Function of BK$_{Ca}$ Channels Is Reduced in Human Vascular Smooth Muscle Cells From Han Chinese Patients With Hypertension

Yan Yang, Peng-Yun Li, Jun Cheng, Liang Mao, Jing Wen, Xiao-Qiu Tan, Zhi-Fei Liu, Xiao-Rong Zeng

Abstract—Chronic hypertension is associated with an impaired vascular relaxation caused by an increased vascular tone; however, the underlying mechanisms are not fully understood in human patients. The present study was to investigate whether large-conductance Ca$^{2+}$- and voltage-activated K$^+$ (BK$_{Ca}$) channels are involved in dysfunctional relaxation of artery in Han Chinese patients with hypertension using the perforated patch clamp, inside-out single-channel, and macromembrane patch recording techniques to determine whole-cell current, spontaneous transient outward current, open probability, and Ca$^{2+}$ sensitivity and the reverse transcription polymerase chain reaction and Western blot analysis to examine the gene and protein expression of α-subunit (KCa1.1) and β1-subunit (KCNMB1) of BK$_{Ca}$ channels in isolated human vascular smooth muscle cells and mesenteric arteries from normotensive and hypertensive patients. It was found that whole-cell current density, spontaneous transient outward current, and Ca$^{2+}$ sensitivity, but not single-channel open probability and slope conductance, were significantly decreased in vascular smooth muscle cells from patients with hypertension. Interestingly, mRNA and protein levels of KCNMB1, but not KCa1.1, were reduced in the arterial tissue from patients with hypertension. These results demonstrate for the first time that whole-cell current, spontaneous transient outward current, and Ca$^{2+}$ sensitivity of BK$_{Ca}$ channels are reduced in human vascular smooth muscle cells, which resulted from downregulation of β1-subunit of the channel. This may account, at least in part, for the dysfunction of artery relaxation in Han Chinese patients with primary hypertension. *(Hypertension. 2013;61:519-525.)* ● Online Data Supplement

Key Words: hypertension ■ potassium channels ■ human mesentery artery

It is well known that chronic hypertension is associated with increased morbidity and mortality from stroke, coronary artery disease, congestive heart failure, renal disease, and so on. Arterial tone is persistently increased as a result of mal-function of vessel relaxation in chronic hypertension. It is well recognized that arterial tone is regulated by functional balance of the ion channels responsible for cellular depolarization and hyperpolarization. The increased arterial tone is mainly related to depolarization of smooth muscle, which may have resulted from dysfunction of ion channels responsible for cell membrane hyperpolarization. The membrane depolarization activates voltage-dependent l-type Ca$^{2+}$ channels, induces an increase in Ca$^{2+}$ influx and global intracellular Ca$^{2+}$ level, and causes vessel constriction. It is believed that large-conductance Ca$^{2+}$-activated K$^+$ (BK$_{Ca}$) channels play an important role in hyperpolarization of vascular smooth muscle cells (VSMCs). BK$_{Ca}$ channels are activated by intracellular local Ca$^{2+}$ release events through ryanodine receptors (Ca$^{2+}$ sparks) from the sarcoplasmic reticulum and subsequently induce a hyperpolarization that opposes vasoconstriction.

The Ca$^{2+}$ sparks are highly localized and short-lived Ca$^{2+}$ transients, which is a local Ca$^{2+}$ signaling to induce spontaneous transient outward currents (STOCs) in VSMCs and neurons, and mediate different physiological functions. In VSMCs, functional coupling of sparks to STOCs hyperpolarizes the membrane potential, which in turn closes the voltage-dependent l-type Ca$^{2+}$ channels, decreases global [Ca$^{2+}$], and induces vascular relaxation. Studies from animal models demonstrated that a reduced activity of Ca$^{2+}$ sparks and STOCs is involved in an increase of vascular tone in hypertension. However, little information is available in literature regarding BK$_{Ca}$ channel activity in human arterial cells/tissue from patients with hypertension. The present study was therefore to investigate whether BK$_{Ca}$ activity is altered in VSMCs isolated from mesentery arterial tissues of Han Chinese patients with hypertension using approaches of electrophysiology and molecular biology.
Materials and Methods

