ORIGINAL RESEARCH

Magnesium Sulfate–Mediated Vascular Relaxation and Calcium Channel Activity in Placental Vessels Different From Nonplacental Vessels

Jiaqi Tang, PhD;* Axin He, MD;* Na Li, PhD;* Xueyi Chen, MD;* Xiuwen Zhou, PhD; Xiaorong Fan, MD; Yanping Liu, MD; Mengshu Zhang, MD; Linglu Qi, MD; Jianying Tao, PhD; Miao Sun, PhD; Zhice Xu, PhD

***Background*—**Magnesium sulfate ($MgSO_4$) has been used as a common therapy for preeclampsia and eclampsia for many years. $MgSO_4$ decreases peripheral vascular resistance so as to reduce maternal blood pressure. Whether placental blood vessels react to $MgSO_4$ in the same patterns as that in maternal vessels is largely unknown.

***Methods and Results*—**This study compared placental vessels (PV) versus nonplacental vessels (non-PV) in human and animal models. $MgSO_4$-caused vascular dilation was significantly weaker in PV than that in non-PV. Prostaglandin I_2 synthetase affected $MgSO_4$-mediated vasodilatation in PV, not in umbilical vessels, while cyclooxygenase did not influence $MgSO_4$-induced relaxation in both PV and non-PV. Mg^{2+}-caused vasodilatation was mainly through calcium channels. In PV, calcium channel activities were significantly weaker in PV than that in non-PV. Relative mRNA expression of *CACNA1D*, *CACNB2*, and *CACNB3* was significantly higher in PV than those in umbilical vessels, despite the fact that the expression of *CACNA1F* was less in PV. The contractile phenotype of smooth muscle cell marker (CALD1) was less and the synthetic phenotype (MYH10) was more in PV than that in UV.

***Conclusions*—**These results demonstrated that PV were characterized by much weaker responses to $MgSO_4$ compared with nonplacental vessels. The difference was related to weaker calcium channel activity and minor contractile phenotype smooth muscle cells in PV, providing important information for further understanding treatments with $MgSO_4$ in preeclampsia. **(*J Am Heart Assoc*. 2018;7:e009896. DOI: 10.1161/JAHA.118.009896.)**

Key Words: calcium channel • MgSO4 • nonplacental vessels • placental vessels • preeclampsia/pregnancy • vasodilation

Magnesium sulfate ($MgSO_4$) is the most widely used as an anticonvulsant medicine in treatments of preeclampsia-eclampsia,[1–3] because $MgSO_4$ can lower maternal blood pressure significantly.[4] It is reasonable that $MgSO_4$ acts on maternal blood vessels for direct vascular relaxation in preeclampsia. Fetal–placental circulation is not innervated and vascular tone is mainly controlled by autocrine or paracrine factors.[5] Previous studies reported that $MgSO_4$ had significant direct effects on fetal–placental circulation.[4,6–8] However, whether $MgSO_4$ produces the same or different vascular dilations in the placenta is largely unknown. Addressing such questions is important to further understanding of the mechanisms of $MgSO_4$ in treatments of preeclampsia, as well as the development of hypertension in preeclampsia.

Placental vessels (PV) stay in a high perfusion and low resistance state, consequently facilitating interchange of oxygen and nutrients. Umbilical cord vessels (UV) are regarded as part of fetal circulation and the only normal blood vessels that can be collected in healthy humans. Several studies reported that $MgSO_4$ affected PV or UV, respectively.[4,7,9] However, no comparisons for $MgSO_4$-mediated vascular dilation were made between PV and non-PV. The present study determined whether or not $MgSO_4$-induced vascular dilation was the same between the placenta and other organs. Further, it is very important to understand precise pharmacological effects of $MgSO_4$ on different vascular systems and precise physiology of placental blood vessels. The present study focused on such

From the Institute of Fetology, First Hospital of Soochow University, Suzhou, China (J. Tang, A.H., N.L., X.C., X.Z., X.F., Y.L., M.Z., L.Q., J. Tao, M.S., Z.X.); Center for Perinatal Biology, Loma Linda University, Loma Linda, CA (Z.X.); Obstetrics and Gynecology Department, Suzhou Municipal Hospital, Suzhou, China (J. Tao).

*Dr Tang, Dr He, Dr Li, and Dr Chen contributed equally to this work.

Correspondence to: Zhice Xu, PhD, First Hospital of Soochow University, Renmin Rd 708, Suzhou 215000, China. E-mail: xuzhice@suda.edu.cn

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Clinical Perspective

What Is New?

- An important finding is the demonstration that $MgSO_4$-mediated vascular dilatations in placental vessels were different from nonplacental vessels in both humans and animals.
- Calcium channel activity played a significantly different role in regulating vascular tone between placental and nonplacental vessels.

What Are the Clinical Implications?

- A new view is offered on placental vascular pharmacology regarding clinical use of $MgSO_4$.
- Important information is provided for further understanding placental vascular physiology and pathophysiology in normal and preeclampsia pregnancy.

comparisons between PV and non-PV in humans, as well as in animal models.

In the study of mechanisms underlying $MgSO_4$-mediated vasodilatation, multiple factors had been considered. Magnesium (Mg^{2+}) was suggested to stimulate prostacyclin I_2 (PGI_2) synthesis in vascular cells, then directly regulating the vascular tone.[10–12] PGI_2 is regarded as a vasodilator and plays a role in Mg^{2+}-induced relaxation. Its synthesis is regulated by PGI_2 synthase (PGIS) and cyclooxygenase.[11] Meanwhile, Mg^{2+} is an important cation that can influence the vascular tone.[13,14] Pharmacologically, Mg^{2+} is a calcium antagonist and mediates vascular relaxation via regulating calcium influx.[14,15] Some research reported that Mg^{2+} did not affect the release of intracellular stored calcium.[16] Thus, this study tested extracellular Ca^{2+} influx in vascular smooth muscle cells. Extracellular Ca^{2+} influx was mainly via voltage-gated Ca^{2+} channels, especially L-type calcium channels, which played a major role in regulating intracellular calcium.[17,18] Although the present study also determined vascular PGIS, cyclooxygenase, and Ca^{2+} channels, our testing focused on comparisons between placental and nonplacental vessels, which was a new experimental design not shown before.

In human placental vessels, there are 2 phenotypes of smooth muscle cells: synthetic phenotype (ie, proliferative phenotype) and contractile phenotype.[19] The proportion of the 2 phenotypes would determine the vascular tension in the placenta. In study of the effects of $MgSO_4$ on placental and nonplacental vessels, the present study also investigated the expression of the 2 phenotypes in human PV and non-PV.

