Apamin Does Not Inhibit Human Cardiac Na\(^+\) Current, L-type Ca\(^{2+}\) Current or Other Major K\(^+\) Currents

Chih-Chieh Yu\(^{1,2}\), Tomohiko Ai\(^{1,3}\), James N. Weiss\(^4\), Peng-Sheng Chen\(^1\)*

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 \(I_{Kd}\)) were inhibited by 500 nM of apamin (KCQ1+KCNE1, from 28 [20;37] to 23 [18;32] pA/pF; KCN2+KCNE2, from 28 [24;30] to 27 [24;29] pA/pF; KCN1J, from –46 [–48;–40] to –46 [–51;–35] pA/pF; KCND3, from 608 [505;748] to 606 [454;684]). Apamin did not inhibit the \(I_{Ks}\), or \(I_{Kas}\) in isolated rabbit ventricular myocytes (\(I_{Kas}\) from –67 [–75;–59] to –68 [–71;–59] pA/pF; \(I_{Kas}\), 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.

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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.

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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 follows: for I_Na, 1.5 μg of CACNA1c/pDNA3.1 and 2.0 μg of CACNB2b/pIRES2-DsRed-Express were co-transfected; for I_Ka, 1 μg of KCNQ1/pIRES2-EGFP and 1 μg of KCNQ1/pIRES-CD8 were co-transfected; for I_Kr, 3 μg of KCN2/pIRES-hyg and 1 μg of KCNE2/pIRES2-DsRed-Express were co-transfected; for I_Ks, 2 μg of KCNJ2/pCMS-EGFP were transfected; and for I_L, 2 μg of KCND3/pIRES2-DsRed-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, mounted onto a Langendorff perfusion apparatus and perfused for 4 minutes with 37°C oxygenated Ca²⁺-free buffer. The composition of the buffer was (in mM) NaCl 136, KCl 5.4, MgCl₂ 1.0, HEPES 10, and glucose 10, adjusted to pH 7.4 with NaOH. After the blood was washed out, the heart was recirculated with enzyme solution, containing 1 mg/ml collagenase type II (Worthington, Lakewood, NJ) and 0.1 mg/ml protease (Sigma-Aldrich, St. Louis, MO, USA) in the same buffer for 28 minutes, followed by another 4 minutes of washing with Ca²⁺-free buffer. The heart was then removed from the apparatus and the ventricle was triturated. The isolated cardiomyocytes were washed and titrated up with Ca²⁺-containing Tyrode’s solution until the Ca²⁺ level reaches 1.8 mM.

Patch-clamp Experiments

Whole cell configuration of the voltage-clamp technique was used in this study as described elsewhere [25]. Briefly, whole-cell configuration was made in Tyrode’s solution. Pipette resistances were 1.5–3 MΩ. After achieving a gigaseal, the test-pulse current was null by adjusting the pipette capacitance compensator with both fast and slow components. After break-in, the whole-cell charging transient was null by adjusting whole cell capacitance and series resistance. Voltage control protocols were generated with Axopatch 200B amplifier/Digidata 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 144, 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) NaF 10, CsF 110, CsCl 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]. For measuring I_Ka, 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, Na₂GTP 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_L were carried out at 37°C. For measuring I_Ks, 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 Na₂GTP 0.1 (pH 7.4 adjusted with KOH). Chromanol 293B 50 μM was used as the positive control [30]. For measuring I_Kr, I_Kr and I_L, 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 mM, CsCl 5 mM and 4-aminoptyridine 10 mM were used as the positive control, respectively [31–33]. To measure I_L and I_L in rabbit cardiomyocytes, we used Tyrode’s solution as the bath solution (see above), and the pipette solution contained (in mM) aspartate 85, TEACI 20, MgCl₂ 2, EGTA 10, HEPES 10, Mg-ATP 5, and Na₂GTP 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#1808) 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_K_a 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 IKr. 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). Figure 2A and 2B show the representative tracings and time course of IKr 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 INa. Figures 3A and 3B show the representative tracings and time course of INa at a frequency of 20/min. The INa 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 average current density after flecainide was –57 [-70;–47] pA/pF (n = 7; p = 0.018 compared to post apamin, p = 0.018 compared to baseline). Figure 3C shows the summary data before and after apamin.

Apamin does not inhibit IK1 and IKs. Figure 4A shows that in transfected HEK 293 cells expressed very low levels of endogenous potassium currents. IK1 and IKs were studied using 1.8 mM Ca2+ in the external solution. However, it was difficult to study the effects of apamin on IK1 due to a marked rundown phenomenon. Apamin did not show significant effects during rundown (Figure S1).

Apamin does not inhibit ICa. A rundown phenomenon was also observed in the study of ICa (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 (τ) 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 ICa. ICa was induced with a 4s depolarization pulse protocol (to +40 mV) from a holding potential of –80 mV. The baseline current density of ICa was 28 [20;37] pA/pF. After apamin, the average 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 [4.1;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 IKr. Figures 5D and 5E represent tracings and the time course of apamin effect on IKr. The current was induced by a 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 IKr 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 tested. No significant effects of apamin were observed on ICa. The baseline current density of ICa was –16 [-20;–10] pA/pF. The current density after apamin was 17 [-19;–13] (n = 8; p = 0.953, compared to baseline), and –1.6 [-3.2;–1.3] pA/pF after nifedipine (n = 8; p = 0.008 compared to post apamin, p = 0.008 compared to baseline). ICa had also been studied using 1.8 mM Ca2+ in the external solution. However, it was difficult to study the effects of apamin on ICa due to a marked rundown phenomenon. Apamin did not show significant effects during rundown (Figure S1).

