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ORIGINAL ARTICLE

Impairment of BKca channels in human placental chorionic plate arteries is potentially relevant to the development of preeclampsia

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Preeclampsia has known associations with insufficient placental perfusion. The large-conductance Ca^{2+}-activated K^+ (BKca) channels that have recently been found to play important roles in cellular growth and vasodilatation could potentially participate in the development of preeclampsia. However, the mechanisms by which downregulated BKca channels are involved in the development of preeclampsia remain unknown. In this study, we investigated the mechanism(s) underlying the impairment of vascular tone regulation by BKca channels in human placental chorionic plate arteries (CPAs) in preeclampsia. The levels of BKca channel α and β1 subunits were compared using immunohistochemistry, western blotting, and RT-PCR in CPAs of normal and preeclamptic pregnant women. To explore the role of BKca channels in the regulation of proliferation and apoptosis in human placental CPA smooth muscle cells (SMCs), a specific BKca opener, NS1619, was used to investigate proliferative reduction and apoptotic induction in human placental chorionic plate arterie smooth muscle cells (CPASMCs) collected from normal pregnancies. The vasodilator effects of BKca channels and their response to SNP (an NO donor) in both groups were also evaluated by wire myography. We found that BKca channel β1 subunits were less expressed in preeclamptic CPAs. After pretreatment with NS1619, cellular proliferation was significantly suppressed, and cellular apoptosis was dramatically promoted in cultured CPASMCs, demonstrating a relationship between increased Bax expression and decreased Bcl-2 expression in CPASMCs. Downregulated BKca is also associated with decreased vasodilatation and reduced susceptibility to NO donors. In conclusion, the decreased expression or activation of BKca channels may induce pathologic remodeling of human CPAs, weaken the vasodilation response, and decrease vascular sensitivity to vasoactive substances, thereby reducing fetal–placental blood flow and leading to the future development of preeclampsia.
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Keywords: human placental chorionic plate artery; large-conductance Ca^{2+}-activated K^+ channels; preeclampsia; vascular smooth muscle cells; vascular dysfunction

INTRODUCTION

Preeclampsia is a pregnancy-associated disease characterized by reduced organ perfusion secondary to abnormal spiral artery remodeling and microvascular vasospasm.[1,2] It affects 2–7% of all healthy pregnancies worldwide and is still one of the leading causes of maternal and fetal morbidity and mortality.[3] The causative and initiating mechanisms of preeclampsia remain largely unknown. It is clear, however, that the central dysfunctional organ is the placenta, and the only truly effective intervention is delivery of the placenta.[4,5] Many studies have shown that maintenance of a successful pregnancy is mostly dependent upon the ability of the trophoblast to invade the uterus and remodel the spiral arteries, which includes promoting apoptosis of the endothelium and smooth muscle cells and inducing a loss of elasticity.[2,6] The physiologic process of remodeling converts these vessels into high-flow, low-resistance conduits that lack maternal vasomotor control.[7] However, the placental chorionic plate arteries are an important component of placental microvessels, which are rarely remodeled by the trophoblast.[7] Whether the placental chorionic plate arteries are pathologically remodeled is an intriguing question, *i.e.,* whether the placental chorionic plate arterial smooth muscle cells show abnormal proliferation and are resistant to apoptosis when the placenta is underperfused.

The large-conductance Ca^{2+}-activated K^+ (BKca) channels are ubiquitously expressed in the vasculature, especially in vascular smooth muscle cells (VSMCs).[8] In VSMCs, BKca channels are formed by ion-conducting α subunits and regulatory β1 subunits.[9] BKca

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channels are essential for the regulation of many fundamental physiologic processes, including the regulation of cellular apoptotic processes[10,11] and the maintenance of vascular tension in microvessels.[12] Many investigators have found that activated BKca channels can inhibit proliferation and promote apoptosis in hippocampal neurons,[13] cerebrovascular SMCs,[14] and so on. In addition, the reduced expression or activation of BKca channels in the vasculature, commensurate with defective vasodilatation, has been identified as a cause of pathogenic hypertension in spontaneously hypertensive rats.[14] Furthermore, vasoactive substances such as phenylephrine,[15] endothelin-1,[16] and NO[17] can also interact with BKca channels, inducing vasomotion. However, the influence of BKca channels in placental vascular structure and function in preeclampsia has not yet been elucidated.

Placental chorionic plate arteries (CPAs) lack autonomic innervation. Therefore, the control of vascular tone must rely predominantly on local and humoral factors.[18] CPAs have the size characteristics of resistance vessels and may contribute significantly to determining overall placental vascular resistance and blood flow.[19] We hypothesized that BKca channels may influence CPA vascular remodeling and vasodilative functions relative to the development of preeclampsia. We used *in vitro* experiments with CPASMCs that focused on the mechanisms of cellular proliferation and apoptosis that may be related to the pathologic remodeling of CPAs in preeclamptic placental vessels. In addition, wire myography was used to test vessel vasomotor function and the sensitivity of CPAs to SNP (an NO donor) in the placental arteries of preeclamptic women.

MATERIALS AND METHODS

Patients

Thirty healthy pregnant women and 30 women diagnosed with severe preeclampsia were recruited in Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (China). The study was approved by the ethics committee of Tongji Hospital. All patients were fully informed about the study and provided written consent. Severe preeclampsia was defined as systolic blood pressure $\geqslant$ 160 mm Hg and/or diastolic pressure $\geqslant$ 110 mm Hg, combined with either a urine dipstick showing 3+ or 4+ in a random urine sample or greater than 5.0 g of proteinuria over 24 h. Severe preeclampsia was also defined when there was evidence of the development of renal insufficiency or pulmonary edema, new onset of cerebral or visual disturbance, or severe persistent epigastric pain unresponsive to medication.[20] Gestations complicated by multiple pregnancies, diabetes, chronic hypertension, chronic renal disease, cardiovascular diseases, or fetal growth restriction were excluded. The relevant clinical characteristics of the two groups are presented in Table 1.