In addition, the vascular effects of $MgSO_4$ were tested and compared between preeclampsia and control pregnancy. $MgSO_4$-induced relaxation was also compared between preeclampsia PV and UV. The information gained would provide new insight into $MgSO_4$ treatment in preeclampsia.

Materials and Methods

The authors declare that all supporting data are available within the article.

Ethics Committee Approval

All procedures were approved by the Institute's Ethics Committee. Informed consent was signed. All experimental procedures were approved by the Institutional Animal Care Committee and were in accordance with the *Guide for the Care and Use of Laboratory Animals*.

Human and Sheep Samples

Umbilical cords and placenta of normal pregnancy as the control (N=35) and preeclampsia (N=12) were obtained from the local hospitals, Suzhou, China. Following natural labor or cesarean delivery, umbilical cords and placenta were collected and kept at 4°C in HEPES-PSS solution (mmol/L: NaCl 141.85, KCl 4.7, $MgSO_4$ 1.7, EDTA 0.51, $CaCl_2{\cdot}2H_2O$ 2.79, KH_2PO_4 1.17, glucose 5.0, and HEPES 10.0, pH 7.4). Human UV and PV were gently isolated from connective tissue.

Pregnant sheep at gestational days 130 to 135 (term: 145±5 days, N=8) were euthanatized and underwent a cesarean operation. PV, UV, and fetal mesenteric arteries were collected.

Measurement of Vascular Tension

Human UVs and sheep UVs were cut into rings ≈3 to 5 mm in length and mounted in an organ bath containing Krebs solutions (mmol/L): 119 NaCl, 4.7 KCl, 25 $NaHCO_3$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 1.0 $MgSO_4$, and 11 D-glucose, gassed with 95% O_2 and 5% CO_2. Human PVs, sheep PVs, and sheep mesenteric arteries were mounted in a M4 myograph system (Radnoti Glass Technology, Inc., USA) filled with HEPES-PSS solution gassed with 95% O_2, 5% CO_2 at 37°C.

Isometric tension was continuously recorded. Vascular rings were allowed to equilibrate for 60 minutes, and potassium chloride (KCl, 0.12 mol/L) was added to achieve optimal resting tension and was considered as a reference. A contraction platform was formed by thromboxane A_2-mimetic U46619 (10^{-6} mol/L), KCl (0.12 mol/L) or 5-hydroxytryptamine (5-HT, 10^{-4} mol/L), and application of dose-dependent $MgSO_4$ (0.01–0.05 mol/L) induced relaxation. Cyclooxygenase inhibitor (indomethacin, indo, 10^{-6} mol/L) and PGIS inhibitor (tranylcypromine, 10^{-6} mol/L) were incubated for 30 minutes followed by application of $MgSO_4$. L-type calcium channel inhibitor (nifedipine, 10^{-6} mol/L) was added when the

contraction platform was achieved. Cumulative concentrations of Bay K8644 (L-type calcium channels agonist) were applied to test L-type calcium channel functions. All drugs were prepared freshly and purchased from Sigma-Aldrich.

Electrophysiological Measurement in Vascular Smooth Muscle Cells of PV and UV

Isolation of smooth muscle cells: intermediate villus vessels (PV in normal pregnancy) and UV were maintained in oxygenated ice-cold PSS (mmol/L: NaCl, 120.9; $NaHCO_3$, 25.0; KCl, 4.6; NaH_2PO_4, 1.2; Na_2HPO_4 1.2, $MgCl_2$, 1.2; $CaCl_2{\cdot}2H_2O$, 2.8; and glucose, 5.0; pH, 7.4), then cut into 1 mm pieces. Vascular smooth muscle cells (VSMC) were isolated enzymatically as described.[20] Single cells were obtained by gentle trituration and stored at 4°C, and used within 6 hours.

Whole-cell calcium channel current: the suspension of VSMC was added into the bath tank of the microscope (Leica, Germany). VSMC adhered to the wall, and those with slender morphology and good refractivity were chosen. $BaCl_2$ 20 mmol/L was used as a charge carrier to limit current rundown. Ba^{2+} current was elicited by 250 ms voltage steps from a holding potential of −60 mV to test potentials in the range from −60 to +70 mV with 10-mV increments. Whole-cell Ca^{2+} channel currents were recorded in conventional whole-cell configuration, voltage-clamp mode using an Axon Multiclamp 700B with Clampex10.1 and normalized to cell capacitance as picoampere per picofarad (pA/pF). Voltage-dependent Ca^{2+} channel current densities were assessed using standard pulse protocols and a patch-clamp station.

Activation curve: For activation of the inward current, Ba^{2+} current was elicited by 250 ms voltage steps from a holding potential of −60 mV to test potentials in the range from −60 to +70 mV with 10-mV increments. Activation data were fit to the Boltzmann distribution. Data were collected after the whole-cell configuration was obtained and current amplitude stabilized. Only cells with an input resistance >2 GΩ without substantial run-down were analyzed.

Quantitative Real-Time Polymerase Chain Reaction and Western Blot Analysis

Total RNA was isolated from vessel tissue using RNA plus (RNA extraction reagent, TaKaRa). RNA was reverse transcribed using the Revert Aid First-Strand cDNA Synthesis Kit (TaKaRa). Q-polymerase chain reaction was performed in 20 μL of the reaction mixture with SYBR Premix Ex Taq (TaKaRa). Before real-time polymerase chain reaction, Nanodrop and the agarose gel electrophoresis were used to detect RNA purity and integrity. Only the samples with A260/A280 ratio ranged from 1.8 to 2.0 and 3 clear bands of the RNA (28s/18s/5s) that appeared were used for further studies. The reaction was analyzed on iCycler Real-Time Polymerase Chain Reaction PCR Detection System (Bio-Rad) with the following protocol: 95°C for 5 minutes and 45 cycles of 95°C 5 s/62°C 15 s/72°C 15 s. The analysis was repeated 3 times for each sample and expression of mRNA was normalized by each *ACTIN* or *GAPDH* and used the $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in Table. The protein abundance of CALD1 and MYH10 were assessed by Western blotting normalized to β-ACTIN with Tanon (Tanon Science & Technology Co, Ltd). Antibodies against CALD1 (Cat. No. 19673-1-AP), MYH10 (Cat. No. 20887-1-AP), and β-ACTIN (Cat. No. D190606-0100) were from Proteintech Group and BBI life sciences, respectively.