Human Arterial Tissue Collection and Cell Preparation

Mesenteric arterial tissues were collected from the removed tissues by abdominal operations in Han Chinese normotensive and primary hypertensive patients. The human tissue collection protocol was approved by the Ethics Committee of Luzhou Medical College. The characterization of the patients is shown in Table S1 in the online-only Data Supplement. Hypertension was defined as systolic blood pressure ≥140 mm Hg and systolic blood pressure ≥90 mm Hg according to the Chinese and international diagnostic criteria of hypertension guidelines. In the present study, informed consent was obtained from the patients for the use of vascular tissue (which is usually discarded).

Preparation of Human VSMCs

Signal VSMCs were enzymatically isolated using a procedure as described previously and in the online-only Data Supplement Methods.

Electrophysiology

Whole-cell current, STOCs, single-channel current, and macromembrane current of BK<sub>c</sub> channels were recorded in human VSMCs with the techniques as described previously and in the online-only Data Supplement Methods.

Real-Time Reverse Transcription Polymerase Chain Reaction and Western Blot Analysis

Gene and protein expression of the BK<sub>c</sub> α-subunit KCa1.1 and the β1-subunit KCNMB1 were determined using the procedure as described in the online-only Data Supplement Methods.

Statistical Analysis

Data were expressed as means±SEM. The term n represented the number of cells or patients. Student t tests for independent test were used for statistical analysis. P<0.05 was considered statistically significant.

Results

Macroscopic Current of BK<sub>c</sub> Channels in Human VSMCs

Figure 1 shows the whole-cell macroscopic current recorded in representative cells with 400-ms voltage steps to between −50 and +60 mV from a holding potential of −60 mV. The oscillatory current was inhibited by the BK<sub>c</sub> channel blocker iberiotoxin (200 nmol/L, Figure 1A), indicating typical BK<sub>c</sub> current. BK<sub>c</sub> current was greater in the cell from normotensive patient than that from hypertensive patients (Figure 1B). The amplitude of whole-cell BK<sub>c</sub> current in response to the step-voltage depolarization was variable in individual cells, and we therefore normalized the current with cell membrane capacitance. Figure 1C illustrates the voltage-current (I-V) relationships of normalized BK<sub>c</sub> current in VSMCs from normotensive and hypertensive patients. The current density (at 0 to +60 mV) was lower in VSMCs from hypertensive patients than that from normotensive patients (P<0.05 or P<0.01, hypertensive versus normotensive: n=90 cells/38 normotensive patients; n=51 cells/27 hypertensive patients). The cell membrane capacitance was 32.4±1.1 pF in cells from normotensive patients (n=90) and 34.0±1.5 pF in cells from hypertensive patients (n=51, P=NS). These results suggest that BK<sub>c</sub> currents are downregulated in VSMCs from hypertensive patients.

Single-Channel Activity of BK<sub>c</sub> Channels in Human VSMCs

To examine whether the decreased BK<sub>c</sub> current is related to the reduced single-channel activity in human VSMCs from hypertensive patients, the single-channel activity was recorded in cell-attached or inside-out recording mode. Figure 2A and 2B shows the single-channel current of BK<sub>c</sub> recorded at potentials from 0 to +60 mV in cell-attached mode and inside-out mode, respectively, in cells from normotensive or hypertensive patients. No difference was observed in single-channel activity, open probability (Figure 2C), or single-channel conductance (Figure 2D) in VSMCs from normotensive patients and hypertensive patients, either in cell-attached recording or inside-out recording. These results indicate that single-channel activity is not involved in the decreased BK<sub>c</sub> current in hypertensive patients.

STOCs in Human VSMCs

It is well recognized that STOCs, as Ca<sup>2+</sup> sparks, represent a local Ca<sup>2+</sup> signaling in activation of BK<sub>c</sub> channels mediating...
smooth muscle relaxation.\textsuperscript{7,8} We recorded STOCs in whole-cell voltage clamp mode to determine whether alteration in STOCs is involved in the decreased whole-cell BK\textsubscript{Ca} current in VSMCs from hypertensive patients.