Tissue acquisition

All CPAs were collected within 1 h of cesarean delivery. CPAs were identified at the umbilical cord insertion point and traced across the placental surface and collected and washed in ice-cold phosphate-buffered saline (PBS). Tissues surrounding the arteries were then dissected. To prevent interference by endothelial cells, we removed them by gently rubbing the intimal surface with fine forceps. The tissues used for western blotting and RT-PCR were immediately frozen in liquid nitrogen. Another portion of the tissue was preserved in 4% formaldehyde solution for immunohistochemical analysis. CPAs from normotensive women served as primary culture tissues and were transferred to the laboratory under sterile conditions.

Table 1 Clinical characteristics of pregnant women recruited for the study

Characteristics	*Normal* (n = *30*)	*Preeclampsia* (n = *30*)
Maternal age (y)	26.5 ± 3.2	27.3 ± 2.5
BMI (kg m^{-2})	23.9 ± 3.4	25.6 ± 2.1
Gestational age (weeks)	38.2 ± 2.2	36.5 ± 2.1
Systolic BP (mm Hg)	110.1 ± 10.3*	163.3 ± 12.5*
Diastolic BP (mm Hg)	75.3 ± 9.6*	105.9 ± 13.5*
Proteinuria (g per 24 h)	0.08 ± 0.05*	8.57 ± 0.02*

Abbreviations: BMI, body mass index; y, years. The data are expressed as means ± s.e.m.
*P<0.05.

Immunohistochemistry

CPAs were paraffin-embedded, cut into 5-μm-thick sections on slides, and stained with Masson trichrome stain. The slides were incubated at 4 °C overnight with primary antibodies against α-smooth muscle actin (α-SMC, 15 μg ml^{-1}; Sigma, Munich, Germany), BKca α (0.8 μg ml^{-1}, Alomone Labs, Israel), and BKca β1 (0.8 μg ml^{-1}, Alomone Labs, Israel). Negative controls were processed following substitution of the primary antibody with the corresponding concentration of non-immunized IgG. The reaction was developed with diaminobenzidine (DAB). Images were analyzed by Image-Pro Plus 6.0 (Media Cybernetics, Denver, CO, USA), and the mean values for area-integrated optical densities were analyzed.

Protein and mRNA expression of BKca α and β1 subunits in CPAs

CPAs were homogenized in lysis buffer and concentrations were measured with a protein assay kit (Bio-Rad, Hercules, USA). Equal amounts of proteins were separated in 8% SDS-PAGE gels and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Burlington, MA, USA). After blocking with 5% milk for 1 h in Tris-buffered saline containing 0.1% Tween-20 (TBST), the membranes were individually incubated overnight at 4 °C with BKca α and BKca β1 diluted 1:400 in 5% milk in TBST. The membranes were then washed in TBST, followed by incubation with anti-rabbit IgG peroxidase-conjugated secondary antibody (Santa Cruz, USA, 1:2000 in 5% BSA in TBST) for 1 h. GAPDH was used as a control for lane loading and was incubated with anti-GAPDH primary antibody (Santa Cruz, USA, 1:2000) overnight and anti-mouse secondary antibody (Santa Cruz, USA, 1:2000) for 1 h. The membranes were developed using Supersignal ECL (Amersham Biosciences, Piscataway, NJ, USA). The resulting band intensities were quantified using Image-Lab software (Bio-Rad Laboratories, Hercules, CA, USA).

Total RNA was extracted from isolated CPAs using Trizol reagent (Invitrogen, Tokyo, Japan) according to the manufacturer's protocol. In addition, cDNA was synthesized from equal amounts of RNA by a reverse kit (TOYOBO, Osaka, Japan). Gene expression was normalized to the housekeeping gene β-actin. The primers for BKca α were 5′-AGTAGCAATATCCACGCGAACC-3′ (forward) and 5′-CCAGGAAAGCCCACCACAG-3′ (reverse), and the primers for BKca β1 were 5′-AGAGCAACCAGTACCTGTCC-3′(forward) and 5′-AGAAGACAGCGTGGATTGGA-3′ (reverse). Each expression level was normalized to the housekeeping gene β-actin using the primers 5′-AGAGCTAC GAGCTGCCTGAC-3′(forward) and 5′-AGCACTGTGTTGGCGTACAG-3′ (reverse). Real-time PCR was performed on a CFX96 Real-Time System (Bio-Rad Laboratories, Franklin Lakes, NJ, USA) using a Quantitative SYBR Green PCR Kit (DBI, Ludwigshafen, Germany). The relative mRNA expression levels of target genes were calculated according to the $2^{-\Delta\Delta Ct}$ method.

TUNEL staining

Vessel sections were fixed using 4% paraformaldehyde in PBS for 30 min at room temperature, washed in PBS for 30 min, and allowed to air dry. The slides were then incubated with permeabilization solution for 10 min and washed in PBS. Vessel sections were incubated with TUNEL reagent and mounted using Vectashield mounting medium containing propidium iodide and stored at 4 °C in the dark. Fluorescent staining was visualized using an Olympus IX70 (OLYMPUS, Tokyo, Japan) inverted fluorescence microscope and TUNEL$^+$ (%) was evaluated with Image-Pro Plus 6.0.

Isolation and identification of CPASMCs

CPASMCs from normotensive women were cultured using explants of CPAs based on the methodology of Leik *et al.*.[21] CPAs without endothelium were cut longitudinally. After the vessels were fragmented, explants were placed in a

culture flask. Dulbecco's modified Eagle's medium F12 (DMEM-F12) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin-glutamine (Beyotime, Shanghai, China) was carefully added to culture dishes to avoid disturbing the adhered explants. Culture plates were placed in a 37 °C, 5% CO_2 humidified atmosphere. Cells started growing from explants within 1 week and reached confluence within 3 weeks. CPASMCs were used between passages 2 and 6.

