ORIGINAL RESEARCH

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# Different Effects of Hypertension and Age on the Function of Large Conductance Calcium- and Voltage-Activated Potassium Channels in Human Mesentery Artery Smooth Muscle Cells

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***Background***—Large-conductance calcium- and voltage-activated potassium channels ($BK_{Ca}$ channels) play important roles in the maintenance of vascular tone, and their dysregulation is associated with abnormal vascular relaxation and contraction. We tested the changes in $BK_{Ca}$ channel properties in patients at different ages to assess the effects of hypertension and aging on the functional changes of $BK_{Ca}$ channels.

***Methods and Results***—Patch clamp was performed to detect the activities of $BK_{Ca}$ channels in freshly isolated human mesenteric artery smooth muscle cells from younger patients (aged ≤45 years) without hypertension, older patients (aged ≥65 years) without hypertension, and older patients with hypertension. The expression of mRNA and protein from $BK_{Ca}$ channels was evaluated by reverse transcription polymerase chain reaction and Western blot analysis, respectively. Results showed that the whole-cell current density, spontaneous transient outward current, and $Ca^{2+}$ sensitivity of the artery smooth muscle cells were significantly decreased in the older patients with hypertension; the decreases were insignificant in the older patients without hypertension, although a clear tendency to have spontaneous transient outward current was detected in these patients. The expression of both mRNA and protein of $BK_{Ca}$ subunits α and β1 was significantly decreased in the older patients with hypertension but not in the older patients without hypertension compared with the younger patients without hypertension.

***Conclusions***—Our findings demonstrate for the first time that hypertension is an important factor for the pathological alteration of the properties of $BK_{Ca}$ channels in human mesenteric artery smooth muscle cells, and aging itself may also be a factor in these changes in the cells. **(*J Am Heart Assoc*. 2016;5:e003913 doi: 10.1161/JAHA.116.003913)**

**Key Words:** age • $BK_{Ca}$ channels • human mesentery artery smooth muscle cells • hypertension • patch clamp

Hypertension is one of the most common diseases and is a major risk factor for stroke, myocardial infarction, and other diseases.[1,2] In hypertension, aberrant expression and dysfunction of the ion channels in artery smooth muscle cells (ASMCs) are the main reasons for the continuing increase in blood pressure and the higher responsibility to the vasoconstrictors.[3,4] Large-conductance calcium- and voltage-activated potassium channels ($BK_{Ca}$ channels) in the ASMCs help maintain the homeostatic vascular tone by counteracting vascular tone. In addition to their membrane voltage and $Ca^{2+}$ sensitivity, these channels are activated by intracellular local $Ca^{2+}$ release events through ryanodine receptors (termed "$Ca^{2+}$ sparks") on the sarcoplasmic reticulum to produce local $Ca^{2+}$ signaling for inducing spontaneous transient outward currents (STOCs) in the vascular smooth muscle cells (VSMCs).[5] Contrary to reports that hypertension caused the increased functional expression of $BK_{Ca}$ channels in vasculature,[2,6] we recently reported that the function of $BK_{Ca}$ channels was reduced in ASMCs from Han Chinese patients with hypertension. Our results supported the theory that reduced expression of the $BK_{Ca}$ β1 subunit would uncouple $BK_{Ca}$ channels (STOCs) from $Ca^{2+}$ sparks in the VSMCs, resulting in disruption of the $BK_{Ca}$ channel–mediated homeostatic function to counteract vascular tone during hypertension.[7]

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Aging is associated with changes in the structure and function of blood vessels that lead to cardiovascular diseases. Hyperactivity of arteries is commonly observed in older adults, and the abnormal expression and activity of $BK_{Ca}$ channels are possible mediators of functional changes in aged blood vessels.[8–10] Although it is recognized that the prevalence and severity of hypertension are markedly increased with age, the changes in cardiovascular system during the aging process seem to occur independently of other risk factors such as arterial hypertension.[9] To date, the functionality and role of $BK_{Ca}$ channels in the cardiovascular system of older adults are not fully understood. In the present study, we observed significant alteration of $BK_{Ca}$ channels in ASMCs from older hypertensive patients (OHPPs; aged ≥65 years). Interestingly, we also noticed a decreased tendency of $BK_{Ca}$ channels in older normotensive patients (ONMPs; aged ≥65 years). To date, however, no report has addressed the age-related changes of $BK_{Ca}$ channels in human artery, to say nothing of OHPPs. To address these issues, we focused on (1) characterization of $BK_{Ca}$ activity in ASMCs freshly isolated from the mesentery arterial tissues of young normotensive patients (YNMPs; aged ≤45 years), ONMPs, and OHPPs during surgical procedures, using electrophysiological and molecular biological studies, and (2) identification of the different effects of hypertension and aging on alteration of $BK_{Ca}$ channel function.

**Table 1.** Demographic Characterization of Patients

| Characteristic | YNMPs | ONMPs | OHPPs |
|---|---|---|---|
| Patients, n (m/f) | 29 (18/11) | 52(39/13) | 44 (30/14) |
| Age, y | 40.0±1.0 | 70.6±0.7* | 73.0±.9* |
| Systolic BP, mm Hg | 125.6±2.0 | 121.6±1.5 | 157.3±2.2* |
| Diastolic BP, mm Hg | 78.4±1.3 | 74.7±1.2 | 91.6±1.4* |
| Diseases, n | | | |
| Rectal cancer | 15 | 36 | 32 |
| Gastric cancer | 5 | 5 | 1 |
| Colon cancer | 8 | 8 | 9 |
| Polyps | — | 2 | 1 |
| Intestinal obstruction | 1 | 1 | 1 |
| Medication, n | | | |
| ACEIs | — | — | 3 |
| Calcium channel blockers | — | — | 5 |
| Angiotensin receptor antagonists | — | — | 3 |

ACEI indicates angiotensin-converting enzyme inhibitor; BP, blood pressure; OHPP, older hypertensive patient; ONMP, older normotensive patient; YNMP, young normotensive patient.
*$P<0.01$ vs YNMPs.

## Materials and Methods

### Human Arteriole Tissue Collection

Mesenteric arteriole tissues were collected from the removed tissues of Han Chinese YNMPs, ONMPs, and OHPPs during abdominal operations. We used the arterioles obtained from grade 3 to 4 mesenteric artery of the patients. According to the Chinese and international diagnostic criteria of hypertension guidelines,[11,12] hypertension is defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg. The human tissue collection protocol was approved by the ethics committee of Southwest Medical University. Informed consent was obtained from all participants. The demographic characterization of the patients is shown in Table 1.