Statistical Analysis

Concentration–response curves were analyzed by computer-assisted nonlinear regression (GraphPad Prism software) to fit the data. Data were presented as mean±SEM. Two-way repeated-measures ANOVA analysis followed by Bonferroni post-hoc test or *t* test, when appropriate, was used to determine statistical significance among groups (*P<0.05; **P<0.01; ***P<0.001). N presented the number of pregnancies.

Table. Primer Sequences Information

Gene	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
CACNA1C	CCGTTGGCAAGTTCTACGCC	GCCCGATGTCATGCAGTGTG
CACNA1D	CATCATTCTTCCCACAAGCAT	GGGAGCTGTTCATCTCCTGAGT
CACNA1F	GTGTGCCATCCTGAAGAGCC	CACATTGCTCTGGACATTCATCA
CACNB1	CAATTGGTTCTCATCCAGGATG	GGATGCTGACACCATCAATCA
CACNB2	ATGCGACCAGTGGTCCTAGT	TTGGCAAGCGAGATGTCA
CACNB3	CTCTTCGACTTCCTCAAACACAGA	AGCGTCCAACACTACTAGCTGC
CALD1	GAGCAGAAGCTGCTGAGAAAC	ACTGGTATACTGCTCCAGTCTGCT
MYH10	ATCCGACTGCTCAGGTTTAGTTTC	AGCTCCATGCCAAGGTCTC
ACTIN	CATGGAGTCCTGTGGCATCCA	CAGGAGGAGCAATGATCTTG
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

Results

$MgSO_4$-Induced Vasodilatation in PV and UV From Normal Pregnant Women

Normal pregnant PV and UV were dilated by dose-dependent $MgSO_4$ based on the precontracted platform by U46619 (Figure 1A). At the same concentration, $MgSO_4$-mediated vascular relaxation in PV was significantly weaker than that in UV. U46619 produced weaker constriction platforms in PV when compared with that in UV (Figure 1C). KCl was also used to maintain the constriction platform and the constriction was stronger in PV than that in UV (Figure 1D). Similar weaker vascular relaxation by $MgSO_4$ was observed in PV (Figure 1B). These meant that no matter whether the platforms were caused by U46619 or KCl, $MgSO_4$ mediated significantly weaker vasodilatation responses in normal PV than that in UV.

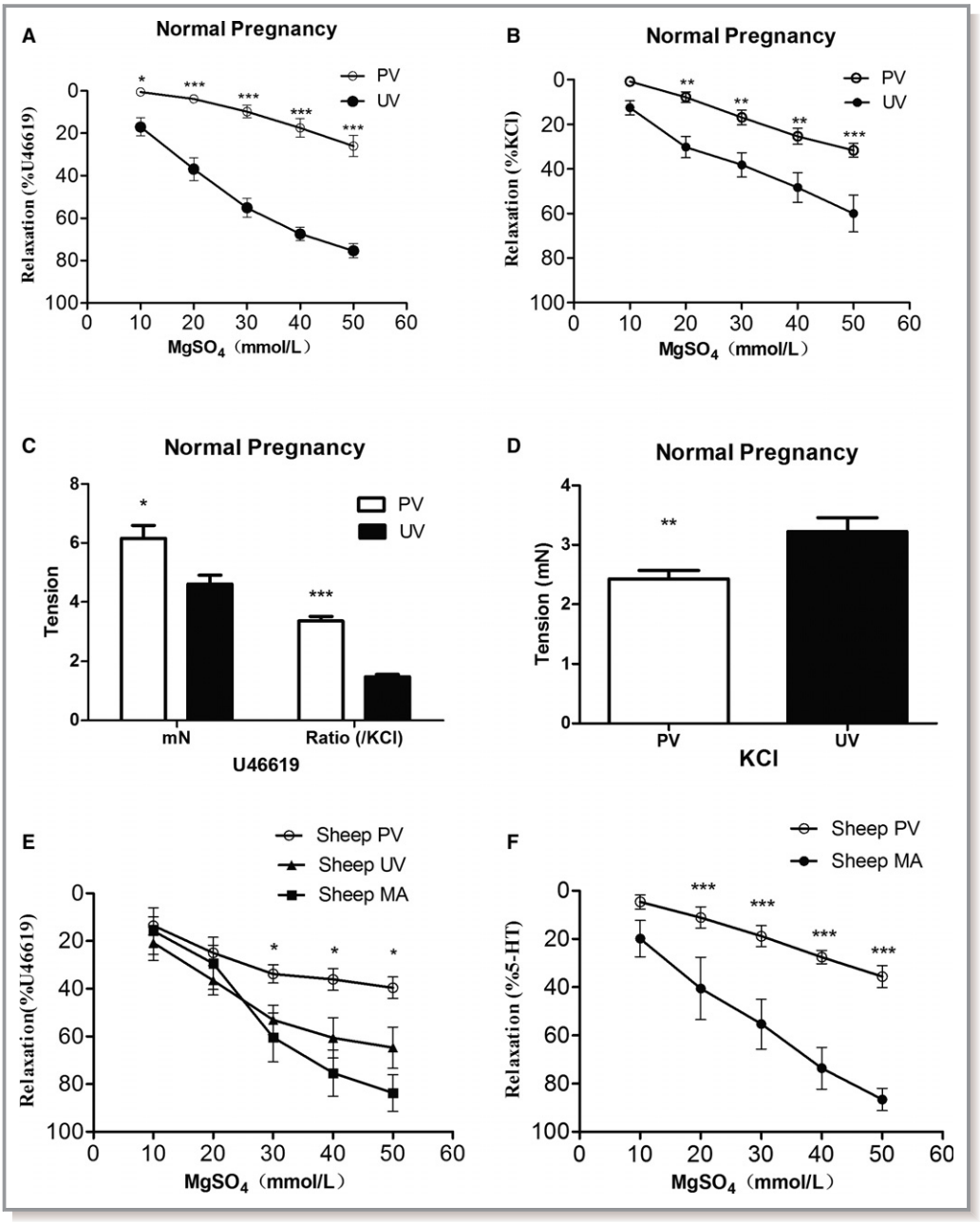


Figure 1. $MgSO_4$-induced dilation in normal PV and non-PV of humans and sheep. A and B, $MgSO_4$ induced vasodilatation following U46619 (10^{-6} mol/L) or KCl (120 mmol/L) was weaker in PV (placental vessel) than that in UV (umbilical vessels), N=14 control pregnant women. C and D, Vascular tension platform caused by U46619 and KCl in normal PV and UV. E and F, $MgSO_4$-mediated dilation in sheep PV and non-PV (UV and MA, mesenteric artery). N=8 pregnant sheep. *P<0.05; **P<0.01; ***P<0.001.