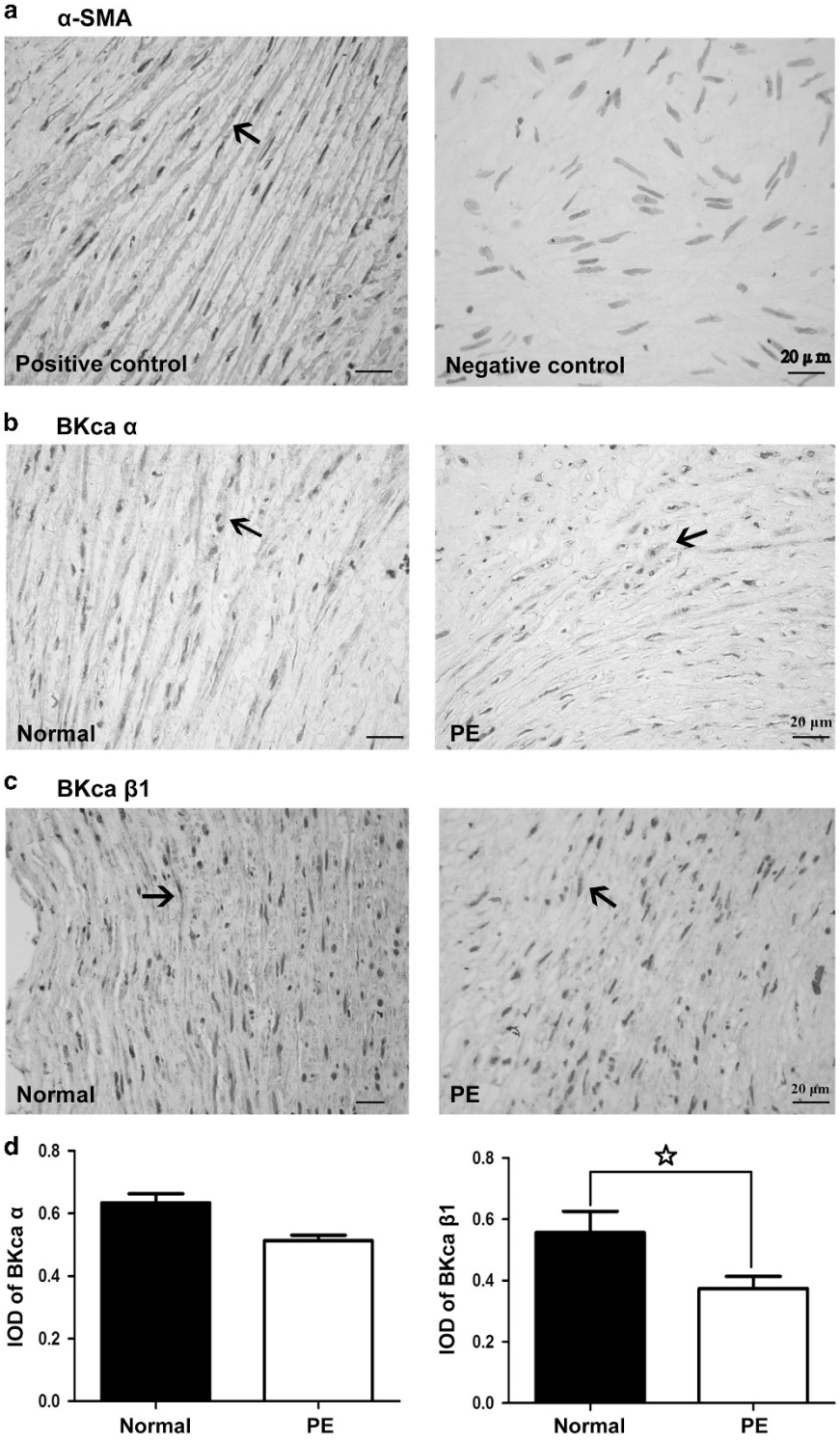

Figure 1 Immunohistochemistry of CPAs. (**a**) smooth muscle α-actin with a negative control (primary antibody substituted with non-immunized IgG), (**b**) BKca α, (**c**) BKca β1 in CPAs from normal pregnant women and preeclamptic women are shown. The brown particles represent positive expression of α-SMC, BKca α, and BKca β1 (arrows). Each sample was analyzed by averaging integrated optical densities (IOD) from 5 different areas; scores were averaged, and statistical analyses were performed. (**d**) Data are expressed as means ± s.e.m. ($n=5$, $^*P<0.05$). A full color version of this figure is available at *Hypertension Research* online.