### Preparation of Human ASMCs

A single ASMC was isolated from the mesentery arteriole tissues of patients during surgical procedure, as described previously.[7] Briefly, the tissues were transported immediately to the laboratory in ice-cold Tyrode solution after surgical removal. After the surrounding tissues of the arterioles were removed, the blood vessels were cut into 1-mm pieces and incubated in $Ca^{2+}$-free Tyrode solution containing 1.0 mg/mL papain, 2.0 mg/mL albumin, and 2.0 mg/mL dithiothreitol for 8 to 10 minutes, followed by incubation in $Ca^{2+}$-free Tyrode solution containing 1.25 mg/mL collagenase XI (Sigma-Aldrich) for another 6 to 8 minutes at 37°C with gentle agitation. The prepared single ASMC was kept in 0.1 mmol/L $[Ca^{2+}]$ Tyrode solution at 4°C for further experiments.

### Electrophysiology and Solutions

Freshly isolated human ASMCs were placed in an open perfusion chamber (0.5 mL) mounted on the stage of an inverted microscope (IX71; Olympus). After adhering to the bottom of the chamber, the cells were superfused with Tyrode solution containing (in mmol/L) NaCl 137.0, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 1.8, glucose 12.0, and HEPES 10.0 (pH 7.4).

For whole-cell recording, tip resistance was ≈3 MΩ filled with the pipette solution containing (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). For perforated-patch whole-cell recording, the pipette solution contained amphotericin B (250 μg/mL). After the whole-cell configuration was formed, the cell membranes were perforated by amphotericin B, and series resistance was compensated by 70% to minimize voltage errors after access resistance reached <10 MΩ. Current signals were acquired

using an EPC-10 amplifier and Pulse software (Heka Elektronik). The data were stored on a PC computer for offline analysis. All experiments were conducted at room temperature (22–23°C).

For spontaneous transient outward $K^+$ current (STOC) recording, current events were measured at −60 to +30 mV with the procedure described previously.[7] The parameters of STOCs were analyzed using Mini Analysis software (Synaptosoft Inc). "Frequency" indicates STOC events per second, and "amplitude" is the peak amplitude.

For single-channel recording, currents were recorded in cell-attached or inside-out patch mode using patch pipettes with tip resistance of 10 MΩ, whereas macropatch inside-out recording was performed using patch pipettes with 1- to 2-MΩ tip resistance. Recording was conducted under symmetrical $K^+$ conditions with pipette and bath solutions containing (in mmol/L) $KMeSO_3$ 118, *N*-methyl-glucamine-$MeSO_3$ 20, KCl 2, EGTA 5, and HEPES 2 (pH 7.2). An appropriate amount of total $CaCl_2$ (100 mmol/L $CaCl_2$ standard solution; Fluka) was added to the base internal solution containing 5 mmol/L EGTA to yield the desired free $Ca^{2+}$ concentration. Free $Ca^{2+}$ concentration was calculated using the Maxchelator program (Stanford University). Data were sampled at 20 kHz and filtered at 1 kHz, and 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.

## Arteriole Membrane Potential Measurement

For membrane potential detection, sharp microelectrode measurement was used. After the surrounding tissues of the arterioles were removed, segments of the arterioles were cut open. After rubbing off the endothelial layer, a hard borosilicate glass sharp microelectrode filled with 3 mol/L KCl (tip resistance 80–100 MΩ) was inserted into smooth muscle cells from the lumen side. The ME-200A (Chengdu Technology and Market Co Ltd) was used for recording the membrane potential. Analog output from the amplifier was recorded using BL-420S biological signal acquisition system (Chengdu Taimeng Software Co. Ltd., China) software (sample frequency 10 kHz). The criteria for the acceptance of the membrane potential recording data were stable membrane potential for at least 10 minutes after an abrupt negative deflection of potential as the microelectrode was inserted into a cell.

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

Expression of the $BK_{Ca}$ α subunit KCNMA1 and β1 subunit KCNMB1 were determined at both the mRNA and protein levels using the following procedure. For reverse transcription polymerase chain reaction (RT-PCR), total RNA was prepared from the human mesentery arterioles using a total RNA extraction kit (Tiangen), following the manufacturer's instructions. To begin, 1 μg total RNA was used to generate cDNA with the ReverTra Ace qPCR RT Kit (Toyobo) in a 20-μL reaction. Of the 20 μL cDNA, 1 μL was used to analyze the expressions of KCNMA1 and KCNMB1 by RT-PCR in a 25-μL reaction containing PCR Master Mix (Qiagen) and gene-specific primers shown in Table 2. RT-PCR was carried out on the 7900 Real Time PCR System (Thermo Fisher Scientific). Expression of the mRNA of KCNMA1 and KCNMB1 normalized to GAPDH was analyzed using the $2^{-\Delta\Delta CT}$ method. All experiments were performed in triplicate.

**Table 2.** Sequence of Primers Used for Quantitative Reverse Transcription Polymerase Chain Reaction

| Gene | Primer Sequence | Product |
|---|---|---|
| $BK_{Ca}$ α | F: 5′-TCTCCAGTGCCTTCGTG-3′<br>R: 5′-GGTGTTGGGTGAGTTCC-3′ | 353 bp |
| $BK_{Ca}$ β1 | F: 5′-TTGAGACCAACATCAGGGA-3′<br>R: 5′-GGTGTTGGGTGAGTTCC-3′ | 250 bp |
| β-actin | F: 5′-ACACTGTGCCCATCTACG-3′<br>R: 5′-TGTCACGCACGATTTCC-3′ | 153 bp |

bp indicates base pairs; F, forward; R, reverse.

### *Western blot*

Human mesentery arteriole samples were snap frozen in liquid nitrogen and homogenized using precold mortar and pestle 100- to 500-μL lysis buffer (in mmol/L: MOPS 20, dithiothreitol 1, sucrose 250, 1% protease inhibitor cocktail). Homogenate was spun down at 10 000*g* for 10 minutes at 4°C, and the supernatants were used for Western blot analysis. Protein concentration was determined using the BCA protein assay kit (Bio-Rad). Next, 30 μg of lysate was resolved on SDS-PAGE and transferred to polyvinylidene difluoride membranes. The polyvinylidene difluoride membranes were washed with PBS for 5 minutes, fixed in 0.5% glutaraldehyde/PBS for 45 minutes, and blocked with 5% nonfat milk for 2 hours at room temperature. The membranes were incubated with an anti-$BK_{Ca}$ α polyclonal (APC-107, 1:1000 dilution in 5% milk/TBST; Alomone Labs), anti-$BK_{Ca}$ β polyclonal (PA1-924, 1:1000 dilution in 5% milk/TBST; Pierce), and anti-GAPDH polyclonal (sc-25778, 1:3000 dilution in 5% milk/TBST; Santa Cruz Biotechnologies) overnight at 4°C, washed 3 times with 0.1% TBST for 5 minutes, and then incubated with secondary goat anti–rabbit IgG (AP132B; Millipore) at 1:10 000 dilution in 5% milk/TBST for 1 hour, followed by horseradish peroxidase–streptavidin antibody (N200, 1:50 000 dilution in 5% milk/TBST; Pierce) for 30 minutes. The membranes were washed again with 0.1% TBST and developed with an electrochemiluminscence kit (Millipore). The data were analyzed with

Quantity One 4.6.2 (Bio-Rad) and SPSS 17.0 software (IBM Corp).