$MgSO_4$-Induced Dilation in Sheep Placental Vessels and Nonplacental Vessels

Sheep PV and non-PV (including UV and fetal mesenteric arteries) were compared. In contrast to sheep non-PV, $MgSO_4$ induced weaker concentration-dependent relaxation following U46619 or 5-HT in sheep PV (Figure 1E and 1F). No reliable platform could be achieved by 5-HT in sheep UV, so only fetal mesenteric arteries were tested to compare with sheep PV. Similar to normal human sample results, $MgSO_4$-mediated vasorelaxation in sheep PV was significantly weaker than that in sheep non-PV.

$MgSO_4$-Mediated Vasodilatation in PV and UV From Preeclampsia Pregnancy

$MgSO_4$-mediated relaxation was also detected in PV and UV from preeclampsia pregnant women. Similar to the control pregnant women and sheep, the vasodilatation in PV from preeclampsia was weaker than that in UV (Figure 2A). Vascular relaxation induced by $MgSO_4$ in PV and non-PV was summarized in Figure 2B. When setting 60% as a reference level for vascular relaxation degree from the maximum vascular tension induced by U46619, $MgSO_4$-caused relaxation of $\geq$60% was 3.85% in PV and 82.69% in non-PV vessels. These data further proved that the relaxation induced by $MgSO_4$ was significantly weaker in PV than that in non-PV. Relaxation responses were compared between the control and preeclampsia pregnancies (Figure 2C and 2D). There were no significant differences between the control and preeclampsia pregnancy in UV or in PV.

Effects of Indo and Tranylcypromine on Vascular Tension of UV and PV From Normal Pregnancy

Vascular rings were incubated with indo (indomethacin, cyclooxygenase inhibitor) or tranylcypromine (PGIS inhibitor) for 30 minutes. Then, accumulated $MgSO_4$ was added following U46619. Indo did not affect the $MgSO_4$-mediated dilation in PV, nor in UV (Figure 3A). However, tranylcypromine diminished the vasodilator responses by $MgSO_4$ in PV, but not in UV (Figure 3B), indicating that PGIS played a critical role in regulating vascular tone of PV.

Calcium Channels in UV and PV

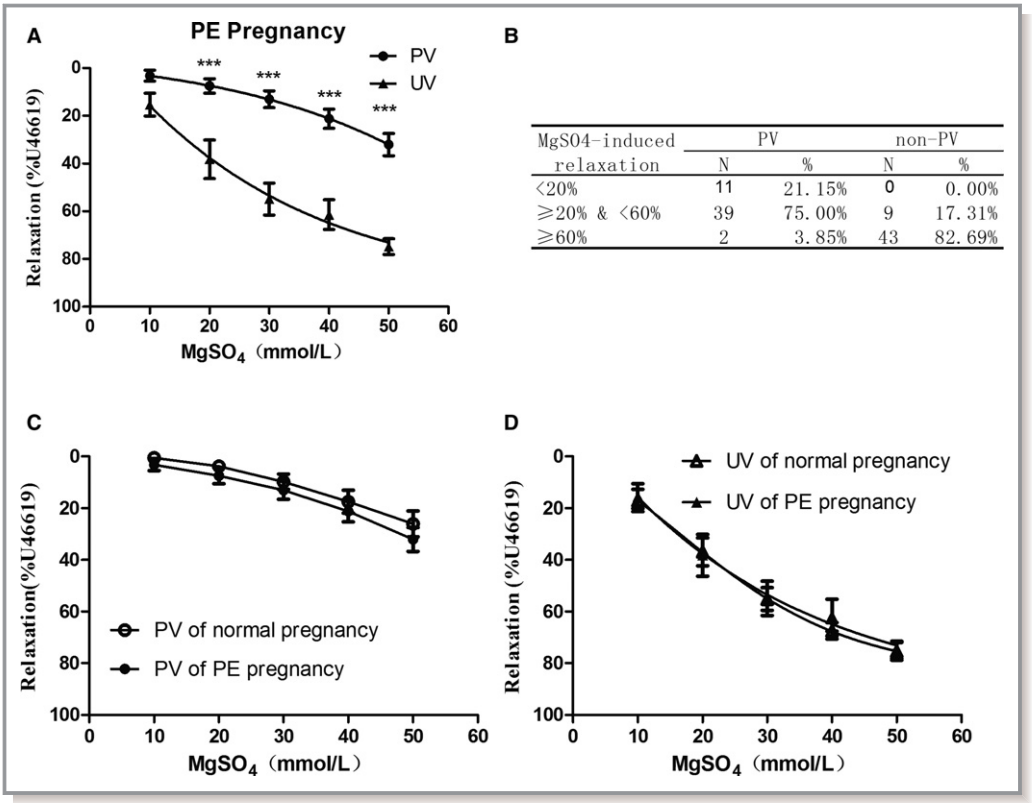
Nifedipine (an L-type calcium channel inhibitor) induced dilatory responses following U46619. The dilatory responses were remarkably weaker in normal PV than that in UV (Figure 3C). In preeclampsia pregnancy, nifedipine-caused relaxation was also weaker in PV than that in UV (Figure 3D). In normal pregnancy, Bay K8644, an L-type calcium channel agonist, mediated weaker vascular contraction in PV than that in UV (Figure 3E). Figure 3F shows that in PV of normal pregnancy, Bay K8644-caused contractile responses occurred only in 3 of 25 tests. However, Bay K8644-mediated potent constrictions were observed in most of normal UV (20 in 22 tests), which further suggested that the $MgSO_4$-mediated weaker relaxation in normal PV was associated with calcium channel functions.

Whole Cell-Ca^{2+} Channel Currents in Smooth Muscle Cells of PV and UV From Normal Pregnancy

Whole-cell calcium channel currents were recorded using patch clamp. The real-time records of calcium channel currents are shown in Figure 4A. The amplitude of calcium channel currents in PV was significantly less than that in UV (Figure 4B). The comparison in LogEC50 and slope of the activation curves between normal PV and UV are shown in Figure 4C. The activation curve shifted to the left significantly in PV (Figure 4D). At the VSMC level, calcium channels showed weaker activities in PV than in UV. Figure 4 shows calcium activities at baseline level between placental and nonplacental VSMCs, and there were significant differences in calcium currents measured at cell levels between PV and UV, showing that the differences of $MgSO_4$-induced vascular dilation were associated with the diversities of calcium channel activities. Notably, an association relationship may or may not be a cause–result relationship between the differences in vascular tone in microvessel testing and calcium currents in cellular observation.