Figure 3 displays the STOCs recorded in human VSMCs from normotensive or hypertensive patients. STOCs were remarkably inhibited by 200 nmol/L iberiotoxin (Figure 3A). The activity of STOCs at potentials of 0 to −50 mV was lower in a cell from hypertensive patient than that from normotensive patient (Figure 3B). Mean values of voltage-dependent STOC frequency and amplitude were reduced in cells from hypertensive patients than those from normotensive patients. At 0 mV, STOC frequency was 5.0±0.4 Hz in cells from normotensive patient and 2.6±0.3 Hz in cells from hypertensive patient, indicating a reduced Ca\textsuperscript{2+} sensitivity. The effect of intracellular free Ca\textsuperscript{2+} on the \(G/G_{\text{max}}\) of BK\textsubscript{Ca} channels was determined using variable concentrations of free Ca\textsuperscript{2+} from 0.05, 0.1, 1.0 to 17.0 \(\mu\text{mol/L CaCl}_2\) in bath solution, respectively.

The variables of \(G/G_{\text{max}}\) were fitted to a Boltzmann function in individual inside-out macropatch recordings with different concentrations of free Ca\textsuperscript{2+} concentrations. Figure 4C illustrates the mean values of \(V_{1/2}\) of BK\textsubscript{Ca} conductance in response to variable concentrations of free Ca\textsuperscript{2+} in VSMCs from normotensive and hypertensive patients. The \(V_{1/2}\) of BK\textsubscript{Ca} conductance was negatively shifted with an increase of free Ca\textsuperscript{2+} in bath solution in cells from normotensive patients or hypertensive patients. However, the \(V_{1/2}\) values of BK\textsubscript{Ca} conductance were more positive at each concentration of free Ca\textsuperscript{2+} exposure in cells from hypertensive patients than those in cells from normotensive patients.

**Ca\textsuperscript{2+} Sensitivity of BK\textsubscript{Ca} Channels in Human VSMCs**

Inside-out macropatch recording was used to determine whether alteration of Ca\textsuperscript{2+} sensitivity is involved in the reduced whole-cell BK\textsubscript{Ca} current. Figure 4A illustrates the representative current traces recorded with 100 nmol/L Ca\textsuperscript{2+} in bath solution in a cell from normotensive patient and a cell from hypertensive patient. The current was elicited by 200-ms voltage steps to between −100 and +140 mV from a holding potential of −150 mV, then to −80 mV (to record the tail current). The inward tail current was measured in cells from normotensive and hypertensive patients. The normalized tail current (\(G/G_{\text{max}}\)) was plotted against testing potentials (Figure 4B) and fitted to a Boltzmann function. The half activation potential (\(V_{1/2}\)) of BK\textsubscript{Ca} channels was clearly different in the cell from hypertensive patient than that from normotensive patient: the \(V_{1/2}\) of \(G/G_{\text{max}}\) was positively shifted in the cell from hypertensive patient, indicating a reduced Ca\textsuperscript{2+} sensitivity. The effect of intracellular free Ca\textsuperscript{2+} on the \(G/G_{\text{max}}\) of BK\textsubscript{Ca} channels was determined using variable concentrations of free Ca\textsuperscript{2+} from 0.05, 0.1, 1.0 to 17.0 \(\mu\text{mol/L CaCl}_2\) in bath solution, respectively.

The variables of \(G/G_{\text{max}}\) were fitted to a Boltzmann function in individual inside-out macropatch recordings with different concentrations of free Ca\textsuperscript{2+} concentrations. Figure 4C illustrates the mean values of \(V_{1/2}\) of BK\textsubscript{Ca} activation conductance in response to variable concentrations of free Ca\textsuperscript{2+} in VSMCs from normotensive and hypertensive patients. The \(V_{1/2}\) of BK\textsubscript{Ca} conductance was negatively shifted with an increase of free Ca\textsuperscript{2+} in bath solution in cells from normotensive patients or hypertensive patients. However, the \(V_{1/2}\) values of BK\textsubscript{Ca} conductance were more positive at each concentration of free Ca\textsuperscript{2+} exposure in cells from hypertensive patients than those in cells from normotensive patients.
Hypertension
February 2013