## Statistical Analysis

All data were expressed as mean±SEM. The normally distributed data were analyzed with 1-way ANOVA. The abnormally distributed data were logarithmically transformed to normal distribution and then analyzed with 1-way ANOVA. The correlations among cells from the same patient as independent units were analyzed with Pearson correlation. $P<0.05$ was considered statistically significant.

# Results

## Macroscopic Current of $BK_{Ca}$ Channels in Human VSMCs

Figure 1 shows the whole-cell macroscopic current recorded in representative cells from YNMPs, ONMPs, and OHPPs. Recording was conducted with holding potential of −60 mV and depolarizing pulses to +60 mV (10-mV step and 400-ms duration) (Figure 1A). The current exhibited was typical of $BK_{Ca}$ current, as reported previously.[13] To eliminate the variations of whole-cell $BK_{Ca}$ in individual cells, the currents were normalized with cell membrane capacitance to obtain the current density. The cell membrane capacitance was 32.9±1.8, 31.6±4.4, and 30.3±1.6 pF in cells from YNMPs (n=21), ONMPs (n=13), and OHPPs (n=44, $P$ value not significant), respectively. $BK_{Ca}$ current density was greater in the cells from the YNMP and ONMP groups than from OHPPs (Figure 1A). Figure 1B illustrates the current–voltage (log i–v) relationships of normalized $BK_{Ca}$ currents in the VSMCs from YNMPs, ONMPs, and OHPPs. The current density data were logarithmically transformed to normal distribution and then analyzed statistically. The results indicated that the current density (at +20 to +60 mV) was significantly lower ($P<0.05$) in VSMCs from the OHPP group (44 cells, 12 patients) compared with the YNMP groups (21 cells, 7 patients). No significant differences were observed between the YNMP and ONMP groups (13 cells, 8 patients) or between the ONMP and OHPP groups.

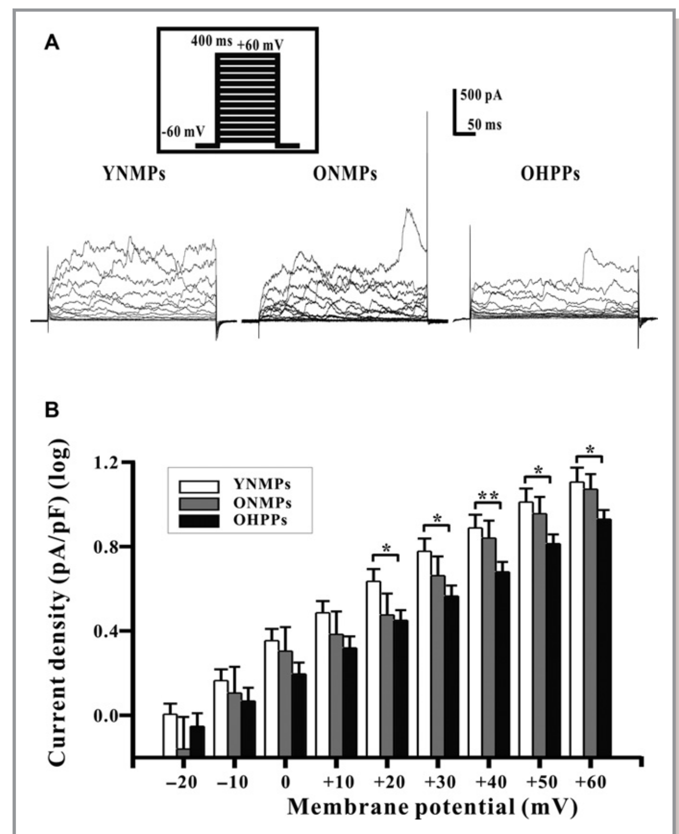


**Figure 1.** Whole-cell current of $BK_{Ca}$ channels in human VSMCs. A, $BK_{Ca}$ currents recorded in the representative cells from YNMPs, ONMPs, and OHPPs. The voltage protocol is shown in the inset. B, Histogram of the log i–v relationship of $BK_{Ca}$ current density in VSMCs from YNMPs (21 cells, 7 patients), ONMPs (13 cells, 8 patients), and OHPPs (44 cells, 12 patients). Data on the *y*-axis were displayed as the logarithmic value and statistically analyzed. Significant differences are marked, $^{*}P<0.05$, $^{**}P<0.01$, OHPPs vs YNMPs. $BK_{Ca}$ indicates large-conductance calcium- and voltage-activated potassium; OHPP, older hypertensive patient; ONMP, older normotensive patient; VSMC, vascular smooth muscle cell; YNMP, young normotensive patient.

## Single-Channel Activity of $BK_{Ca}$ Channels in Human VSMCs

$BK_{Ca}$ single-channel activity was detected to examine whether a change of activity had occurred. Single-channel current was recorded in cell-attached or inside-out recording mode. Figure 2A and 2B show the typical single-channel current traces of $BK_{Ca}$ channels in the cells from YNMPs, ONMPs, and OHPPs (at representative potentials +20, +40, and +60 mV). As shown in Figure 2C and 2D, the results from either cell-attached or inside-out recording demonstrated that no significant difference was observed in single-channel activity, including the open probability and single-channel conductance in VSMCs from the 3 groups. These results indicate that no significant changes occurred in single-channel activity in ONMPs and OHPPs.

## STOCs in Human VSMCs

It is believed that STOCs generated from the activation of $BK_{Ca}$ channels by $Ca^{2+}$ sparks have a key role in the control of vascular tone.[14] In the present study, STOCs were detected in whole-cell voltage clamp mode to determine whether alteration of STOCs occurred in VSMCs from ONMPs and OHPPs.

