L-type Ca2+ current or other major K+ currents.
Apamin Does Not Inhibit Human Cardiac Na\(^+\) Current, L-type Ca\(^{2+}\) Current or Other Major K\(^+\) Currents

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Abstract

**Background:** Apamin is commonly used as a small-conductance Ca\(^{2+}\)-activated K\(^+\) (SK) current inhibitor. However, the specificity of apamin in cardiac tissues remains unclear.

**Objective:** To test the hypothesis that apamin does not inhibit any major cardiac ion currents.

**Methods:** We studied human embryonic kidney (HEK) 293 cells that expressed human voltage-gated Na\(^+\), K\(^+\) and Ca\(^{2+}\) currents and isolated rabbit ventricular myocytes. Whole-cell patch clamp techniques were used to determine ionic current densities before and after apamin administration.

**Results:** Ca\(^{2+}\) currents (CACNA1C+CACNB2b) were not affected by apamin (500 nM) (data are presented as median [25\(^{th}\) percentile;75\(^{th}\) percentile] from –16 [–20;–10] to –17 [–19;–13] pA/pF, \(P = NS\)), but were reduced by nifedipine to –1.6 [–3.2;–1.3] pA/pF (\(p = 0.008\)). Na\(^+\) currents (SCN5A) were not affected by apamin (from –261 [–282;–145] to –268 [–379;–132] pA/pF, \(P = NS\)), but were reduced by flecainide to –57 [–70;–47] pA/pF (\(p = 0.018\)). None of the major K\(^+\) currents (\(I_{Ks}, I_{K1}\) and \(I_{h}\)) were inhibited by 500 nM of apamin (KCNQ1+KCN1E, from 28 [20;37] to 23 [18;32] pA/pF; KCNH2+KCNE2, from 28 [24;30] to 27 [24;29] pA/pF; KCNJ2, from [–46 [–48;–40] to [–46 [–51;–35] pA/pF; KCND3, from 608 [505;748] to 606 [454;684]). Apamin did not inhibit the \(I_{h}\) or \(I_{Ks}\) in isolated rabbit ventricular myocytes (\(I_{h}\) from [–67 [–75;–59] to [–68 [–71;–59] pA/pF; \(I_{Ks}\), from [–16 [–17;–14] to [–14 [–15;–13] pA/pF, \(P = NS\) for both).

**Conclusions:** Apamin does not inhibit human cardiac Na\(^+\) currents, L-type Ca\(^{2+}\) currents or other major K\(^+\) currents. These findings indicate that apamin is a specific SK current inhibitor in hearts as well as in other organs.

Introduction

Small-conductance calcium activated potassium (SK) channels, which are abundantly present in the central nervous system [1], were first cloned in 1996 by Kohler et al [2]. Study of this channel is facilitated by the use of apamin, which has been thought to be a specific inhibitor of SK current in the nervous system [1,3,4]. Subsequent investigations showed that the apamin-sensitive potassium current (\(I_{KAS}\)) is present in the atria [5–12]. In addition, while normal ventricles paced at physiological cycle lengths do not express significant \(I_{KAS}\) [13], we and others found that \(I_{KAS}\) expression is upregulated in failing, ischemic or infarcted human, rabbit and rat ventricles and in normal rabbit ventricles with complete atrioventricular block [14–19]. A common criticism of all these studies is that the specificity of apamin in cardiac type ion channels has not been well established. Some previous studies have shown that apamin inhibits fetal L-type Ca\(^{2+}\) currents [20–22] and Na\(^+\) currents [23] in the chick heart, suggesting that apamin may have off target effects on other cardiac ion channels. However, there is no information on the effects of apamin on Na\(^+\), Ca\(^{2+}\) and K\(^+\) currents that are responsible for adult human cardiac activation and repolarization. Because \(I_{KAS}\) is potentially important in human cardiac arrhythmogenesis, it is important to establish whether apamin is a specific SK current inhibitor as apamin is used to define \(I_{KAS}\). The purpose of the present study was to test the hypothesis that apamin is a specific inhibitor of \(I_{KAS}\) in adult human cardiac ion channels. We tested that hypothesis by performing patch clamp studies of major cardiac ion channels expressed in human embryonic kidney (HEK) 293 cells and by testing the effects of apamin on Na\(^+\) and Ca\(^{2+}\) currents in rabbit ventricular myocytes.
Apamin Specificity

Materials and Methods

The study was approved by the Institutional Biosafety Committee and Institutional Animal Care and Use Committee of the Indiana University and the Methodist Research Institute, Indianapolis, Indiana.