![Figure 1. The endogenous K+ currents of HEK 293 cells.](https://example.com/figure1.png)
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 \(I_{K1}\).** Figures 6A and 6B show the representative time course and tracings of \(I_{K1}\) in the absence and presence of apamin \((500 \text{ nM})\). The \(I_{K1}\) was induced by a ramp pulse protocol between \(-120 \text{ mV} and 40 \text{ mV}\) \((1\text{ s}\) in duration, every 5 s) from a holding potential of \(-80 \text{ mV}\). The current at \(-100 \text{ mV}\) was monitored and shown in Figure 4B. No significant effects were observed after adding apamin. The baseline current density of \(I_{K1}\) was \(-46 \text{ [–48;–40]} \text{ pA/pF}\). After apamin administration, the average current density was \(-46 \text{ [–51;–35]} \text{ pA/pF}\) \((n = 7; p = 0.612\) compared to baseline). CsCl reduced the current density to \(-18 \text{ [–27;–15]} \text{ pA/pF}\) \((n = 7; p = 0.018\) compared to post apamin, \(p = 0.018\) compared to baseline). Figure 6C shows the summary of drug effects normalized to baseline.

**Studies in Rabbit Cardiomyocytes**

**Apamin does not inhibit \(I_{Na}\).** Figures 6D and 6E show the effect of apamin on \(I_{Na}\). The current was induced by a repetitive depolarization pulse \(+20 \text{ mV}\) for 500 ms in duration) from a holding potential of \(-80 \text{ mV}\). Apamin had no significant effect. The baseline current density of \(I_{Na}\) was \(608 \text{ [505;748]} \text{ pA/pF}\), and was \(606 \text{ [454;684]} \text{ pA/pF}\) after apamin \((n = 13, p = 0.052\) compared to baseline). The current density was reduced to \(247 \text{ [228;323]} \text{ pA/pF}\) 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**

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**Figure 2. Effect of apamin on \(I_{SK2}\) in transfected HEK 293 cells.** (A) The representative \(I_{SK2}\) 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 \(I_{SK2}\) at 0 mV. (C) The summary of current density before and after apamin.

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**Figure 3. Effects of apamin on \(I_{Na}\) and \(I_{Ba}\) in transfected HEK 293 cells.** (A) The representative \(I_{Na}\) 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 \(I_{Na}\) measured at \(-10 \text{ mV}\). (C) The summary of drug effects normalized to baseline. (D) The representative \(I_{Ba}\) 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 \(I_{Ba}\) measured at 0 mV. (F) The summary of drug effects normalized to baseline.

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Apamin had no significant effects on $I_{\text{Ca}}$. The current density of $I_{\text{Ca}}$ 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 $I_{\text{Na}}$. In the same experiments, the native cardiac $I_{\text{Na}}$ was also measured during the prepulse to $-40$ mV. Figure 7D and 7E showed the representative tracings and time course of $I_{\text{Na}}$ before and after apamin. There was no significant change after adding apamin. The baseline current density of $I_{\text{Na}}$ 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 ($I_{\text{ks}}, I_{\text{k}}, I_{\text{c}}, I_{\text{k}}$). 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...
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. doi:10.1371/journal.pone.0096691.g006

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. doi:10.1371/journal.pone.0096691.g007
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 Ca\(^{2+}\) 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 be in question. For example, if apamin can inhibit any of the major repolarization currents, such as \(I_{Ks}\) or \(I_{Kr}\), 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 \(I_{Kr}\), then the observed effect of apamin in atria may be explained by \(I_{Kr}\) inhibition since that current is abundantly present in the atria [38–41]. If apamin could affect \(I_{K1}\), then the change of arrhythmia burden after apamin administration could in part come from resting membrane potential shift due to \(I_{K1}\) inhibition [17,42,43]. If apamin could affect \(I_{Ca,L}\), then apamin would affect propagation velocity and excitability of heart tissue and thereby influence the arrhythmogenesis. In addition, if apamin inhibits \(I_{Ca,L}\), 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 experiments 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 IC\(_{50}\) (0.027–0.095 nM) of the SK2 currents in HEK 293 cells [33–35]. Five hundred nM is also higher than the dose used to block Ca\(^{2+}\) and Na\(^{+}\) currents in chick embryonic reaggregates by Bkaily et al [20,21,23]. In addition to HEK 293 cells, we also performed studies in isolated rabbit ventricular myocytes and showed that apamin failed to block either \(I_{Ca}\) or \(I_{Ca,L}\). The differences between our results and those reported by Bkaily et al. might have come from species differences or the differences of isoforms between adult and fetal ion channels.

Limitations of the study. Because we did not test the fetal isoform-encoded ionic currents or ionic currents of various possible splicing isoforms in all animal species, our results are only applicable to the most common isoforms of adult human cardiac cells. It is also possible that in native cardiac myocytes, some of these channels have different subunit combinations that we did not test, or their regulation may be different. In the intact heart, ionic currents are also affected by autonomic nerves sensitive to apamin. Since cell environments of HEK cells and rabbit cardiomyocytes are 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 \(I_{Ca}\) 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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