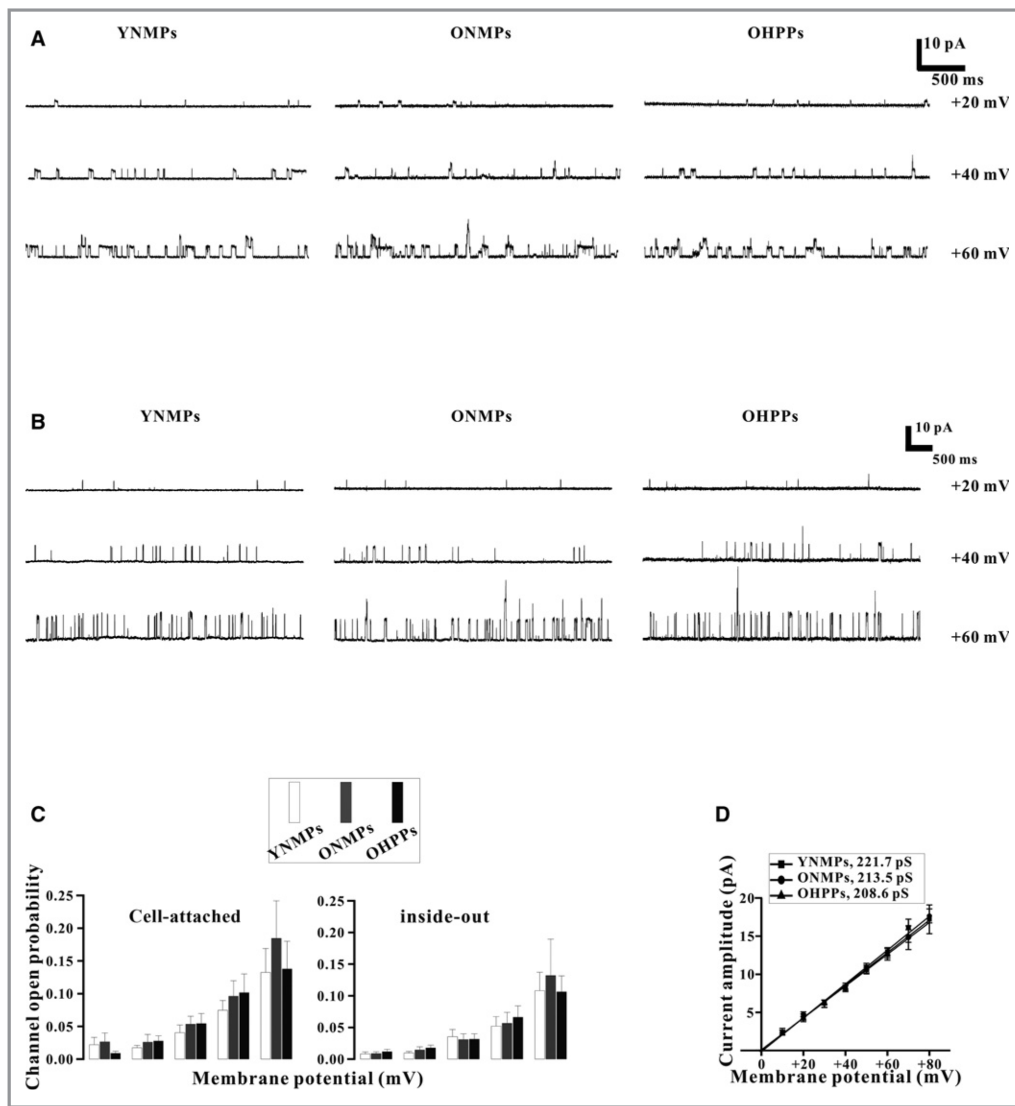


**Figure 2.** Single-channel currents of BK$_{Ca}$ channels in human VSMCs. Single-channel current recorded in cell-attached configuration (A) and inside-out configuration (B) (≈0 $Ca^{2+}$ in bath solution) in the representative cells from a YNMP, an ONMP, and an OHPP, respectively. C, The mean values of open probability of single-channel currents recorded in cell-attached configuration (YNMP: 21 cells, 5 patients; ONMP: 11 cells, 5 patients; OHPP: 9 cells, 5 patients) or inside-out configuration (YNMP: 14 cells, 8 patients; ONMP: 14 cells, 7 patients; OHPP: 15 cells, 9 patients). D, Log i–v relations of BK$_{Ca}$ single-channel conductance in the VSMCs from the YNMP, the ONMP, and the OHPP (YNMP: 14 cells, 7 patients; ONMP: 14 cells, 8 patients; OHPP: 15 cells, 9 patients). The data were obtained from inside-out configurations. BK$_{Ca}$ indicates large-conductance calcium- and voltage-activated potassium; OHPP, older hypertensive patient; ONMP, older normotensive patient; VSMC, vascular smooth muscle cell; YNMP, young normotensive patient.

The results (Figure 3) obtained from the human VSMCs of YNMPs, ONMPs, and OHPPs demonstrated that STOC activity at membrane potentials more positive than −20 mV were significantly lower in cells from the OHPP group than from the YNMP group, mainly reflecting decreases of the mean values of voltage-dependent STOC frequency (Figure 3B and 3C). OHPP cells also showed a significant decrease in STOC frequency ($P<0.05$) (Figure 3B) compared with ONMP cells. Although age-dependent change was much weaker in cells from ONMPs than from OHPPs, it still showed significance at membrane potentials of −10 and 0 mV (Figure 3B). The data also clearly showed the downregulation of STOCs in aging. At 0 mV, the STOC frequency was 0.81±0.01 Hz (log) in YNMP cells (11 cells, 6 patients), 0.57±0.09 Hz (log) in ONMP cells (12 cells, 9 patients), and 0.31±0.06 Hz (log) in OHPP cells (32 cells, 10 patients; ONMPs versus YNMPs, $P$=0.046; OHPPs versus YNMPs, $P$=0.000; ONMPs versus OHPPs, $P$=0.012), whereas STOC amplitude was 1.33±0.04 pA (log)