Cell Culture and Gene Transfection

Human embryonic kidney (HEK) 293 cells were cultured in Iscove’s Modified Dulbecco’s Medium (Gibco) with 10% fetal bovine serum and 1% penicillin/streptomycin in 5% CO2 at 37°C. To study human Nav1.5, a stable HEK 293 cell line expressing consistent sodium currents (I_{Na}) was used [24]. Other than I_{Na}, 35 mm dishes of HEK 293 cells were transiently transfected using Effectene Transfection Reagent (Qiagen) according to the manufacturer’s protocol and were harvested for patch clamp experiment 48–72 hours later. The amount and content of plasmids transfected for each channel were described as followings: for I_{Na}, 1.5 μg of CACNA1c/pDNA3.1 and 2.0 μg of CACNB2b/pIRE2-DsRed-Express were co-transfected; for I_{Kur}, 1 μg of KCNQ1/pIRE2-EGFP and 1 μg of KCNE2/pIRE2-CD8 were co-transfected for I_{Kr}, 3 μg of KCNH2/pires-hyg and 1 μg of KCN2/pIRE2-DES-Express were co-transfected; for I_{K1,2}, 2 μg of KCNJ2/pCMS-EGFP were transfected; and for I_{tot}, 2 μg of KCND3/pIRE2-Des-Express were transfected.

Rabbit Cardiomyocyte Isolation

The rabbits were intravenously injected with 1,000 units of heparin and anesthetized with sodium pentobarbital (100 mg/kg). After a median sternotomy, the hearts were rapidly excised, heparin and anesthetized with sodium pentobarbital (100 mg/kg). Whole-cell configuration of the voltage-clamp technique was made in Tyrode’s solution. Pipette resistances were 1.5–3 MΩ. After achieving a gigaseal, the test-pulse current was nulled by adjusting the pipette capacitance compensator with both fast and slow components. After break-in, the whole cell charging transient was nulled by adjusting whole cell capacitance and series resistance. Voltage control protocols were generated with Axopatch 200B amplifier/Digitida 1440A acquisition system using pCLAMP-10 software (Molecular Devices/Axon, Sunnyvale, CA). Whole-cell recording was analyzed using Clampfit 10.2. To measure I_{SK2}, we used Tyrode’s solution as the bath solution containing (in mM) NaCl 140, KCl 5.4, MgCl2 1.2, HEPES 5, Na2HPO4 0.33, CaCl2 1.8 and Glucose 10 (pH 7.4 adjusted with NaOH). The pipette solution contained (in mM) K-Gluconate 44, MgCl2 1.15, EGTA 1, HEPES 10 and free Ca2+ 1 μM (pH 7.2 adjusted with KOH). All experiments for I_{SK2} were carried out at 37°C. For measuring I_{Na}, we used Tyrode’s solution (see above) as the bath solution. The pipette solution contained (in mM) NaCl 10, CsCl 10, CsGlu 20, EGTA 10, and HEPES 10 (pH 7.35 adjusted with CsOH). After testing apamin 500 nM, flecainide 100 μM was used as a positive control [26]. To measuring I_{Kur}, we replaced extracellular calcium with barium to lessen the rundown phenomenon [27,28]. The bath solution contained (in mM) BaCl2 5, NaCl 130, MgCl2 1.0, HEPES 10, and Glucose 11 (pH 7.4 adjusted with NaOH). The pipette solution contained (in mM) CsCl 120, MgCl2 2, EGTA 10, HEPES 10, Mg-ATP 5, Na2-GTP 1.5 and cAMP 1 (pH 7.24 adjusted with CsOH). Nifedipine 2 μM was used as the positive control [29]. All experiments for I_{Na} were carried out at 37°C. For measuring I_{Kur}, we used Tyrode’s solution as the bath solution (see above). The pipette solution contained (in mM) KC1 130, KOH 20, EGTA 5, Mg-ATP 5, HEPES 5, CAMP 0.05 and Na2-GTP 0.1 (pH 7.4 adjusted with KOH). Chromanol 293B 50 μM was used as the positive control [30]. For measuring I_{Kur}, I_{Kr} and I_{Kto}, we used Tyrode’s solution (see above) as the bath solution. The pipette solution contained (in mM) KC1 150, KOH 20, EGTA 5, Mg-ATP 5 and HEPES 5 (pH 7.4 adjusted with KOH). E4031 100 nM, CsCl 5 mM and 4-aminoptyridine 10 mM were used as the positive control, respectively [31–33]. To measure I_{Kur} and I_{Na} in rabbit cardiomyocytes, we used Tyrode’s solution as the bath solution (see above), and the pipette solution contained (in mM) aspartate 5, TEACl 20, MgCl2 2, EGTA 10, HEPES 10, Mg-ATP 5, and Na2-GTP 5 (pH 7.2 adjusted with KOH).