Relative mRNA Expression and Protein Expression

The mRNA expression of *CACNA1C*, *CACNA1D*, *CACNA1F*, *CACNB1*, *CACNB2*, *CACNB3*, *CALD1*, and *MYH10* was detected in normal PV and UV (Figure 5A through 5C). *ACTIN* and *GAPDH* were used as references, respectively. *CACNA1D*, *CACNB2*, *CACNB3*, and *MYH10* were significantly higher in PV than that in UV. *CACNA1F* mRNA expression was significantly less in normal PV than that in UV (Figure 5A). Ratio of *CALD1* to *MYH10* in mRNA expression was remarkably lower in normal PV than that in UV. The protein expression of CALD1 was significantly less in PV than that in UV (Figure 5D). MYH10 protein expression in PV was more abundant than that in UV (Figure 5D). The ratio of CALD1 to MYH10 in protein expression was also notably lower in PV than that in UV. In spite of the complicated mRNA expression of L-type calcium channels, the results indicated that the contractile phenotype of smooth muscle cells was minor and only played a weaker role in the regulation of PV tone.



MgSO4-induced relaxation	PV		non-PV	
	N	%	N	%
<20%	11	21.15%	0	0.00%
≥20% & <60%	39	75.00%	9	17.31%
≥60%	2	3.85%	43	82.69%

Figure 2. $MgSO_4$ induced vasodilatation in preeclampsia. A, $MgSO_4$-mediated relaxation in PV and UV in preeclampsia (PE). B, Summary of $MgSO_4$-mediated vasodilation and the vessel counts used. C and D, Comparison between normal and PE pregnancy. N=12, each group. PV indicates placental vessel; UV, umbilical vessels. ***P<0.001.

Discussion

Although $MgSO_4$ is a classic drug for treatment of pregnancy hypertension in practice for many years because of its vasorelaxation effects, this is the first time it has been demonstrated that the effect of $MgSO_4$-mediated vasodilatory responses was much weaker in PV than that in non-PV. This is an important finding for further understanding of the mechanisms of $MgSO_4$ in treatment of pregnancy hypertension. It is very interesting that the same dose of $MgSO_4$-mediated vasodilatory responses could be quite different in the placenta versus other organs in the body. This means when $MgSO_4$ is used for reducing maternal high blood pressure, vasorelaxation effects by the drug are relatively weaker in the placenta, which may benefit the maintenance of placental vascular tension for a sufficient local perfusion and circulation. Notably, a number of studies reported that preeclampsia is accompanied by poor placental perfusion and ischemia.[21,22]

In fact, the finding mentioned above demonstrates that vascular physiology and pharmacology are not the same for placental versus nonplacental blood vessels. Although a relatively large sample size was considered for the human tissue analysis, the present study also confirmed the new results in animal models. This was the first study to compare effects of $MgSO_4$ on PV and non-PV using various species. Since human tissue sample analysis could be affected by huge individual variations, including genetic, dieting, body size, nutrition, and many other factors, using relatively pure species of animals could be helpful to avoid those variations in confirming important research findings in human studies.

In human PV, $MgSO_4$ induced about 40% relaxation based on U46619-induced vasoconstrictions (100 μmol/L).[6] In human umbilical arteries, $MgSO_4$ appeared to exert a stronger vascular relaxation.[7] However, those 2 independent studies have not been looked at together before for comparing the effects of $MgSO_4$ in human PV versus umbilical blood vessels. In the present study, $MgSO_4$-caused vasodilatation levels <20% of the U46619-generated maximum vascular tension was 21.2% in PV, and 0% in UV; ≥20% and <60% was 75.0% in PV, and 17.3% in UV; ≥60% was only 3.9% in PV, and 82.7% in UV. Obviously, $MgSO_4$-induced vasodilation was significantly weaker in PV than in UV in humans. One question that was raised is: was the difference that was observed related to the preconstriction induced by the vascular agonist? Thus, we also used KCl to constrict vessels. The results showed that $MgSO_4$-mediated vasodilation also was significantly weaker in