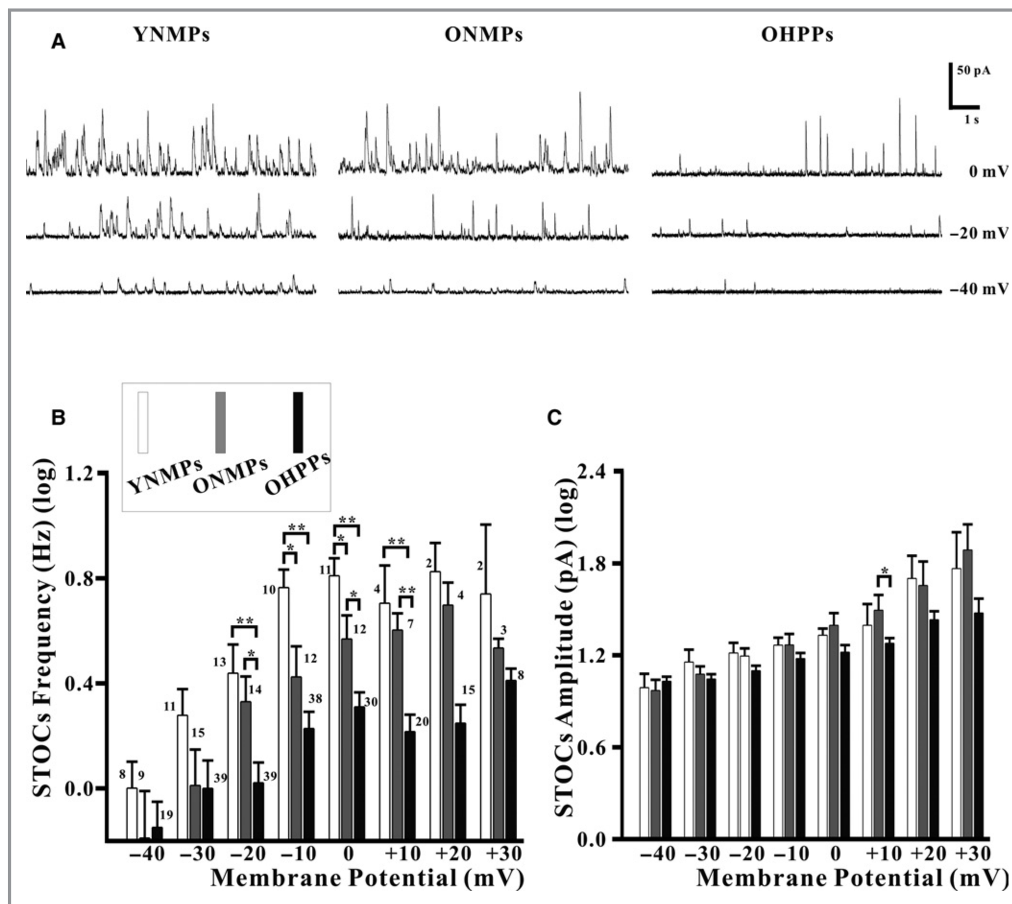


**Figure 3.** The STOCs of $BK_{Ca}$ channels in human VSMCs. A, Representative figures show the typical STOCs recording in the cells from a YNMP, an ONMP, and an OHPP at −40, −20, and 0 mV membrane potential, respectively. The mean values of the STOC frequency (B) and amplitude (C) of $BK_{Ca}$ channels in the VSMCs of YNMPs, ONMPs, and OHPPs. The data were displayed as the logarithmic value and statistically analyzed. Significant differences of STOCs were found with the membrane potentials among the 3 groups, $*P<0.05$, $**P<0.01$. Numbers of cells are labeled near the relative bar. The data were from 6 YNMPs, 11 ONMPs, and 13 OHPPs. $BK_{Ca}$ indicates large-conductance calcium- and voltage-activated potassium; OHPP, older hypertensive patient; ONMP, older normotensive patient; STOC, spontaneous transient outward current; VSMC, vascular smooth muscle cell; YNMP, young normotensive patient.

in YNMP cells, 1.40±0.08 pA (log) in ONMP cells, and 1.22±0.05 pA (log) in OHPP cells (*P* value not significant). These results suggest that human hypertension syndrome substantially altered the properties of $BK_{Ca}$ in the mesenteric ASMCs, whereas age itself has only a potentially weak impact on changes in $BK_{Ca}$ channels. Dysfunction in $BK_{Ca}$ channels started to appear in the older population and was worse in those with hypertension.

## $Ca^{2+}$ Sensitivity of $BK_{Ca}$ Channels in Human VSMCs

$Ca^{2+}$ sensitivity of $BK_{Ca}$ channels is required for proper regulation of smooth muscle tone. To determine whether alteration of $Ca^{2+}$ sensitivity was involved in the regulation of $BK_{Ca}$ channels, inside-out macropatch recording was used for the study. The current was elicited by 200-ms voltage steps to between −100 and +140 mV (10-mV increment) from a holding potential of −150 mV and then to −80 mV (typical recordings are shown in Figure 4A). The inward tail current was measured in cells from the 3 groups. The normalized tail current ($G/G_{max}$) was plotted against testing potentials and fitted to a Boltzmann function. No significant difference in half activation potential ($V_{1/2}$) of $BK_{Ca}$ channels was found between cells from the YNMP and ONMP groups, but significant differences were observed in the cells from OHPPs compared with both YNMPs and ONMPs. The data showed that the $V_{1/2}$ of $G/G_{max}$ is positively shifted in the cells from the OHPP group (Figure 4B), indicating reduced voltage sensitivity in OHPPs. In addition, using the G–V relations over a series of $Ca^{2+}$ concentrations, the $Ca^{2+}$ sensitivity of $BK_{Ca}$ channels was determined. The effect of intracellular free