Stable current density during baseline solution superfusion was measured immediately before the addition of apamin to define baseline current density. This was followed by superfusion with 500 nM apamin for at least 3 minutes until the current became stable. Following apamin exposure, stable blockers of each current were used as positive controls.

Drugs and Reagents

Apamin (catalog#1652), was purchased from Tocris Bioscience (Minneapolis, MN) and was dissolved in water for a 250 μM stock solution. Apamin was freshly diluted with bath solution daily before experiment. Flecainide (catalog#1470), chromanol 293B (catalog#1412) and E4031 (catalog#1008) were purchased from Tocris. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Statistical Analysis

Summary data following apamin or positive controls were normalized to baseline. Nonparametric tests were used in this whole experiment. Related-samples Friedman’s Two-Way Analysis of Variance by Ranks was conducted to compare continuous variables among baseline, post apamin and post specific blockers. Related-Samples Wilcoxon Signed Rank Test was performed for post-hoc analysis. I_{Kur} rundown was quantified by the time constant (τ) of a single exponential fit of the current. Independent-samples Mann-Whitney U test was performed to compare τ of rundown with and without apamin. P value less than 0.05 was considered statistically significant. Statistical analyses were performed using SPSS (IBM, Chicago, IL, USA, version 21). Data in text and figures are presented as median [25th percentile;75th percentile].
Results

Studies in HEK 293 Cells

Apamin’s effects on \(I_{SK2}\). Figure 1 shows that untransfected HEK 293 cells expressed very low levels of endogenous potassium currents (<100 pA) compared to the nA levels of currents observed after transfection with various ion channels (see figure legends and subsequent figures). Figures 2A and 2B show the representative tracings and time course of \(I_{SK2}\) in transfected HEK 293 cells, induced by a repetitive voltage-ramp pulses (from +40 to –100 mV, 400 ms duration) from a holding potential of –50 mV. A total 8 cells were tested at 37°C. The currents became stable 4–8 minutes after the whole-cell configuration was made. Subsequent application of apamin (500 nM) reduced the currents by 99±4%. Figure 2C shows the summary data before and after apamin.

Apamin does not inhibit \(I_{Na}\). Figures 3A and 3B show the representative tracings and time course of \(I_{Na}\) at a frequency of 20/min. The \(I_{Na}\) was induced by a repetitive depolarization pulse (to –10 mV for 300 ms) from a holding potential of –140 mV. All experiments were carried out in room temperature. A total of 9 cells were tested and no significant inhibition or enhancement was observed after adding 500 nM apamin. The median baseline current density was –261 [–282;–145] pA/pF. The averaged current density after apamin was –268 [–379;–132] pA/pF (n = 9, p = 0.767, compared to the baseline). The averaged current density after flecainide was –57 [–70;–47] pA/pF (n = 7; p = 0.018 compared to baseline). The averaged current density after apamin was –268 [–379;–132] pA/pF (n = 9, p = 0.767, compared to the baseline). The averaged current density after apamin was –261 [–282;–145] pA/pF. The averaged current density after flecainide was –57 [–70;–47] pA/pF (n = 7; p = 0.018 compared to baseline). Figure 3C shows the summary of drug effects normalized to the baseline.

Apamin does not inhibit \(I_{Kv1}\). Figures 3D and 3E show the representative tracings and time course of \(I_{Kv1}\) in the presence of apamin 500 nM or nifedipine 2 µM. \(I_{Kv1}\) was induced by a step pulse protocol (to 0 mV for 500 ms) from a holding potential of –80 mV and a brief prepulse at –40 mV. A total of 8 cells were tested. No significant effects of apamin were observed on \(I_{Kv1}\). The baseline current density of \(I_{Kv1}\) was –35 [–40;–30] pA/pF. The current density after apamin was 28 [24;30] pA/pF, and was 27 [24;29] pA/pF after nifedipine (n = 6, p = 0.345, compared to baseline). The current density was reduced

Apamin does not inhibit \(I_{K1}\). No significant effects of apamin were observed on \(I_{K1}\). The baseline current density of \(I_{K1}\) was –20 [–25;–15] pA/pF. The current density after apamin was 20 [15;25] pA/pF (n = 6, p = 0.014, compared to the baseline).