Patients. These results indicate that the sensitivity of BKCa channels to Ca2+ is reduced in VSMCs from hypertensive patients.

mRNA and Proteins of BKCa α- and β1-Subunits
To investigate the molecular mechanisms underlying the reduced whole-cell current, STOCs, and Ca2+ sensitivity of BKCa channels in VSMCs from hypertensive patients, mRNA and proteins of the α-subunit KCa1.1 (KCNMA1 or Slo1) and the β1-subunit KCNMB1 of BKCa channels were determined using reverse transcription polymerase chain reaction and Western blot analysis in human mesenteric arterial tissues. The mRNA expression of KCNMB1, but not KCa1.1, was reduced in mesenteric arterial tissues from hypertensive patients (Figure 5A and 5B). Figure 5C displays the Western blots of KCa1.1 and KCNMB1 proteins in human mesenteric arterial tissues from normotensive or hypertensive patients. As the observation in reverse transcription polymerase chain reaction for gene expression, the protein of KCa1.1 was not altered, whereas the KCNMB1 protein level was reduced in human mesenteric arterial tissues from hypertensive patients.
hypertensive patients. Figure 5D illustrates the mean values of relative levels of mRNA to β-actin and protein levels to GAPDH of KCa1.1 and KCNMB1 in human mesenteric arterial tissues from normotensive or hypertensive patients. No difference in KCa1.1 mRNA or protein was observed in VSMCs from normotensive or hypertensive patients. No difference in human mesenteric arterial tissues of KCa1.1 and KCNMB1 in normotensive patients and hypertensive patients (P>0.05 vs normotensive patients; patient numbers as shown in the inset bar).

Discussion

In the present study, we demonstrate for the first time to our knowledge that whole-cell current of BKCa channels is decreased in VSMCs from hypertensive patients, which is associated with reduced STOCs and Ca2+ sensitivity of the channel, but not single-channel open probability. Biochemical analysis reveals that the reduced whole-cell current, STOCs, and Ca2+ sensitivity of BKCa channels likely result from the downregulation of the β1-subunit KCNMB1, but not the α-subunit KCa1.1 (or Slo) of the channel in Han Chinese patients with hypertension.

It is well recognized that BKCa channels are highly expressed in vascular smooth muscles and play a crucial role in vascular relaxation via an endogenous compensatory mechanism to buffer vasoconstriction, particularly in the intense myogenic constriction of resistance vessels exposed to high intraluminal pressures. The Ca2+-dependent relaxation is mediated by local Ca2+ release from the sarcoplasmic reticulum, termed as calcium sparks, which activates BKCa channels to induce STOCs. Therefore, BKCa channels in VSMCs are the key regulator in the vasoregulation by tuning vascular smooth muscle tone. BKCa channels may open in response to the depolarization of VSMCs and the rise in cytosolic Ca2+ concentration that occurs during arterial contraction, and the Ca2+ then enhances the open-state probability of the BKCa channel to produce VSMC relaxation by hyperpolarizing membrane potential, thereby preventing further Ca2+ influx. The physiological role of BKCa channels is protective against excessive vasoconstriction by a Ca2+-dependent relaxation mechanism.

The function of BKCa channels in VSMCs is finely tuned by its regulatory β1-subunit KCNMB1 via enhancing the channel sensitivity to intracellular local Ca2+. Animal experiments have demonstrated that dysfunction of KCNMB1 is associated with elevated blood pressure and left ventricular hypertrophy in mice. In spontaneously hypertensive rats, it has been observed that the β1-subunit of BKCa channels is reduced and the sensitivity of the channel to physiological changes in Ca2+ is decreased in VSMCs. These results support the notion that changes in the molecular composition of β1-subunit of BKCa channels may be a fundamental event contributing to the development of vascular dysfunction in hypertension.