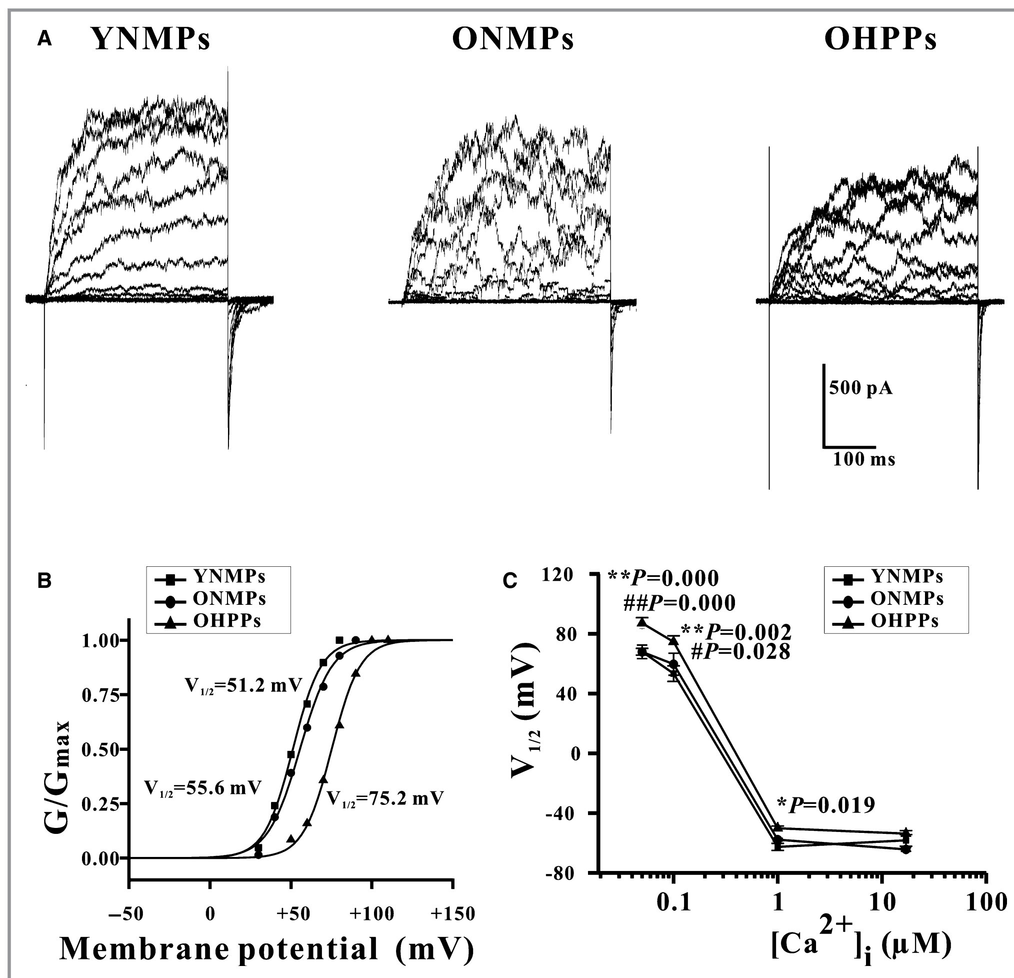


**Figure 4.** Activation conductance ($G/G_{max}$) of $BK_{Ca}$ and $Ca^{2+}$ sensitivity. A, Typical inside-out macromembrane current traces recorded in the VSMCs from YNMPs, ONMPs, and OHPPs. The voltage protocol is shown in the inset. B, Normalized tail currents ($G/G_{max}$) of $BK_{Ca}$ channels are fitted to the Boltzmann function: $y=1/(1+\exp((V-V_{1/2})/k))$, in which V is the tested membrane potential, $V_{1/2}$ is the potential of half-maximal activation, and k is the slop factor. The data are fitted from the same recordings as panel (A). C, The mean values of $Ca^{2+}$ concentration-dependent $V_{1/2}$ of $BK_{Ca}$ activation conductance in the VSMCs from YNMPs, ONMPs, and OHPPs. YNMPs vs OHPPs: $^{*}P<0.05$, $^{**}P<0.01$;ONMPs vs OHPPs: $^{\#}P<0.05$, $^{\#\#}P<0.01$. No differences in spontaneous transient outward currents were found between YNMPs and ONMPs (*P* value not significant). The cells were from YNMPs (6–10 cells, 8 patients), ONMPs (5–13 cells, 4 patients), and OHPPs (5–16 cells, 4 patients). $BK_{Ca}$ indicates large-conductance calcium- and voltage-activated potassium; OHPP, older hypertensive patient; ONMP, older normotensive patient; VSMC, vascular smooth muscle cell; YNMP, young normotensive patient.

$Ca^{2+}$ ($[Ca^{2+}]_i$) on the $G/G_{max}$ of $BK_{Ca}$ channels was tested using various concentrations of free $Ca^{2+}$ at 0.05, 0.1, 1.0, and 17.0 mmol/L $CaCl_2$ in bath solution. The relations of $G/G_{max}$–V were established at each $[Ca^{2+}]_i$, and the individual $V_{1/2}$ was obtained after fitting each relation curve to Boltzmann function. Figure 4C illustrates the mean values of $V_{1/2}$ of $BK_{Ca}$ channels in response to various $[Ca^{2+}]_i$ obtained from YNMPs, ONMPs, and OHPPs. The results indicate that the $V_{1/2}$ of $BK_{Ca}$ conductance negatively shifted with the increase of $[Ca^{2+}]_i$ in the cells from the 3 groups; however, the $V_{1/2}$ values of $BK_{Ca}$ channels were more positive at each $[Ca^{2+}]_i$ exposure in the cells from OHPPs than from YNMPs and ONMPs. The study demonstrated that the sensitivity of $BK_{Ca}$ channels to $Ca^{2+}$ was decreased in VSMCs from OHPPs but not from YNMPs and ONMPs.

## Influence of $BK_{Ca}$ Channels by Cancer

Because most tissues were derived from cancer patients in our study, we tried to retrieve the vessel tissues as far away as possible from the site of the cancer. To show whether $BK_{Ca}$ current is affected by cancer, we measured current in tissues

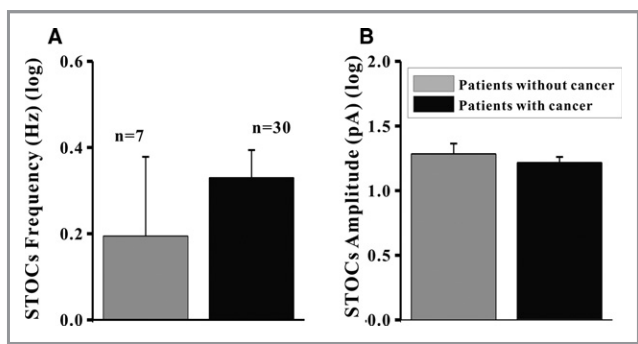


**Figure 5.** Influence of cancer on large-conductance calcium- and voltage-activated potassium channels. The data are displayed as the logarithmic value and were statistically analyzed. No influence of cancer was observed on STOC frequency (A) and amplitude (B). The arteriole smooth muscle cells were from older hypertensive patients with and without cancer. Test membrane potential was 0 mV. STOC indicates spontaneous transient outward current.

from both noncancer and cancer patients. The results showed that at 0 mV membrane potential, STOC frequencies (log) were 0.19±0.18 Hz (7 cells, 3 noncancer patients with hypertension) and 0.33±0.06 Hz (30 cells, 11 cancer patients with hypertension), and STOC amplitude (log) was 1.28±0.08 pA (7 cells, 3 noncancer patients with hypertension) and 1.22±0.04 pA (30 cells, 11 cancer patients with hypertension) The results indicated that cancer had no influence on BK$_{Ca}$ current in our study (Figure 5).

## Human Mesentery Arterioles Membrane Potential

To detect whether membrane potential is different between younger and older patients and between hypertensive and nonhypertensive patients, arteriole tissues were used to verify the effects. The arteriole strips were bathed in PSS solution, and the sharp microelectrode method was applied to measure the membrane potential of VSMCs directly in intact mesenteric arteries. The membrane potential was 44.7±1.9 mV in YNMPs (n=18), 38±1.6 mV in ONMPs (n=14), and 40.4±1.1 mV in OHPPs (n=20). These data indicated that no significant changes in resting membrane potential of patients' arterioles were observed among these groups (*P* value not significant).

## Messenger RNAs and Proteins of the α and β1 Subunits of BK$_{Ca}$

To quantitate the molecular changes of BK$_{Ca}$ channels in ONMPs and OHPPs, the expression of BK$_{Ca}$ subunits α (KCNMA1) and β1 (KCNMB1) in human mesenteric arterial cells was determined using RT-PCR and Western blot analysis, respectively. The expression of the *KCNMA1* and *KCNMB1* genes were markedly reduced in OHPPs compared with YNMPs. The expression of the *KCNMB1* gene had no statistical significance in ONMPs (Figure 6A), although it showed a decreasing trend compared with YNMPs.