Apamin does not inhibit \(I_{Ca}\). A rundown phenomenon was also observed in the study of \(I_{Ca}\) (Figure 4A). Various concentrations of apamin (from 0.5 nM to 500 nM) were applied during rundown, but the time course of rundown was not affected (Figure 4B). Figure 4C summarizes the time constant (t) of rundown with and without apamin. There were no significant differences between the two. Figures 5A and 5B show the representative tracings and time course of \(I_{Ca}\). \(I_{Ca}\) was induced with a 4s depolarization pulse protocol (to +40 mV) from a holding potential of –80 mV. The baseline current density of \(I_{Ca}\) was 28 [20;37] pA/pF. After apamin, the current density was 23 [18;32] pA/pF (n = 10, p = 0.037, compared to the baseline). After adding 50 µM Chromanol 293B, the current density was reduced to 4.7 [1.6;7] pA/pF (n = 6; p = 0.028 compared to post apamin, p = 0.028 compared to baseline). Figure 5C shows the summary of drug effects normalized to the baseline.

Apamin does not inhibit \(I_{Ks}\). Figures 5D and 5E show the representative tracings and time course of \(I_{Ks}\) at a frequency of 20/min. The \(I_{Ks}\) was induced by a repetitive depolarization pulse (to +20 mV for 4 s in duration) from a holding potential of –80 mV, and measured as the peak tail current at –50 mV, repeated every 10 s. Apamin had no significant effect. The baseline current density of \(I_{Ks}\) was 28 [24;30] pA/pF, and was 27 [24;29] pA/pF after apamin (n = 6, p = 0.345, compared to baseline). The current density was reduced

![Figure 1. The endogenous K⁺ currents of HEK 293 cells](https://doi.org/10.1371/journal.pone.0096691.g001)
to 10 [8;14] pA/pF by E4031 (n = 5; p = 0.043 compared to post apamin, p = 0.043 compared to baseline). Figure 5F shows the summary of drug effects normalized to baseline.

**Apamin does not inhibit IK1.** Figures 6A and 6B show the representative time course and tracings of IK1 in the absence and presence of apamin (500 nM). The IK1 was induced by a ramp pulse protocol between –120 mV and 40 mV (1 s in duration, every 5 s) from a holding potential of –80 mV. The current at –100 mV was monitored and shown in Figure 4B. No significant effects were observed after adding apamin. The baseline current density of IK1 was –46 [–48;–40] pA/pF. After apamin administration, the average current density was –46 [–51;–35] pA/pF (n = 7, p = 0.612, compared to baseline). CsCl reduced the current density to –18 [–27;–15] pA/pF (n = 13, p = 0.052, compared to baseline). The current density was reduced to 247 [228;323] pA/pF by 4-aminopyridine (n = 12; p = 0.001 compared to apamin’s effect, p = 0.002 compared to baseline). Figure 6F shows the summary of drug effects normalized to baseline.

**Studies in Rabbit Cardiomyocytes**

**Apamin does not inhibit Ito.** Figures 6D and 6E show the effect of apamin on Ito. The current was induced by a repetitive depolarization pulse (+20 mV for 500 ms in duration) from a holding potential of –80 mV. Apamin had no significant effect. The baseline current density of Ito was 608 [505;748] pA/pF, and was 606 [454;684] pA/pF after apamin (n = 13, p = 0.052, compared to baseline). The current density was reduced to 247 [228;323] pA/pF by 4-aminopyridine (n = 12; p = 0.001 compared to apamin’s effect, p = 0.002 compared to baseline). Figure 6F shows the summary of drug effects normalized to baseline.

**Apamin Specificity**

Figure 2. **Effect of apamin on ISK2 in transfected HEK 293 cells.** (A) The representative ISK2 tracings obtained by the descending voltage ramp protocol shown in the inset before (a) and after (b) apamin at time points indicated by arrows a and b in (B). (B) The time course of ISK2 at 0 mV. (C) The summary of current density before and after apamin.