Genetic epidemiological studies from a Spanish human population demonstrate that a gain of function of the β1-subunit KCNMB1 (E65K mutant) is associated with a low prevalence of diastolic hypertension. Their functional analysis of BKCaE65K channel currents revealed a further negative shift in the G-V relationships, which becomes larger with progressive increases in intracellular Ca2+, presenting an increased activity of the channel at equivalent voltage and Ca2+ concentrations compared with the wild-type BKCa channel. However, interestingly, a recent study from Han Chinese population shows that a reduced function of BKCa channels with KCNMB1-rs11739136 is associated with essential hypertension susceptibility. The present study provides the novel direct evidence that function of BKCa channels is downregulated in VSMCs from Han Chinese patients with essential hypertension.

It is believed that Ca2+ sparks are tightly coupled to BKCa channels to create a hyperpolarization K+ current known as STOCs that oppose further vascular constriction. However, a sustained increase in arterial tone is always an essential component in the development of hypertension. Generally speaking, higher level of [Ca2+]i could induce a higher activity...
of BK<sub>Ca</sub> channels so that the vascular dysfunction in hypertension might implicate a reduction in [Ca<sup>2+</sup>]<sub>i</sub>-BK<sub>Ca</sub> channel coupling. Amberg et al<sup>12</sup> found that the Ca<sup>2+</sup> sparks-BK<sub>Ca</sub> activation coupling was reduced in spontaneously hypertensive rats and that the sparks-STOC coupling efficiency was decreased as a result of the modulation of the subunit stoichiometry of BK<sub>Ca</sub> channels in VSMCs in this animal model. This also happened in BK<sub>Ca</sub> β1<sup>−/−</sup> mice<sup>11</sup> and in insulin-resistant rats with hypertension.<sup>22</sup> Similarly, in the present study we found that dysfunction of BK<sub>Ca</sub> channels is related to reduced STOCs and the decreased sensitivity to physiologically relevant changes in [Ca<sup>2+</sup>]<sub>i</sub> in VSMCs from Han Chinese patients with hypertension, which resulted from the downregulation of the β1-subunit KCNMB1 of the channel. This result at least suggests that the decreased whole-cell current may be partly due to the reduced STOC activity of BK<sub>Ca</sub> channels. However, it was obvious that the decrease of whole-cell BK<sub>Ca</sub> current in hypertension does not just mean the reduction of STOCs.

Although whether loss of channel activity produces hypertension or whether hypertension causes the reduction in channel activity cannot be currently concluded, the present study provides the novel direct evidence that function of BK<sub>Ca</sub> channels is downregulated in VSMCs from patients with essential hypertension. This study suggests that recovering the activity of BK<sub>Ca</sub> channels by restoring β1 function may be a therapeutic approach to correcting vascular dysfunction in hypertension in humans.

A limitation of the present study was that we did not determine whether alteration of BK<sub>Ca</sub> channel function in endothelial cells is involved in hypertension as a result of the shortage of human arterial specimens. It is interesting to make such observation in the future by collecting sufficient human vascular tissues.

**Perspectives**

The result of the present study demonstrated that BK<sub>Ca</sub> activity is decreased in Han Chinese patients with hypertension. Our data indicate that the mechanism underlying dysfunction of BK<sub>Ca</sub> channels in VSMCs is correlated to human hypertension. The decreased expression of the β1-subunit KCNMB1 is likely a common feature in hypertension. So this study suggests that recovering the activity of BK<sub>Ca</sub> channels by restoring β1 function may be a therapeutic approach to correcting vascular dysfunction in hypertension in humans.

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**Disclosures**

None.

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**References**


Novelty and Significance

What Is New?
• The study demonstrated for the first time that whole-cell current, spontaneous transient outward currents, and Ca\(^{2+}\) sensitivity of BK\(_{ca}\) channels are reduced in human vascular smooth muscle cells from hypertensive patients, which resulted from downregulation of β1-subunit of the channel.

What Is Relevant?
• BK\(_{ca}\) channels in vascular smooth muscle cells are the key regulator in the vasoregulation by tuning vascular smooth muscle tone. The function of BK\(_{ca}\) channels in vascular smooth muscle cells is finely tuned by its regulatory β1-subunit via enhancing the channel sensitivity to intracellular local Ca\(^{2+}\). Changes in the molecular composition β1-subunit of BK\(_{ca}\) channels may be a fundamental event contributing to the development of vascular dysfunction in hypertension.