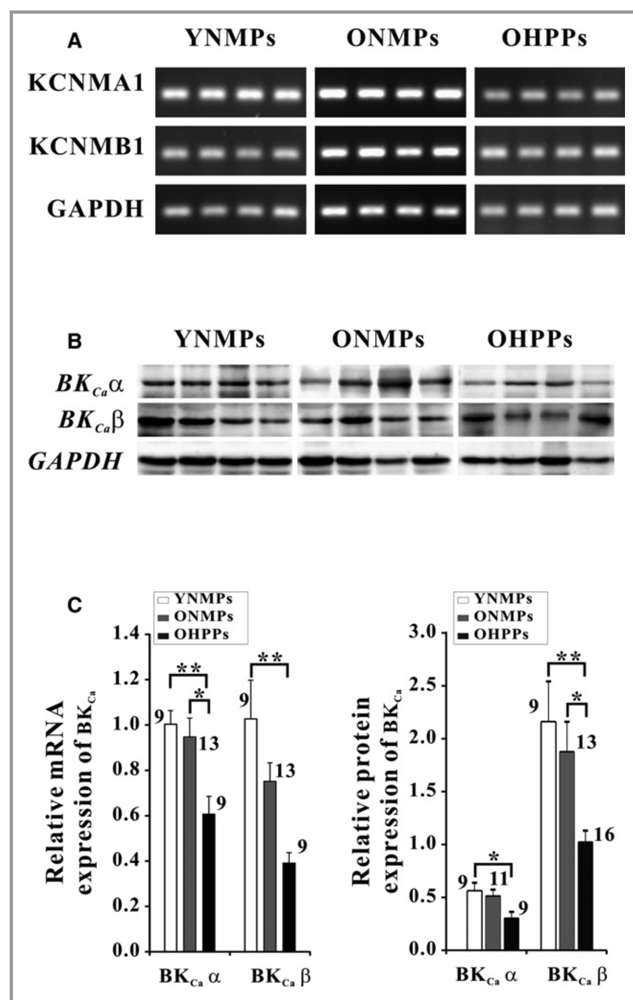


**Figure 6.** Messenger RNAs and proteins of KCNMA1 and KCNMB1 in human mesenteric cells. A, Images from relative quantification in reverse transcripitioin polymerase chain reaction. B, Protein expression of BK$_{Ca}$ subunits α and β1 with Western blot analysis. C, Mean values of mRNA and protein expression of KCNMA1 and KCNMB1 in human mesenteric cells from YNMPs, ONMPs, and OHPPs (**P*<0.05, ***P*<0.01 vs YNMP; the tested numbers are shown in the inset bar). BK$_{Ca}$ indicates large-conductance calcium- and voltage-activated potassium; OHPP, older hypertensive patient; ONMP, older normotensive patient; YNMP, young normotensive patient.

Figure 6B shows the expression of KCNMA1 and KCNMB1 at protein levels in the human mesenteric arterial cells from the 3 groups. Consistent with the gene expression of KCNMB1, the expression of KCNMB1 was markedly reduced at the protein level in the mesenteric arterial cells in OHPPs but not in ONMPs compared with YNMPs (Figure 6B). Figure 6C shows quantitation of the relative expression of KCNMA1 and KCNMB1 mRNA and proteins in the human

mesenteric arterial cells. No significant differences were observed at mRNA or protein levels of both KCNMA1 and KCNMB1 in VSMCs from YNMPs and ONMPs; however, expression of KCNMA1 and KCNMB1 at both mRNA and protein levels was significantly reduced ($P<0.01$ or $P<0.05$) in cells from OHPPs compared with YNMPs. In addition, the expression of KCNMA1 mRNA and KCNMB1 protein was significantly reduced in cells from OHPPs compared with ONMPs ($P<0.05$). These results indicate that downregulation of KCNMA1 and KCNMB1 expression was related to reduced whole-cell currents, STOCs, and $Ca^{2+}$ sensitivity of $BK_{Ca}$ channels in the VSMCs from the OHPPs. Compared with YNMPs, mRNA and protein expression of $BK_{Ca}$ channels did not decrease in VSMCs from ONMPs; this finding is not consistent with the results showing declined function of $BK_{Ca}$ channels.

## Discussion

This paper describes the characteristics of $BK_{Ca}$ channels in YNMPs, ONMPs and OHPPs and provides novel and important findings. First, the activities of $BK_{Ca}$ macroscopic currents were found to be significantly lower in OHPPs but were insignificant in ONMPs compared with YNMPs. Second, the activity of voltage-dependent STOCs was reduced in frequency in the currents of OHPPs and was reduced at some voltages in ONMPs. Moreover, changes in STOCs were much more significant in OHPPs and relatively mild in ONMPs compared with YNMPs, indicating that age itself may not be a key factor for these changes. Third, the G–V relation obtained from the macro–inside-out patch experiment demonstrated decreased voltage and $Ca^{2+}$ dependency of $BK_{Ca}$ in only OHPPs, not in ONMPs. Fourth, changes in $BK_{Ca}$ currents were not related to single-channel activity. Fifth, biochemical analysis revealed that reduced whole-cell current, STOCs, and $Ca^{2+}$ sensitivity of $BK_{Ca}$ channels in OHPPs likely resulted from downregulation of the $\alpha$ and $\beta 1$ subunits of the $BK_{Ca}$ channels. The decreased $BK_{Ca}$ channel STOCs in the ONMPs, however, were not consistent with expressions of $BK_{Ca}$ channels at both mRNA and protein levels.