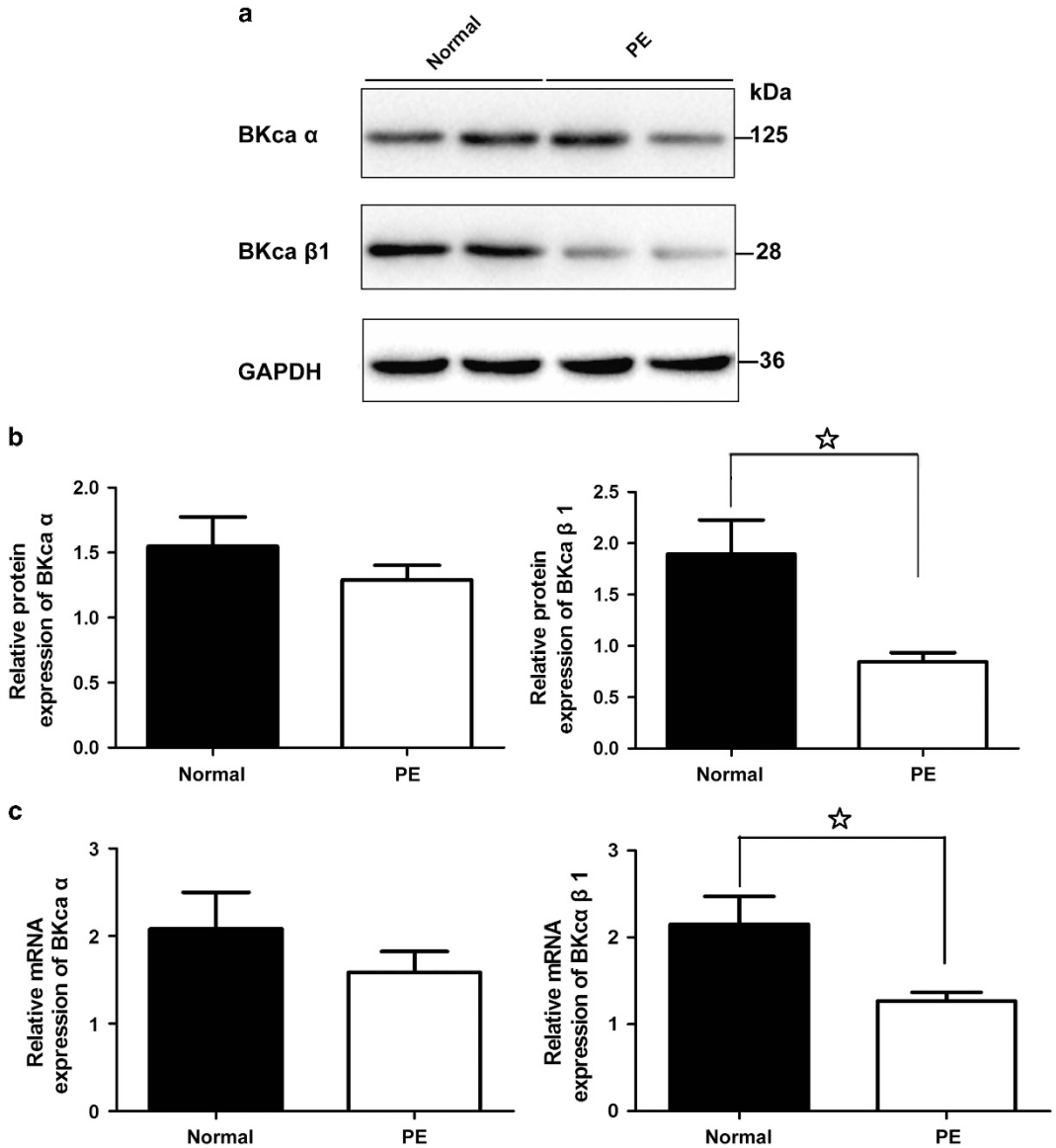

Figure 2 Protein and mRNA expression of BKca channels in CPAs. The protein expression of BKca channels was detected in CPAs from normal pregnant women and preeclamptic pregnant women by western blotting analysis. Blots were stripped and reprobed with antibodies to GAPDH to control for loading consistency. (**a**) The quantification of gray values from the two groups showed that there was a decrease in expression of BKca β1 in the preeclamptic groups. (**b**) mRNA expression of BKca α and BKca β1 in CPAs of the normal and preeclamptic groups. (**c**) β-Actin was used to normalize the signals observed with RT-PCR. Data are expressed as means ± s.e.m. ($n=30$, $^*P<0.05$).

The purity of SMCs isolated from CPAs was confirmed by immunofluorescence through α-SMC (1:100; Sigma). The expression levels of BKca α and β1 were also assessed in CPASMCs on the isolated cells. Negative controls were incubated with non-immunized IgG. Immunofluorescent images were obtained using an Olympus IX70 confocal microscope.

Cell proliferation assay

SMCs were seeded at 5000 cells per well in 96-well plates and the final volume of cell culture medium in each well was 100 μl. After 24 h, the cells were treated with various concentrations (10, 30, 50, 100, or 150 μM) of NS1619 in 100 μl of serum-free medium for 12, 24, 36 or 48 h. At the end of culture, Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) was added to each well. Cells in serum-free medium were used as a negative control. The optical density (OD) values were measured with a microplate reader (Bio-Rad, West Berkeley, CA, USA) at 450 nm. All assays were performed in triplicate. Cellular proliferation at each concentration was calculated as a percentage of the controls.

Measurements of apoptosis

SMCs were seeded and treated in six-well plates. They were harvestedcollected after treatment with 70 μM NS1619 for 12 h, 24 h, 36 h, or 48 h. Cells were washed twice in PBS and exposed to Annexin V-FITC and PI binding buffer for 15 min. The stained cells were then analyzed by a FACScan flow cytometer (BD Bioscience, CA, USA).

Cell protein expression for Bax and Bcl-2

Cells treated with various concentrations (0, 10, 30, 50 or 100 μM) of NS1619 were harvested after 48 h. Bax and Bcl-2 antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Cell proteins were added to 10 or 12% SDS-PAGE gels and proteins were detected and analyzed by immunoblotting as previously described.