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Figure 3. **Effects of apamin on INa and IBa in transfected HEK 293 cells.** (A) The representative INa tracings obtained by the pulse protocol shown in the inset before apamin (a), after apamin (b) and after flecainide (c) at time points indicated by arrows a through c, respectively, in (B). (B) The time course of peak INa measured at –10 mV. (C) The summary of drug effects normalized to baseline. (D) The representative IBa tracings at 0 mV obtained by the pulse protocol shown in the inset before apamin (a), after apamin (b) and after nifedipine (c) at time points indicated by arrows a through c, respectively, in (E). (E) The time course of peak IBa measured at 0 mV. (F) The summary of drug effects normalized to baseline.

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Apamin had no significant effects on $I_{Ca}$. The current density of $I_{Ca}$ averaged $-16 [-17; -14]$ pA/pF at baseline, and $-14 [-15; -13]$ pA/pF after apamin ($n = 7$, $p = 0.091$, compared to the baseline). After adding 2 μM of nifedipine, the current density was reduced to $-3.9 [-5.8; -2.6]$ pA/pF ($n = 7$, $p = 0.018$ compared to post apamin, $p = 0.018$ compared to baseline). Figure 7C shows the summary of drug effects normalized to baseline.

Apamin does not inhibit the native $I_{Na}$. In the same experiments, the native cardiac $I_{Na}$ was also measured during the prepulse to $-40$ mV. Figure 7D and 7E showed the representative tracings and time course of $I_{Na}$ before and after apamin. There was no significant change after adding apamin. The baseline current density of $I_{Na}$ was $-67 [-75; -59]$ pA/pF, and was $-68 [-71; -59]$ pA/pF after apamin ($n = 6$, $p = 0.753$ compared to the baseline). Figure 7F shows the summary of drug effects.

**Discussion**

We found that at a concentration of 500 nM, apamin has no significant effects on major cardiac ion currents that underlie the action potential in human hearts, including L-type Ca$^{2+}$, Na$^{+}$ and the major K$^{+}$ currents ($I_{Ks}$, $I_{Kr}$, $I_{K1}$, $I_{to}$). This finding suggests that apamin at this concentration can be used to study the role of SK currents in human cardiomyocytes.

Apamin as a specific ion channel inhibitor. Apamin is a peptide toxin isolated from Western honey bees [34]. When injected with 0.5 mg/kg or more of apamin, mice develop neurological symptoms including spasms, jerks and convulsions of apparently spinal origin [34]. Subsequent studies showed that apamin is a highly selective SK channel inhibitor in the central nervous system. Because SK channels are the only known targets

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**Figure 4. Effects of different concentrations of apamin on the rundown course of $I_{Ks}$ in transfected HEK 293 cells.** (A) An observation experiment without apamin treatment showing time-dependent rundown, obtained with the pulse protocol shown in the inset with chromanol 293B at the end. (B) The representative time course of $I_{Ks}$ treated with different concentrations of apamin. (C) The time constant ($\tau$) of the rundown curve with ($n = 10$) and without ($n = 3$) apamin. doi:10.1371/journal.pone.0096691.g004

**Figure 5. Effects of apamin on $I_{Ks}$ and $I_{Kr}$ in transfected HEK 293 cells.** (A) The representative tracings of $I_{Ks}$ obtained by pulse protocol shown in the inset before apamin (a), after apamin (b) and after chromanol (c) at time points indicated by arrows a through c, respectively, in (B). (B) The time course of peak $I_{Ks}$ at 40 mV. (C) The summary of drug effects normalized to baseline. (D) The representative tracings of $I_{Kr}$ obtained by a pulse protocol shown in the inset before apamin (a), after apamin (b) and after E4031 (c) at time points indicated by arrows a through c in (E). (E) The time course of peak $I_{Kr}$ at 20 mV. (F) The summary of drug effects normalized to baseline. doi:10.1371/journal.pone.0096691.g005
for apamin, the effects of apamin at the molecular, cellular, and behavioral levels may be ascribed to SK channel blockade [35]. The specificity of apamin in the central nervous system has contributed significantly to the understanding of SK channel function in controlling activation and repolarization of neurons. Since 2003, apamin-sensitive K currents have also been known to

Figure 6. Effects of apamin on $I_{K1}$ and $I_{to}$ in transfected HEK 293 cells. (A) The representative tracings of $I_{K1}$ by ascending voltage ramp protocol shown in the inset before apamin (a), after apamin (b) and after CsCl (c) at time points indicated by arrows a through c, respectively, in (B). (B) The time course of $I_{K1}$ at –100 mV. (C) The summary of drug effects normalized to baseline. (D) The representative tracings of $I_{to}$ obtained by a pulse protocol shown in the inset before apamin (a), after apamin (b) and after 4-AP (c) at time points indicated by arrows a through c, respectively, in (E). (E) The time course of peak $I_{to}$ at 20 mV. (F) The summary of drug effects normalized to baseline.