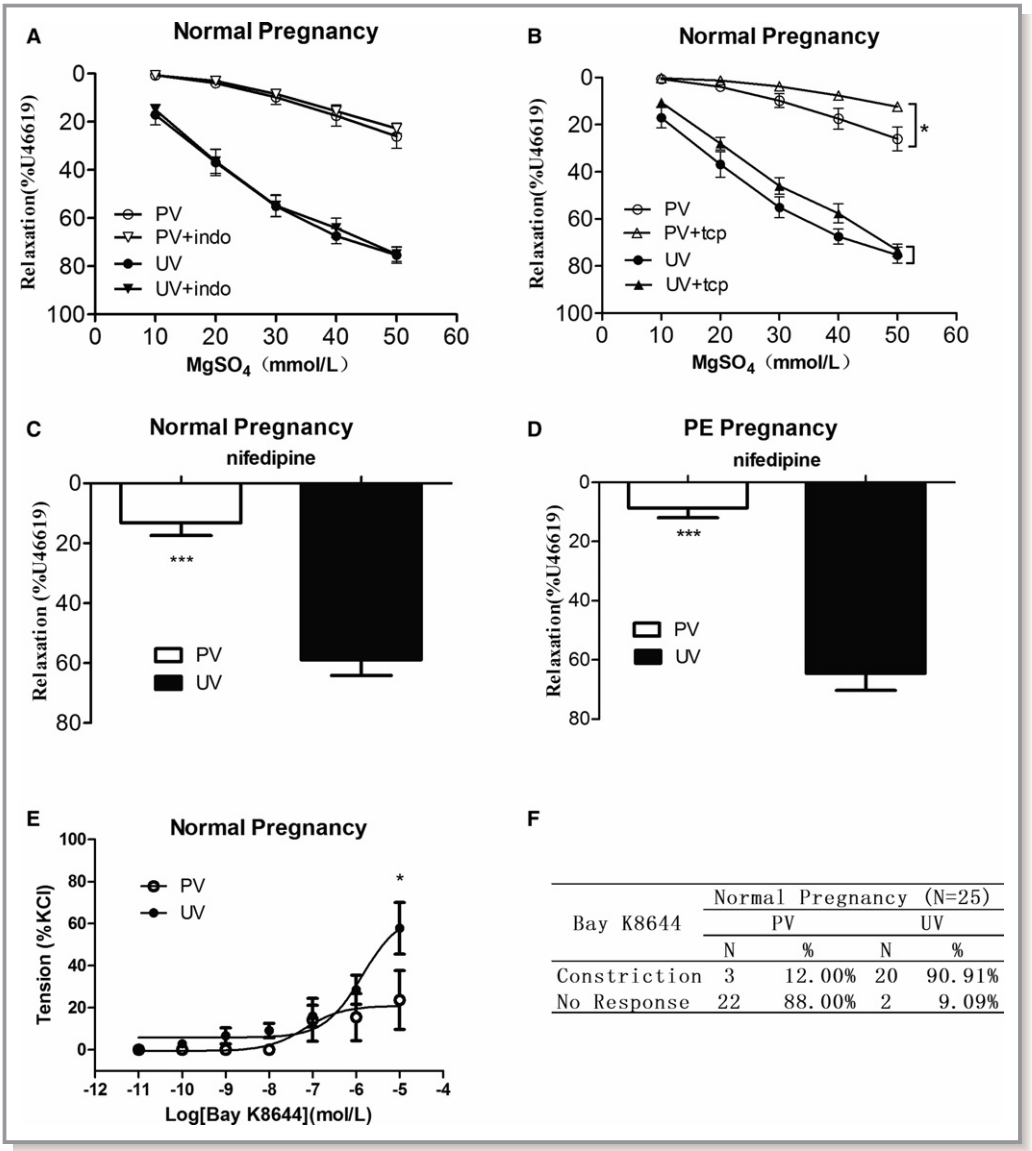


Figure 3. Cyclooxygenase, PGIS, and calcium channels in regulating human UV and PV tone. A and B, $MgSO_4$ induced vasodilatation in the presence or absence of indo (indomethicin, cyclooxygenase inhibitor) or tcp (tranylcypromine, the PGI_2 synthase inhibitor) in normal PV and UV, respectively. C and D, Nifedipine-mediated dilation on U46619-increased platform in PV and UV from the control or PE. E, Bay K8644, a voltage-dependent calcium channel agonist, caused constrictions in normal PV and UV. F, The count of vessel ring and the constriction responses. N=13 to 25, each group. PE indicates preeclampsia; PGI_2, prostacyclin I_2; PGIS, PGI_2 synthase; PV, placental vessels; UV, umbilical cord vessels. **P*<0.05; ***P*<0.01.

human PV following KCl, indicating that the weaker dilation by $MgSO_4$ in the placenta was independent of preconstrictions stimulated by various agonists. In other words, $MgSO_4$-mediated weaker vascular dilation effects are not specific to certain agonist-produced vasoconstrictions.

Because $MgSO_4$ is a common therapy for preeclampsia–eclampsia in clinical practice, it is rational to compare the drug effects between the normal and preeclampsia placenta. No significant differences were found in $MgSO_4$-induced relaxation in either PV or UV between the control and preeclampsia women in our experiments. This suggested that vascular functions in response to $MgSO_4$ in the preeclampsia placenta were similar to that of the control, proving the evidence that the drug only could relieve the hypertension symptoms in the patients by reducing increased blood pressure, while being unable to prevent the fundamental causes of preeclampsia . We noted that a previous study reported that when exposing to $MgSO_4$, the perfusion pressure was decreased in the preeclampsia but not in the control pregnancy.[8] The difference between their results and ours could be caused by different experimental subjects: placental organ perfusion versus isolated vessel tension. The