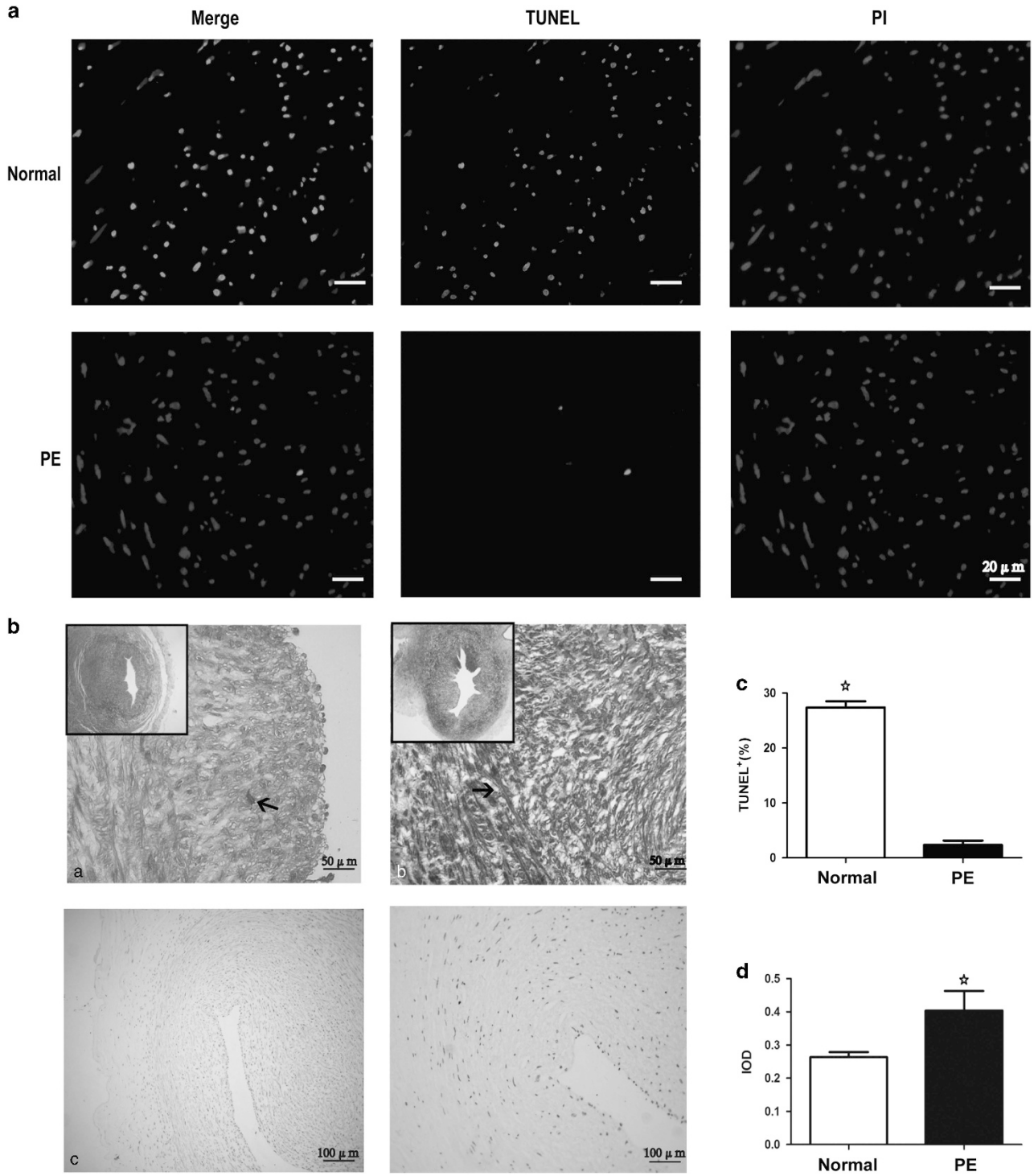

Figure 3 Detection of pathologic remodeling of CPAs using TUNEL assay and Masson trichrome. (**a**) Apoptotic cells were examined using TUNEL assay. TUNEL-positive nuclei are shown in green, and total nuclei stained with DAPI are in blue. (**b**) Masson trichrome showed that the collagen stained blue and the SMCs pink (arrows). (a) control group, (b) preeclamptic groups, (c) and (d) represent smooth muscle α-actin and negative control in CPAs. **c** and **d** represent the quantitative analysis of TUNEL$^+$ (%) and IOD of Masson staining, respectively, from 5 randomly selected fields for each individual experiment ($n=5$, $^*P<0.05$). A full color version of this figure is available at *Hypertension Research* online.

Myography

CPAs (100-500 μm) were cut into rings and placed in ice-cold Krebs Henseleit solution (KH; composition in mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 7.2; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; and glucose, 11.1; pH 7.4). They were then mounted on a wire myograph (Catamount Myotechnology, Catamount R&D, UK) and normalized to 0.9 $L_{5.1kpa}$ as described previously.[22] After this procedure, the vessel rings were equilibrated for 1 h by repeatedly washing in KH solution every 15 min at 37 °C and continuous bubbling with 95% O_2/5% CO_2. Tissue contraction with 60 mM KCl was referenced as the normalization standard for contractile capacity. It was possible to assess the complete removal