It is well known that the physiological role of $BK_{Ca}$ channels is to prevent excessive vasoconstriction by a $Ca^{2+}$-dependent relaxation mechanism. Calcium-dependent relaxation is mediated by local calcium release from the sarcoplasmic reticulum, called "calcium sparks." Normally, $Ca^{2+}$ sparks are tightly coupled to $BK_{Ca}$ channels to create a hyperpolarizing $K^+$ current known as STOCs to oppose further vessel constriction.[14] As in our previous report of the hypertension study,[7] the present study provides direct evidence that the function of $BK_{Ca}$ channels is downregulated in VSMCs in Han Chinese OHPPs and is also associated with reduced STOCs and $Ca^{2+}$ sensitivity of the channels. These results indicate that hypertension is an important factor for the pathological alteration of $BK_{Ca}$ channel properties. Our results appear to conflict with some previous reports that $BK_{Ca}$ current was positively correlated to the blood pressure level of the host animals, suggesting that $BK_{Ca}$ channels in vascular smooth muscle membranes may be dynamically regulated by in situ blood pressure. The authors believed that the enhanced $BK_{Ca}$ current observed in arterial myocytes from hypertensive animals may represent a cellular compensatory mechanism to limit vascular reactivity in this disease. Liu[15] et al reported, for example, that increased expression of $BK_{Ca}$ channels in the cerebral microcirculation of genetically hypertensive rats may provide protection against cerebral vasospasm. Amberg and Santana,[16] however, found that the amplitude of spontaneous $BK_{Ca}$ currents in hypertensive cells was lower than in normotensive cells, indicating that decreased expression of the $BK_{Ca}$ channel subunit $\beta 1$ underscored the lower $Ca^{2+}$ sensitivity of $BK_{Ca}$ channels in hypertensive myocytes, and the lower expression of the $\beta 1$ subunit during genetic borderline and severe hypertension reduced $BK_{Ca}$ channel activity. These results and others[17,18] are consistent with our findings and support the view that changes in the molecular composition of $BK_{Ca}$ channels may be a fundamental event that contributes to the development of vascular dysfunction during hypertension. Generally speaking, the membrane potential of vascular smooth muscle should be positively correlated to the blood pressure level of the host. Accordingly, the resting membrane potential of vascular smooth muscle from hypertension should have greater depolarization; however, although hypertension reduced $BK_{Ca}$ channel activity, no significant changes in the resting membrane potential of arterioles smooth muscle from hypertensive patients were observed in our study. This may be explained by the different reactions of the cells in vitro and in vivo.

Cardiovascular function declines with aging, and hypertension seems to be more prevalent in older participants,[2] which convinced us to focus on another potential variable, age, on the function of $BK_{Ca}$ channels. It is interesting to note that age itself induced a small effect on the changes in $BK_{Ca}$ channels. A decrease in STOC frequency was detected in the ONMPs, suggesting decreased intracellular signaling in the regulation of $BK_{Ca}$ in older adults. Combined with our previous studies[7] on hypertension, we noted that the damage became more serious in OHPPs because of the decreases not only in function but also in expression of the $\alpha$ and $\beta 1$ subunits of $BK_{Ca}$ channels at both the mRNA and protein levels. Evidence supports the role of aging for $BK_{Ca}$ channels. It was reported that age enhanced the contractile responses of vessels to endothelial vasoconstricting factors in the older people, induced a reduction in the density of the $\alpha$ subunit of $BK_{Ca}$ channels in coronary smooth muscle, lowered baseline endothelial release of nitric oxide, and increased the response

to endothelial constrictor factors and $K^+$.[10] The data from Shi et al[19] suggested that age decreased $BK_{Ca}$ channel capability, which was mediated by unparalleled downregulation of α and β1 subunit expression in the rat mesenteric artery; however, conflicting evidence was also reported. Hayoz et al[20] found that age increased the capacitance and STOC amplitude of smooth muscle cells from murine superior epigastric arteries. They concluded that the enhanced functional expression of $BK_{Ca}$-dependent STOCs in older participants represented an adaptation of resistant arteries to maintain functional integrity. Another report indicated no significant difference in $BK_{Ca}$ expression in pulmonary artery in the vessels of older and younger participants.[5] Nishimaru et al[21] also reported that $BK_{Ca}$ channel expression, current density, kinetics, and $Ca^{2+}$ sensitivity were practically identical during cerebral aging, and the sensitivity response to dehydrosoyasaponin I was also the same in myocytes of older and younger participants. The study also showed that the age-dependent changes were not uniform among different vascular beds, indicating that the contribution of $K_V$ and $BK_{Ca}$ channels to myogenic responsiveness varied by fiber type.[22] Our data from human mesentery artery cells confirmed the $BK_{Ca}$ defect in hypertension and aging—for the first time, to our knowledge—and noted that although the change was relatively mild, $BK_{Ca}$ channel dysfunction was already present in older adults and became more serious in those with hypertension.

The incidence of cardiovascular diseases is increased with age, and changes in cardiovascular system that take place during the aging process may occur independently of arterial hypertension.[23] Our results confirm that hypertension and age play different roles in the alteration of $BK_{Ca}$ channels. Although aging does not necessarily cause high blood pressure, it is related to vascular dysfunction, such as vascular hyperactivity to stimulators. Furthermore, we noted a clear tendency of function to decline but no changes in the expressions of $BK_{Ca}$ channels in older adults. The results obtained from older patients indicate that the $BK_{Ca}$ channel functional defect could not be explained solely by molecular evidence of the α and β1 subunits of $BK_{Ca}$ channels, indicating that other factors, such as store-operated $Ca^{2+}$ entry channels triggered by the depletion of intracellular $Ca^{2+}$ stores,[24–26] may play the important roles and should be further investigated; this will be the subject of our next publication. Because STOC activity is a direct function of underlying $Ca^{2+}$ spark behavior, we also tested changes in underlying $Ca^{2+}$ handling in the cells from YNMPs, ONMPs, and OHPPs. The results showed no significant differences in basal $[Ca^{2+}]_i$ under resting condition, and no significant changes among these groups were found with 10 mmol/L caffeine, indicating that the same amount of $Ca^{2+}$ was stored in the sarcoplasmic reticulum of the human mesentery ASMCs in the different groups. Significant differences in rise time and decay time of the $[Ca^{2+}]_i$ changes induced by caffeine were found in the OHPPs and should be considered for further investigation (data not shown).

In the present study, most tissues were obtained from cancer patients, and it is suggested that $K^+$ channels may be affected by cancer, as reported by Bonnet et al.[27] We tried to retrieve vessel tissues from as far away as possible from the site of cancer in our experiments. To determine whether $K^+$ channels are influenced by cancer, we measured tissues from the OHPPs with and without cancer. The results showed that $BK_{Ca}$ currents were not significantly influenced by cancer (Figure 5).

We also analyzed the correlations among cells from the same patient as independent units by Pearson correlation, and the results indicated no correlation among cells from the same patient (data not shown).

A limitation of the present study is that alteration of $BK_{Ca}$ channel function in young patients with hypertension was not determined because of the shortage of human arterial specimens. Such observations will be noted in the future when sufficient human vascular tissues are collected from young hypertensive patients.

## Perspectives

The present studies demonstrated that $BK_{Ca}$ activity is decreased in older adults with hypertension. The dysfunction of $BK_{Ca}$ channels is already present in older patients and is worse in those with hypertension. Changes in $BK_{Ca}$ channels may contribute to the propensity for cardiovascular diseases, strongly suggesting that restoration of the dysfunctional $BK_{Ca}$ channels may prevent the development of cardiovascular diseases and improve outcomes in the hypertensive patients and/or the older population.

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## Disclosures

None.

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