Summary
The result of the present study demonstrated that BK\(_{ca}\) activity is decreased in Han Chinese patients with hypertension. Our data indicate that the mechanism underlying dysfunction of BK\(_{ca}\) channels in vascular smooth muscle cells is correlated to human hypertension. The decreased expression of the β1-subunit KCNMB1 is likely a common feature in hypertension.
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Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2012/12/10/HYPERTENSIONAHA.111.00211.DC1

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Function of BKCa channels is reduced in human vascular smooth muscle cells from Han Chinese patients with hypertension

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Online Supplement

Files in this online supplement: Methods, Table S1, Table S2
Supplemental Methods:

Preparation of human vascular smooth muscle cells (VSMCs)

Single VSMCs were isolated from the mesentery artery tissues as described previously.\(^1\) Briefly, the tissue was transported immediately to the laboratory in an ice-cold Tyrode’s solution upon the dissection. The surrounding tissues of the artery were removed under a microscope in the ice-cold Tyrode’s solution contained (in mmol/L): NaCl 127.0, KCl 5.9, MgCl\(_2\) 1.2, CaCl\(_2\) 2.4, Na-HEPES 10.0, glucose 12.0, pH 7.4), and then cut into 1 mm pieces. The tissue chunks were incubated in a Ca\(^{2+}\)-free Tyrode’s solution contained 1.0 mg/ml papain, 2.0 mg/ml albumin and 2.0 mg/ml dithiothreitol (DTT) for 8~10 min, then in a fresh Ca\(^{2+}\)-free Tyrode’s solution contained 1.25 mg/ml collagenase XI (Sigma) for 6~8 min at 37°C with gentle agitation. The isolated cells were kept in 0.1 mmol/L [Ca\(^{2+}\)] Tyrode’s solution at 4°C, and freshly used for electrophysiological recording.

Electrophysiology and solutions

A small amount of the solution containing the isolated human VSMCs was placed in an open perfusion chamber (0.5 ml) mounted on the stage of an inverted microscope (DMIRE2, Germany) The cells were allowed to adhere to the bottom of the chamber for 10 min and superfused with Tyrode’s solution contained (in mmol/L) NaCl 137.0, KCl 5.9, MgCl\(_2\) 1.2, CaCl\(_2\) 1.8, glucose 12.0, HEPES 10.0, pH 7.4.

For whole-cell recording, borosilicate glass electrodes (0.8-mm OD) were pulled with a Brown-Flaming puller (P-97, Sutter Instrument Co, Novato, CA) and had tip resistances of about 3 M\(\Omega\) when filled with the pipette solution contained (in mmol/L) K-aspartate 110.0, KCl 30.0, NaCl 10.0, MgCl\(_2\) 1.0, EGTA 0.05, and HEPES 10.0 (pH=7.3). A perforated-patch configuration was applied for the whole-cell recording by including amphotericin B (250 µg/ml) in the pipette solution. After the access resistance reached to <10 M\(\Omega\), and series resistance was compensated by 70% to minimize voltage errors. Current signals were acquisitioned using an EPC-10 amplifier and Pulse software (Heka Elektronik, Lambrecht, Germany). Command pulses were generated by a 12-bit digital-to-analog converter controlled by Pulse software. The data were stored on a PC computer for offline data analysis. All experiments were conducted at room temperature (22-23°C).

Spontaneous transient outward K\(^+\) currents (STOCs) measured at -60 to +30 mV
with the procedure as described previously. The parameters of STOCs were analyzed using the Mini Analysis software (Synaptosoft Inc., USA). The “frequency” indicates the events of STOCs per second, while the “amplitude” is the peak amplitude.

