ORIGINAL RESEARCH

Prenatal Hypoxia Induced Dysfunction in Cerebral Arteries of Offspring Rats

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***Background*—**Hypoxia during pregnancy could cause abnormal development and lead to increased risks of vascular diseases in adults. This study determined angiotensin II (AII)-mediated vascular dysfunction in offspring middle cerebral arteries (MCA).

***Methods and Results*—**Pregnant rats were subjected to hypoxia. Vascular tension in offspring MCA by AII with or without inhibitors, calcium channel activities, and endoplasmic reticulum calcium stores were tested. Whole-cell patch clamping was used to investigate voltage-dependent calcium channel currents. mRNA expression was tested using quantitative real-time polymerase chain reaction. AII-mediated MCA constriction was greater in male offspring exposed to prenatal hypoxia. AT1 and AT2 receptors were involved in the altered AII-mediated vasoconstriction. Prenatal hypoxia increased baseline activities of L-type calcium channel currents in MCA smooth muscle cells. However, calcium currents stimulated by AII were not significantly changed, whereas nifedipine inhibited AII-mediated vasoconstrictions in the MCA. Activities of IP_3/ryanodine receptor–operated calcium channels, endoplasmic reticulum calcium stores, and sarcoendoplasmic reticulum membrane Ca^{2+}-ATPase were increased. Prenatal hypoxia also caused dysfunction of vasodilatation via the endothelium NO synthase. The mRNA expressions of AT1A, AT1B, AT2R, Cav1.2α1C, Cav3.2α1H, and ryanodine receptor RyR2 were increased in the prenatal-hypoxia group.

***Conclusions*—**Hypoxia in pregnancy could induce dysfunction in both contraction and dilation in the offspring MCA. AII-increased constriction in the prenatal-hypoxia group was not mainly dependent on the L-type and T-type calcium channels; it might predominantly rely on the AII receptors, IP_3/ryanodine receptors, and the endoplasmic reticulum calcium store as well as calcium ATPase. **(*J Am Heart Assoc*. 2017;6:e006630. DOI: 10.1161/JAHA.117.006630.)**

Key Words: angiotensin II • calcium channel • hypoxia • microvascular dysfunction • pregnancy

The middle cerebral artery (MCA) is a major channel supplying blood and oxygen to the brain. Damage to the MCA could cause stroke and other brain diseases.[1,2] Recent progress has been made in demonstrating that hypoxia during pregnancy can cause vascular diseases and damaged learning/memory ability in the offspring.[3-5] However, it is still unknown whether cerebral vessels would be influenced by prenatal hypoxia. It is worthwhile to investigate whether and how prenatal hypoxia affects central vascular systems.

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Adverse factors during pregnancy could increase the morbidity rate of cardiovascular diseases (coronary heart disease, stroke, etc) in adults,[6,7] and increase susceptibility to cardiovascular diseases.[8-10] This study evaluated whether gestation hypoxia affected vascular tone and ion channels of the MCA. Voltage-gated and receptor-gated calcium channels play central roles in the regulation of vascular tone.[11] The sarcoendoplasmic reticulum store determines the maximal capability to release calcium,[12] and Ca^{2+}-ATPase transports calcium back so as to keep intracellular calcium in balance. Those MCA functional units were tested in vessel tissue and smooth muscle cells in the present study.

Cerebral circulation is regulated by critical hormones, including angiotensin II (AII).[1] There is a local renin-angiotensin system in the cerebral circulation.[13] AII was used in testing the MCA in the present study. Notably, there has been very limited information regarding the influence and underlying mechanisms of prenatal hypoxia–affected calcium channel activities in the MCA. The present study hypothesized that chronic prenatal hypoxia may cause functional and molecular changes in cerebral blood vessels of the offspring,

Clinical Perspective

What Is New?

- Prenatal hypoxia damages middle cerebral artery function and increases cerebrovascular risk in offspring.
- Middle cerebral artery functional disorders were linked to renin-angiotensin system receptors and calcium channels in offspring cerebral arteries.

What Are the Clinical Implications?