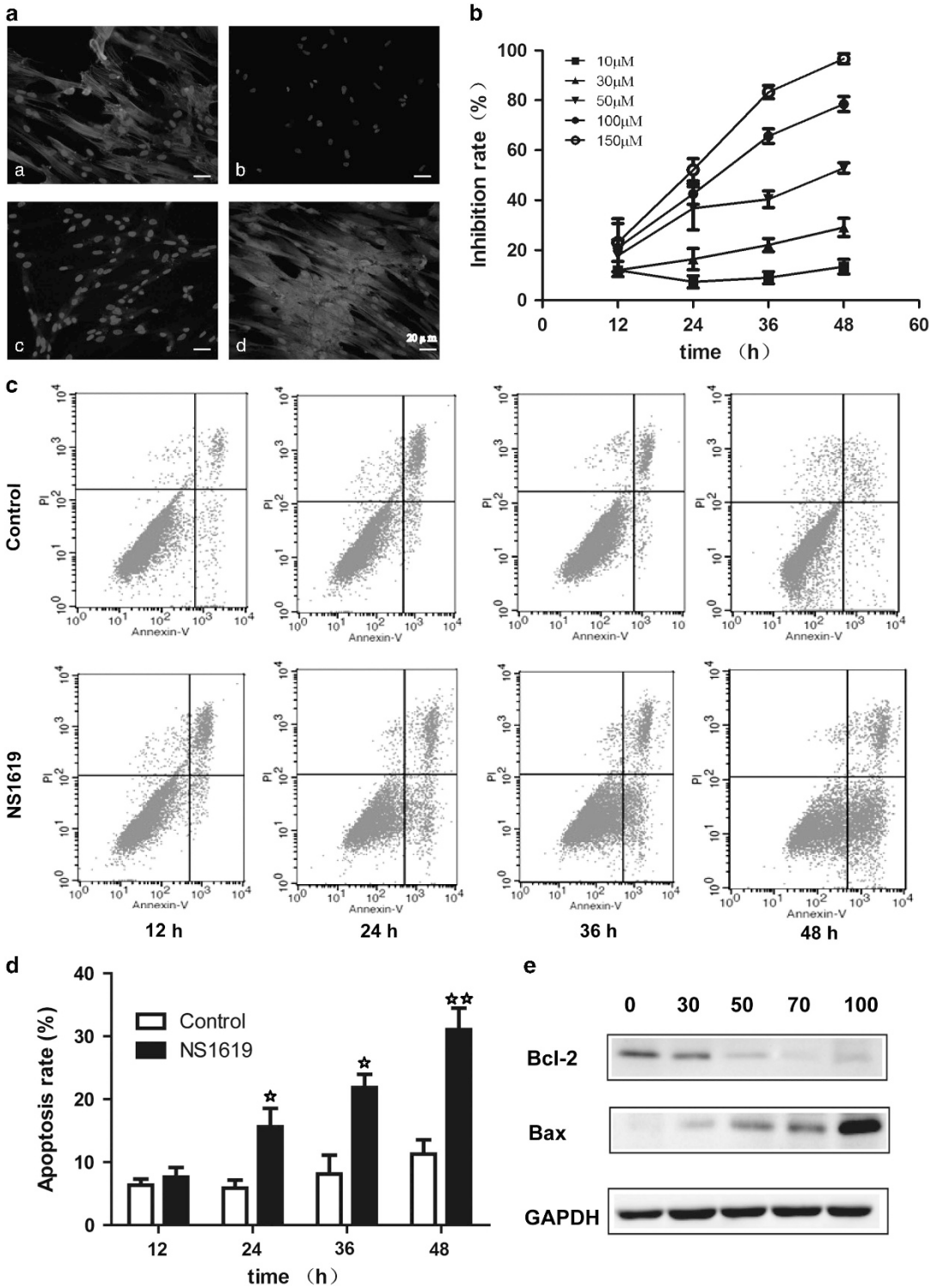

Figure 4 Proliferation and apoptosis of cultured CPASMCs induced by NS1619. (**a**) Immunofluorescent staining of cultured CPASMCs. (a) smooth muscle α-actin, (b) negative control (incubation with non-immune IgG), (c) BKca α, (d) BKca β1. (**b**) CCK-8 assays show that the proliferation of cultured CPASMCs was inhibited by NS1619 (10, 30, 50, 100 or 150 μM) following 48 h exposures to the drug. (**c**) CPASMCs were pretreated with 70 μM NS1619 for 12, 24, 36 or 48 h, and then subjected to FACS analysis through Annexin V-FITC/PI staining. (**d**) The histogram shows that the percentage of apoptotic cells increased significantly after treatment with 70 μM NS1619 for 12, 24, 36 and 48 h. (**e**) Primary cultures of CPASMCs were exposed to 0, 30, 50, 70 or 100 μM NS1619 for 48 h. The expression of Bcl-2 in CPASMCs was significantly decreased and Bax was increased in comparison with controls ($n=5$, $^{*}P<0.05$, $^{**}P<0.01$). A full color version of this figure is available at *Hypertension Research* online.

of the endothelium in CPAs because KCl-pretreated CPAs do not relax in response to acetylcholine (Ach, 10 μM).

After KCl (60 mM)-induced contractions had reached a stable plateau, a concentration-response curve to NS1619 (BKca special opener, 10^{-8}–10^{-3} M) was created to compare the vasodilator effects of BKca in CPAs of normotensive and preeclamptic pregnant women. Sodium nitroprusside (SNP, NO donor, 10^{-8}–10^{-3} M) was applied to paired CPAs to determine the sensitivity of CPAs to SNP after washing, equilibrating and administering KCl (60 mM) to reach a response plateau. To determine whether SNP affected vessel relaxation through BKca, we repeated the SNP-induced concentration-response curves after incubating the tissue with iberiotoxin (IBTX, a selective BKca blocker, 10^{-7} M) or TEA (a relatively selective BKca blocker, 10^{-3} M) for 30 min. Concentration-response curves were recorded and compared between the normal and preeclamptic groups.

Statistical analyses

The data were analyzed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). All results are expressed as the means ± s.e.m., and *n* refers to the number of CPAs in each group. Relaxation (%) was expressed as a percentage of the maximal contraction amplitude observed with an administered dose of 60 mM KCl. Concentration-response curves were analyzed by GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). Values were compared by a *t* test for paired variables or by a two-way ANOVA to determine significance between/among the respective groups. A P-value < 0.05 was considered significant.

RESULTS

Reduced BKca β1 expression in CPAs from preeclamptic pregnancies

We performed immunolocalization studies to determine the presence of SMCs (Figure 1a). Clear and strong immunoreactivity for BKca α and β1 subunits was observed on vascular smooth muscle cells of CPAs (Figures 1b and c) In addition, the expression level of the BKca β1 subunit, but not the α subunit, was significantly downregulated in the preeclamptic group compared with the normal group (Figure 1d). The western blot analysis showed similar results as illustrated in Figures 2a and b. Protein levels of α subunits were almost unchanged in preeclamptic pregnancies, whereas β1 subunit protein expression was reduced in the preeclamptic group compared to the controls. Figure 2c illustrates the mRNA expression levels of BKca α and β1 subunits in normal and preeclamptic pregnancies. After normalization with β-actin, α-subunits were unaffected in preeclamptic pregnancies, but a significant reduction in the β1 subunit transcript was observed.

Pathologic remodeling of CPAs in preeclampsia

The percentage of TUNEL-positive SMCs observed within CPAs was lower in the preeclamptic group compared to the normal group (Figures 3a and c). Masson's trichrome was used to assess muscular fibers; in vessels, muscle fibers in the smooth muscle layer are stained red, and the surrounding connective tissue containing collagen fibers is stained blue. As Figure 3b and Figure 3d show, more smooth muscle cells were present in the CPAs of the preeclamptic group relative to the normal group (0.4040 ± 0.0592 *vs.* 0.2637 ± 0.01515; $n = 5$, $P < 0.05$).

BKca inhibits the proliferation of CPASMCs

VSMCs of CPA sections displayed positive immunostaining for vessel smooth muscle cell markers, as seen in α-smooth muscle actin (a-SMA; Figure 4(a)). No evident staining was recorded following substitution of the primary antibody with non-immunized IgG at an equivalent concentration (Figure 4a and b). Protein expression of the α and β1 subunits of BKca was also evident in isolated CPASMCs (Figure 4a(c, d).