Single channel current was recorded in cell-attached mode or inside-out patch mode using patch pipettes with about 10 MΩ when filled with the solution described below, while macropatch inside-out recording was performed with patch pipettes with 1 ~ 2 MΩ. The recording was conducted under symmetrical K⁺ conditions with pipette and bath solutions contained (in mmol/L) KMeSO₃ 118, N-methyl-glucamine-MeSO₃ 20, KCl 2, HEPES 2, EGTA 5, pH 7.2. The appropriate amount of total CaCl₂ (100 mmol/L CaCl₂ standard solution; Fluka) to add to the base internal solution containing 5 mmol/L EGTA to yield the desired free Ca²⁺ concentration was calculated using the program Max Chelator. Data were sampled at 20 kHz and filtered at 1 kHz, capacity and leak current were subtracted using a P/5 subtraction protocol with a holding potential of -150 mV and leak pulses in opposite polarity to the test pulse.

**Real-time RT-PCR**

Total RNA was isolated from the human mesentery arterial tissues using a total RNA extraction kit (Shanghai Huashun, Shanghai, China) following the manufacturer's instruction, and was quantified with a spectrophotometer. The first strand cDNA was created with 1 µg total RNA using ReverTra Ace® qPCR RT Kit (Toyobo, Japan). Primers were designed using Primer Premier 5.0 program with human genes as shown in the online Supplemental Table S2, and used for real time PCR experiments. Quantitative Real time PCR was carried out using Real time PCR Master Mix (Toyobo, Japan) with the MJ Research PRC-200 Real Time PCR System (Bio-Rad, USA). Each measurement was made in triplicate and expressed relative to the housekeeping gene GAPDH.

**Western blot analysis**

After carefully removing surrounding tissues, the human mesentery arterial tissue was grinded in liquid nitrogen using a pre-cold pestle and mortar, then adding 500-1000 µl lysis buffer (20 mmol/L MOPS, 1 mmol/L DTT, 250 mmol/L sucrose
plus protease inhibitor cocktail). The homogenate was transferred to microcentrifuge tubes and kept on ice for 30 minutes, then centrifuged at 10,000 g for 10 min at 4°C. The proteins was determined and used for Western blot analysis.

The equal amounts of protein were mixed with SDS sample buffer and denatured at 95°C for 5 min. Samples were electrophoresed on SDS-PAGE, and transferred to PVDF membrane. The membrane was washed with PBS for 5 min, fixed in 0.5% glutaraldehyde/PBS for 45 min, and blocked with defatted milk for 2 hours after washing with PBS. Afterward, the membrane was probed with the primary antibody (polyclonal Rabbit anti-KCa1.1 (APC-107, Alomone Labs, Jerusalem, Israel) and polyclonal Rabbit anti-KCNMB1 (PA1-924, Pierce, Illinois, USA) and polyclonal Rabbit anti-GAPDH (sc-25778, Santa Cruz, California, USA) in 0.1% TBST for overnight (4°C). After wash with TBST (0.05%), the membrane was incubated with secondary goat anti-rabbit IgG (AP132B, Millipore, Massachusetts, USA) in TBST (0.1%) for 1 hour or HRP-streptavidin antibody (N200, Pierce, Illinois, USA) in TBST (0.1%) for 30 min. The membrane was washed with TBST (0.05%) and developed by ECL kit (Millipore, Massachusetts, USA). The western blot data was analyzed with software Quantity One 4.6.2 and SPSS17.0.

References


Table S1. Characteristics of the patients

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**P<0.01 vs. normal blood pressure (BP)
### Table S2. Sequence of primers used for quantitative RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCa 1.1</td>
<td>F: 5’-TCTCCAGTGCTTCGTG-3’&lt;br&gt;R: 5’-GGTGTTGGGTGAGTTCC-3’</td>
<td>353bp</td>
</tr>
<tr>
<td>KCNMB1</td>
<td>F: 5’-TTGAGACCAACATCAGGGA-3’&lt;br&gt;R: 5’-GGTGTTGGGTGAGTTCC-3’</td>
<td>250bp</td>
</tr>
<tr>
<td>β-actin</td>
<td>F: 5’-ACACTGTGCCCATCTACG-3’&lt;br&gt;R: 5’-TGTCACGCACGATTTCC-3’</td>
<td>153bp</td>
</tr>
</tbody>
</table>