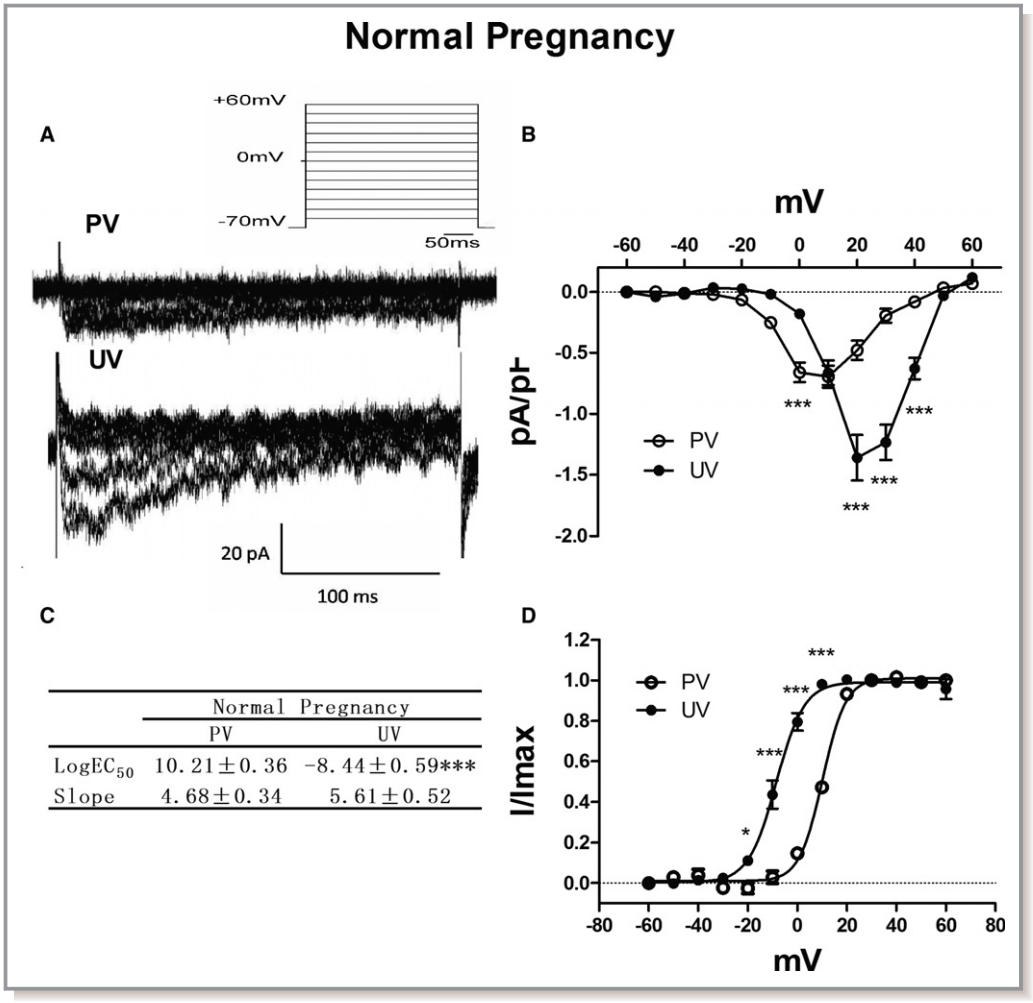


Figure 4. Whole-cell calcium-channel currents in VSMC of normal PV and UV. A, Real-time recording of whole-cell calcium currents in smooth muscle cells (VSMC). B, Whole-cell calcium-current amplitude at depolarizing voltage steps. C, Comparison in LogEC50 and slope of the activation curves in the 2 groups. D, Activation curves of calcium channels in the 2 different VSMCs. N=15 to 20, each group. PV indicates placental vessels; UV, umbilical cord vessels; VSMC, vascular smooth muscle cells. **P*<0.05; ***P*<0.01.

presence or absence of connective tissue surrounding placental vessels was a critical difference in the 2 settings. Combining both results, analysis suggests that $MgSO_4$ also may have indirect special effects via the tissue outside PV, decreasing placental perfusion in preeclampsia as has been reported.[8] However, there is a critical issue that cannot be explained. The organ perfusion results indicated that placental perfusion was reduced by $MgSO_4$, meaning a reduced vascular tension. However, the isolated vessel data suggested that the vascular effects of $MgSO_4$ were relatively weaker in the placenta, meaning that placental vascular tension was less affected by $MgSO_4$. This kind of discordancy should be very interesting for further investigations, because it is very important to know the effects of $MgSO_4$ on the maintenance or improving of placental perfusion via "decreasing" or "protecting" placental vascular tension. Addressing such questions not only benefits further understanding of the mechanisms of $MgSO_4$ in treatments of preeclampsia, but also may contribute to elucidating the precise pathophysiology in the placenta in the development of preeclampsia.

To determine the mechanisms of $MgSO_4$-mediated vasodilatation, we tested cyclooxygenase, PGIS, and calcium channels. Previous report showed that the inhibitor of cyclooxygenase, indo, did not have effects on the $MgSO_4$-mediated vasodilatation in human PV.[23] The present study also demonstrated that indo did not affect $MgSO_4$-induced relaxation in PV, indicating that the cyclooxygenase signaling pathway did not influence the vascular regulations by $MgSO_4$ in human PV and UV. The inhibitor of PGIS could change $MgSO_4$-mediated vasodilatation in PV, but not in UV, suggesting that PGIS might play a role in regulating the vasodilatation in PV. Substantial evidence showed that

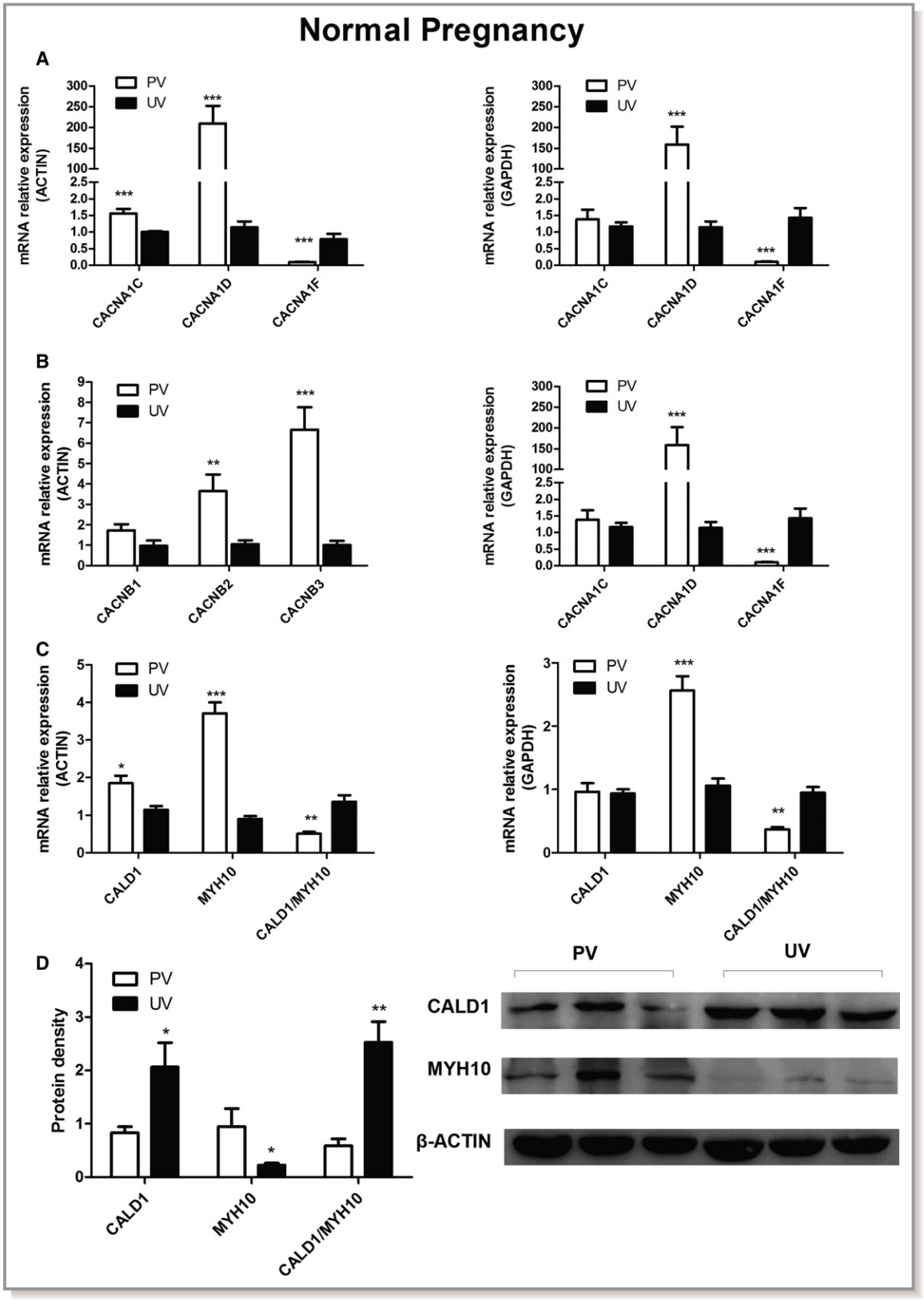


Figure 5. Relative mRNA and protein expression in normal PV and UV. *ACTIN* and *GAPDH* were used as references in the comparison between normal PV and UV. A and B, The mRNA expression of *CACNA1C*, *CACNA1D*, *CACNA1F*, *CACNB1*, *CACNB2*, and *CACNB3* in PV and UV. C and D, The mRNA and protein expression of CALD1 and MYH10 and the ratio of CALD1/MYH10. N=16, each group. PV indicates placental vessels; UV, umbilical cord vessels. **P*<0.05; ***P*<0.01; ****P*<0.001.