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Figure 7. Effects of apamin on $I_{Ca}$ and $I_{Na}$ in rabbit cardiomyocytes. (A) The representative $I_{Ca}$ tracings obtained by a pulse protocol shown in the inset before apamin (a), after apamin (b) and after nifedipine (c) at time points indicated by arrows a through c, respectively, in (B). (B) The time course of peak $I_{Ca}$ measured at 0 mV. (C) The summary of drug effects normalized to baseline. (D) The representative tracings of $I_{Na}$ obtained by a pulse protocol shown in the inset before apamin (a), after apamin (b) at time points indicated by arrows a and b, respectively, in (E). (E) The time course of peak $I_{Na}$ at –40 mV. (F) The summary of current densities before and after apamin.

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be present in cardiac tissues and play an important role in atrial repolarization [5–12]. Apamin also prolongs the action potential duration in diseased ventricles, such as in heart failure, myocardial infarction and after atrioventricular block [14–16,18,19]. However, because previous studies showed that apamin inhibited L-type Ca2+ currents [20–22] and Na+ currents [23] in fetal heart tissue, it is possible that apamin also has non-specific effects on ion channels in adult cardiac tissues. If this is the case, the validity of all research using apamin as a SK inhibitor to explore the role of SK in the heart would in question. For example, if apamin can inhibit any of the major repolarization currents, such as IK or IKs, then the prolongation of action potential duration after apamin demonstrated in all optical mapping or patch clamp studies may be a result of inhibition of those major ionic currents, and not exclusively from the inhibition of SK currents [15,36,37]. If apamin inhibits IKs then the observed effect of apamin in atria may be explained by IKs inhibition since that current is abundantly present in the atria [38–41]. If apamin could affect IK1, then the change of arrhythmia burden after apamin administration could in part come from resting membrane potential shift due to IK1 inhibition [17,42,43]. If apamin could affect IKs, then apamin would affect propagation velocity and excitability of heart tissue and thereby influence the arrhythmogenesis. In addition, if apamin inhibits ICaL, then the latter effect may explain the flattening of action potential duration restitution curve in failing ventricles by apamin [15]. Therefore, if apamin is a non-specific ion channel blocker, the effects of apamin on arrhythmogenesis may not come from SK channel inhibition alone. Because an extensive literature search showed no other studies that have tested the specificity of apamin in human cardiac ion channels, our study is both novel and important for interpreting the antiarrhythmic and proarrhythmic mechanisms of SK current inhibition evaluated using apamin.

In the present study, we used HEK 293 cell line and isolated rabbit ventricular myocytes to study apamin specificity. The HEK 293 cell line was originally derived from human embryonic kidney cells and has the advantage of high transfectability and being easy to culture. This cell line has relatively small endogenous currents compared to the currents expressed in transfected cells (Figure 1), making contamination by endogenous currents insignificant. HEK 293 cells have been widely used to express cloned cardiac ion channels, including Nav1.5 [44,45], Cav1.2 [46–48], Kv7.1 [49], Kv11.1 [31,50], Kir2.1 [51] and Kv4.3 [52] channels. The currents exhibited in the present experiment are consistent with those reports. The concentration of apamin tested most commonly in this study was 500 nM, which is more than 1000 times the reported IC50 (0.027 μM for Kv11.1 [31,50], Kir2.1 [51] and Kv4.3 [52]) channels. The ion channels that we studied in HEK cells were very different from human cardiomyocytes, there is a possibility that apamin may show some effects on the ion channels that we studied in human cardiomyocytes. Further studies using human cells will be warranted.

Conclusions

We conclude that apamin does not have significant effects on the most common isoforms underlying the major human cardiac ion channels. These findings support prior evidence that apamin is a highly selective inhibitor of SK current in the cardiomyocytes.

Supporting Information

Figure S1 A representative time course of ICa in transfected HEK 293 cells measured at 0 mV. Apamin and nifedipine was added during rundown. (TIF)

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Author Contributions

Conceived and designed the experiments: CCY TA PSC. Performed the experiments: CCY TA. Analyzed the data: CCY TA. Contributed reagents/materials/analysis tools: TA PSC. Wrote the paper: CCY TA JW PSC.

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