To investigate the role of the BKca channel in cellular proliferation, CPASMCs were exposed to various concentrations of the BKca channel opener NS1619, ranging from 10 to 150 μM. As shown in Figure 4b, the results from the CCK-8 assays indicated that NS1619 inhibited cell growth in a time- and concentration-dependent manner over 48 h of exposure ($IC_{50} = 66.52$ μM). Our data indicate that the BKca channel exerts anti-proliferative influences on CPASMCs.

BKca promotes CPASMC apoptosis

To further confirm and measure the rate of CPASMC apoptosis, we utilized flow cytometry to evaluate apoptosis after treatment with 70 μM NS1619. A FACS analysis was performed to determine the apoptotic rate of CPASMCs exposed to NS1619 for various time-periods (Figures 4c and d). Our results show that the percentage of apoptosis increased significantly after 24 h, 36 h, and 48 h.

BKca increases the expression of Bax and decreases Bcl-2 expression in CPASMCs

To determine whether the pro-apoptotic protein Bax and the anti-apoptotic protein Bcl-2 are involved in the anti-proliferative effects of NS1619 on CPASMCs, the levels of these proteins were tested by immunoblotting. As shown in Figure 4e, increased levels of Bax proteins and decreased levels of Bcl-2 were observed in cells treated with 0, 30, 50, 70, or 100 μM NS1619. These results suggest that the opening of the BKca channel by NS1619 may mediate apoptosis through the induction of Bax/Bcl-2 proteins in CPASMCs.

Impaired BKca channels impede vascular relaxation in preeclampsia

We also examined whether the role of BKca channels in CPAs was altered in preeclampsia. The attenuated vessel tones were normalized to a maximal contraction triggered by 60 mM KCl. As shown in Figure 5a, NS1619 produced concentration-dependent relaxation in CPAs, which was lower in preeclamptic pregnancies (Emax% $4.76 \pm 0.65\%$; $n = 7$, $P < 0.05$) compared to normal pregnancies (Emax% $12.57 \pm 3.19\%$, $n = 7$). SNP had a prominent effect on the relaxation of CPAs. Figure 5b shows the cumulative concentration-response curves of SNP in CPAs from preeclamptic and control subjects upon pre-incubation with 60 mM KCl. The maximal relaxation percentages were smaller in the pre-eclamptic subjects than in the controls (20.64 ± 3.00 *vs.* $34.44 \pm 5.35\%$, $P < 0.05$), indicating that the sensitivity of CPAs to SNP-induced relaxation was reduced in the preeclamptic group. There was also significant inhibition in the relaxation of CPAs pretreated with IBTX or TEA in the control group (Emax%: SNP, $34.44 \pm 5.35\%$; SNP+IBTX, $5.02 \pm 1.40\%$; SNP+TEA, $11.60 \pm 3.44\%$; $n = 7$, $P < 0.05$; Figure 5c), but no difference was noted in the preeclamptic group (Emax%: SNP, $20.64 \pm 3.00\%$; SNP+IBTX, $12.26 \pm 3.27\%$; SNP+TEA, $13.70 \pm 5.82\%$; $n = 7$, $P > 0.05$; Figure 5d). The vascular tone alterations in CPAs due to the BKca channel opener and blockers demonstrated that the expression or activity of BKca channels was remarkably impaired in preeclampsia.

DISCUSSION

The present study provides evidence for a novel mechanism involved in preeclampsia in which abnormal CPASMC growth and aberrant vascular vasomotor function appear to promote the disease. These structural and functional changes induced by the decreased expression or activation of BKca channels may induce pathologic vascular remodeling, enhance the vasoconstrictor response, and decrease sensitivity to vasoactive substance in CPAs, thereby reducing fetal–placental blood flow and leading to further exacerbation of preeclampsia.

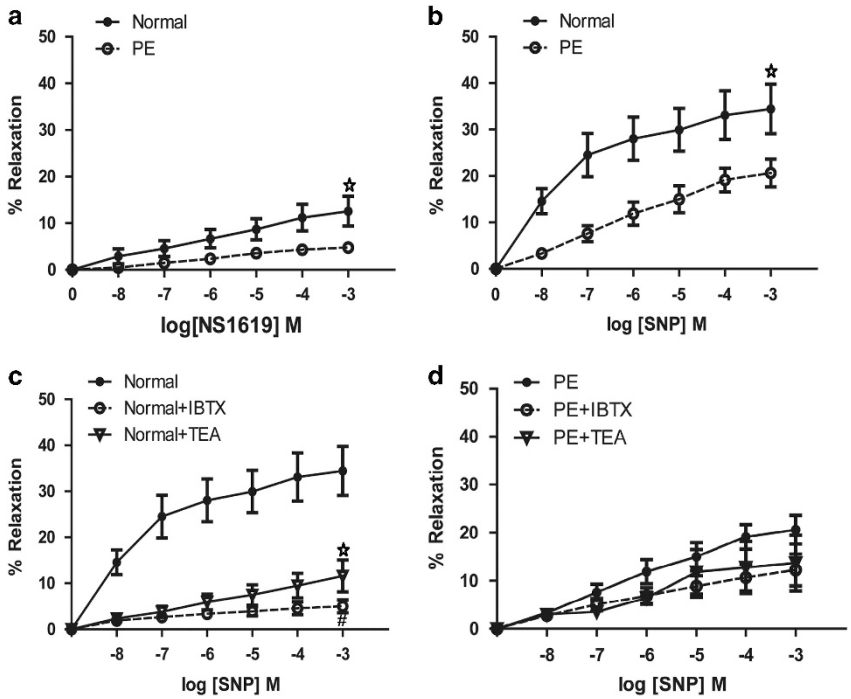

Figure 5 BKca channel-mediated vascular responses in CPAs from the preeclamptic group. (**a**) Graphic representation of the effects of cumulatively increasing concentrations of NS1619 (10^{-8}–10^{-3} M) on isolated CPAs from normal ($n=7$) and preeclamptic ($n=7$) groups. (**b**) The concentration-response curves for SNP (10^{-8}–10^{-3} M) in control ($n=7$) and preeclamptic ($n=7$) CPAs. Cumulatively increasing concentrations of SNP-induced relaxation in the absence or presence of 100 nM IBTX or 1 mM TEA in normotensive (**c**; $n=7$) and preeclamptic pregnant women (**d**; $n=7$). Relaxation data are expressed as a percentage of the maximal contractions observed at a dose of 60 mM KCl. Each point represents the mean ± s.e.m. ($^{*}P<0.05$ *vs* control group, $^{\#}P<0.05$ *vs* control group).