increased PGIS activity could generate more PGI_2, which might induce vasodilatation.[24] The PGIS blocker partially inhibited $MgSO_4$-mediated vasodilation, which suggested that vascular regulations might be partially influenced by the PGI_2 pathway. This is worth further investigation. It is well known that PGI_2 also could influence vasodilatation via regulations of intracellular calcium.[25,26] The present study determined calcium channel activities in smooth muscle cells from human PV and UV.

Previous studies have proved that $MgSO_4$ induced vasodilatory responses through inhibiting calcium influx.[14] In the present study, a new finding was that the calcium channel blocker, nifedipine-mediated vasodilatation, in human PV was significantly weaker than that in human UV, indicating that $MgSO_4$-mediated weaker dilation in PV was related to calcium channels in the vessels. Subsequent experiments certified Bay K8644, a voltage-dependent calcium channel agonist, to be able to cause weaker vasoconstriction in PV compared with UV in normal pregnancy. These novel findings from comparisons between PV and UV prompted ideas to test the ion channels in VSMC between PV and UV. The amplitude of voltage-dependent calcium channel currents was significantly lower in the VSMC from PV than that from UV in normal pregnancy. To the best of our knowledge, this was the first study to compare calcium channel currents between PV and non-PV in humans. Notably, only 12% of placental vessels presented weak vasoconstrictions by Bay K8644, compared with 91% in non-PV. In other words, 88% of PV showed no constriction responses to the calcium channel stimulator, compared with only 9% in non-PV. Taken together, the findings from vascular tissue to cellular studies clarified that activities of voltage-dependent calcium channels were weaker in human PV, which could be a major contribution to the weaker vasodilatation by $MgSO_4$ in PV.

The present study also analyzed molecular expression of the calcium channel subunits and other genes related to vascular functions. Calcium channels consist of $\alpha 1$, β, and other subunits. *CACNA1C*, *CACNA1D*, and *CACNA1F* are the major $\alpha 1$ subunits in the vasculature, which mediate the influx of calcium ions into the cell upon membrane polarization.[27] *CACNB1*, *CACNB2*, and *CACNB3* are β subunits, regulating activities of calcium channels, voltage-dependent activation and inactivation. The mRNA expression of *CACNA1D*, *CACNB2*, and *CACNB3* were significantly higher in PV than UV in normal pregnancy, while the expression of *CACNA1F* was less in PV, showing that some of the calcium channel units were expressed more in PV while *CACNA1F* was less compared with non-PV. Generally, lower levels of functional calcium channel units such as *CACNA1F* should be correlative to the weaker vasodilatation effects by $MgSO_4$. However, why *CACNA1D*, *CACNB2*, and *CACNB3* were higher in PV, and whether their higher expression contributed to the $MgSO_4$-mediated vascular responses in PV required further investigations. To validate the experimental data, we not only used *ACTIN* as the control reference but also used *GAPDH* in confirming the mRNA data.

There are 2 phenotypes of smooth muscle cells, synthetic phenotype and contractile phenotype in human placenta.[19,28] *MYH10* is suggested to be the marker of synthetic phenotype and *CALD1*, as an indicator of contractile phenotype.[29,30] In PV of normal pregnancy, the mRNA and protein expression of MYH10 was higher. The protein expression of CALD1 was significantly less, suggesting that the contractile phenotype was an inferior proportion, indicating that there was $MgSO_4$-mediated weaker vasodilatation in PV.

Finally, what is the significance of the characterized effects of $MgSO_4$ in the placenta being different from that in other organs, and is there any clinical significance? The answer is interesting and important. When using $MgSO_4$ to treat hypertension in pregnancy, it was previously assumed and currently accepted that the pathological basis in vascular systems, including placental circulation, was vasospasm or too much vascular tension that led to placental ischemia. Thus, $MgSO_4$-mediated dilation can relieve the symptom. However, PV from both the normal and preeclampsia vessels behaved very differently with certain resistance in response to $MgSO_4$-mediated dilation if compared with non-PV. This suggested that $MgSO_4$ could significantly reduce nonplacental vascular tension to lower maternal blood pressure, while it also reserved placental vascular tension and perfusion via its weaker responses on the placenta. If such a new theory would be further confirmed in future studies, the findings from this study would contribute significantly to further understanding the mechanisms of the development of hypertension as well as treatments of hypertension in pregnancy with $MgSO_4$. Limitations of this study included that more information is still needed about the calcium activity–related deep mechanisms underlying the differences in vascular relaxation caused by $MgSO_4$ between placental and non-PV. It should be valuable to consider additional and following measurements for the effects of $MgSO_4$ on the calcium-channel currents and the expression of calcium-channel subunits in the PV or UV. Such additional approaches will be very helpful to prove that the observed vascular differences were because of the differences of the calcium activities.

Conclusion

This was the first study to demonstrate that PV respond to $MgSO_4$ with a relatively weaker pattern, quite different from that of non-PV, in both human and animal models. Calcium channels and PGIS pathway might be involved in the characterized effects of $MgSO_4$ in PV. Lower calcium-channel activities were associated with weaker $MgSO_4$ vasodilatation in PV and were because of the minor contractile phenotype

smooth muscle cells. Taken together, those results could contribute to further understanding the pathophysiology of preeclampsia and the pharmacology function of $MgSO_4$.

Author Contributions

Tang wrote the article. Xu and Sun revised the article. Tao and Liu prepared the samples. He and Chen did vascular experiments and prepared Figures 1 through 3. Li, Fan, and Qi did electrophysiological experiments and prepared Figure 4. Zhou and Zhang detected mRNA and protein expression and prepared Figure 5. All authors reviewed the article.

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Disclosures

None.

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