Various studies have demonstrated that BKca channels could be remodeled under different physiologic and pathologic conditions.[23] BKca channels are composed of α and β subunits and the β1 subunit is ubiquitously expressed in VSMCs. Previous studies proved that pregnancy induced a significant increase in BKca β1 subunit expression, leading to increased BKca activity in uterine arteries.[24] Here we demonstrated for the first time that there was a selective down-regulation of the BKca β1 subunit but not the α-subunit in human placental CPAs with preeclampsia. The decreased β1 subunit in CPAs with preeclampsia may impede BKca channel activation and induce the development of preeclampsia.

Using TUNEL staining, we demonstrated that apoptosis of human placental CPASMCs was significantly decreased in preeclamptic pregnancies relative to normal pregnancies. It is well known that K^+ channel-mediated signals play an important role in cellular death or apoptosis in many cell types.[25] For example, Bae *et al.* reported that activation of BKca channels by a BKca agonist mediates early apoptosis in human dermal fibroblasts and influences cellular proliferation.[26] In this study, we found that NS1619, a specific opener of BKca, inhibits CPASMC proliferation and promotes cellular apoptosis in a time- and dose-dependent manner. Although BKca may induce cells toward apoptosis, little is known regarding its intracellular mechanism(s) of action. Therefore, we investigated the expression of Bax and Bcl-2, which are involved in the promotion of caspase-dependent apoptosis. We demonstrated that NS1619 increases levels of the pro-apoptotic protein Bax and decreases levels of the anti-apoptotic protein Bcl-2 in human placental CPASMCs. An imbalance in the Bax/Bcl-2 ratio caused by the activation of BKca channels may then contribute to the activation of a caspase cascade and initiation of the apoptotic mechanism.[27]

As the augmented expression or activation of BKca channels is associated with increased CPASMC apoptosis, we postulated that pro-apoptosis of BKca may inhibit pathologic remodeling of placental CPAs in normal pregnancy. This remodeling process is different from spiral artery remodeling, which requires the invasion of trophoblast cells to induce endothelial and vascular smooth muscle apoptosis to reduce maternal blood flow resistance and increase utero-placental perfusion during the first half of gestation in human pregnancy.[7] Abnormal arterial SMC proliferation and resistance to apoptosis have been proposed to be essential for vascular remodeling in hypertension.[28] The pathologic remodeling of placental CPAs is the key feature of vascular plasticity in hypertension, resulting in a persistent elevation in arterial resistance. As Figure 3 illustrates, there are more SMCs in the vasculature of preeclamptic women compared to controls. The decreased BKca channels in preeclampsia may ultimately lead to pathologic remodeling of placental CPAs and impede arterial blood flow. From our results, we hypothesized that increased BKca channel expression or activation mediated the reduction in CPASMC proliferation and the induction of apoptosis, which could represent an essential mechanism in the formation of dilated, high-flow and low-resistance vessels.

Currently, data on the relationship between BKca channels and myogenic vascular tone in human preeclamptic CPAs are limited. In the present study, vasodilator reactivity of CPAs induced by NS1619 was observed in the preeclamptic and control groups. Relaxation (%) of preeclamptic CPAs due to NS1619 in the preeclamptic group was less than that of the control group, demonstrating that the role of BKca channels in the control of vasodilatation was impaired in preeclampsia.

In non-innervated placental vasculature, vascular tone is regulated by locally released endothelial vasoconstrictors and vasodilators,[29,30] and nitric oxide (NO) is one of the most relevant modulators.[31,32] NO is primarily synthesized in the endothelium and diffuses to VSMCs, causing vasorelaxation.[33] During normal pregnancy, the regulation of vascular tone is believed to result, at least in part, from increased sensitivity to NO derived from endothelial cells.[34] Here we provide evidence that the endothelium-denuded CPAs in preeclampsia were more insensitive to SNP in the presence of reduced NO bioactivity in the relaxation of preeclamptic CPAs. Researchers have reported that NO can directly interact with BKca channel proteins and lead to vasodilation.[35] Therefore, we surmised that decreased NO bioavailability was partially induced by the inactive BKca channels in preeclamptic CPAs. Our results supported this hypothesis, as the efficacy of SNP in inducing CPA relaxation was significantly attenuated by IBTX or TEA pretreatment in the control group compared to the preeclamptic group. We attribute this result to the fact that there is a smaller inhibitory effect of vasodilatation by the BKca channel blockers IBTX or TEA in the preeclamptic group. In other words, there was reduced BKca activity or expression in preeclamptic CPAs, and the sensitivity of endothelium-denuded CPAs to SNP was decreased in preeclamptic women relative to normotensive pregnant women. Our limited data set using CPAs from pregnant women provides insight into the decreased vasodilatory response and sensitivity to NO in preeclamptic pregnancies. However, this study needs to be expanded to further elucidate any apparent discrepancies.

In conclusion, our data indicated that in preeclampsia, the decreased expression or activation of BKca β1 subunits may induce the pathologic remodeling of CPAs, increase CPAs resistance, and inhibit the reactivity of CPAs to NO. These results are expected to deepen our understanding of the pathogenic mechanisms implicated in preeclampsia. The intriguing finding that BKca channels influence CPASMC apoptosis also raises compelling questions concerning BKca channel involvement in the remodeling of spiral arteries in the first trimester, which is worthy of further investigation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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