- The data suggest potential targets for treatment of central vascular disorders related to developmental origins.
- Special attention to cardiovascular health should be considered and suggested for persons exposed to prenatal hypoxia.

determined vascular relaxation and constrictions in the MCA of 5-month-old offspring rats, and investigated intracellular calcium activities via membrane channels, sarcoendoplasmic reticulum calcium store, and Ca^{2+}-ATPase. The data gained would provide important information on chronic gestation hypoxia–affected cerebral arterial functions.

Methods

Animals

Sprague-Dawley rats (Slaccas Laboratory, Shanghai, China) were used. 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. Each female rat was mated with 2 male rats, pregnancy was confirmed the next morning by detecting vaginal mucus plugs, and the day was recorded as the first day of gestation. Pregnant rats were divided into 2 groups randomly (N=25, each group): control and prenatal-hypoxia group (PH). From gestation days 5 to 21, PH rats were kept in a hypoxia cabin (10.5% O_2) and the controls in a normal cabin (21% O_2), with standard rat food and water. At gestational day 21, some pregnant rats were euthanatized, and fetal body weight and brain weight were measured. The others gave birth naturally, and then all rats were kept in the normal environment. Five-month-old male offspring were used. Adult body and brain weight were measured. Vessel diameter and wall thickness of the MCA were measured at 40 mm Hg perfusion pressure using Living Systems Instrumentation (St. Albans, VT). The MCA was mounted on glass electrodes. The chamber was continuously perfused with physiological saline solution (PSS) and maintained at 37°C. Intravascular pressure was monitored using a pressure transducer and kept at 40 mm Hg. The computer automatically traced the vessel diameter and wall thickness based on gray density.

Measurement of Vascular Tone

Before decapitation with a guillotine, rats were weighed and anesthetized with sodium pentobarbital, 60 to 100 mg/kg intraperitoneally. MCA were isolated from connective tissues under a dissecting microscope. Isometric recordings of tension were made using 1- to 2-mm segments of MCA. The segments were mounted on 40-μm stainless steel wires in M series myograph chambers (Radnoti Glass Technology, Inc, Covina, CA) filled with 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), warmed to 37°C and oxygenated with 95% O_2-5% CO_2. Potassium chloride (120 mmol/L) was used to achieve optimal resting tension, and the maximal tension as a reference before addition of drugs. When the difference between 2 adjacent contractions by KCl was less than 10%, then the optimal resting tension was established. Vasoconstrictions induced by the drugs were normalized by comparison with the contraction elicited by KCl. Vasoconstrictions were obtained following cumulative addition of AII (10^{-11} to 10^{-5} mol/L). N^G-nitro-L-arginine (L-Name, a nitric oxide synthase inhibitor, 10^{-4} mol/L), losartan (AT1 receptor inhibitor, 10^{-5} mol/L), or PD123,319 (AT2 receptor inhibitor, 10^{-5} mol/L) was added into the chambers for 30 minutes before application of AII. Mibefradil (antagonist for L- and T-type calcium channels, 10^{-5} mol/L) or nifedipine (antagonist for L-type calcium channels specifically, 10^{-6} mol/L) was incubated in the chamber for 30 minutes followed by accumulative AII. Ryanodine (ryanodine receptor inhibitor, 10^{-5} mol/L), 2-aminoethyl diphenylborinate (2APB, IP3R inhibitor, 10^{-7} and 10^{-5} mol/L), or thapsigargin (sarcoendoplasmic Ca^{2+}-ATPase inhibitor, 10^{-6} mol/L) was incubated for 30 minutes before addition of AII, respectively. Caffeine 10 mmol/L was added into the chambers to test the sarcoendoplasmic reticulum calcium store. All drugs were freshly prepared and purchased from Sigma-Aldrich (St. Louis, MO).

Measurement of Intracellular Calcium Transient in Vascular Smooth Muscle Cells of MCA

Isolation of Vascular Smooth Muscle Cells

Cerebral arteries were dissected gently and cut into small fragments (about 0.5 mm) on 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), and then were placed for 34 minutes at 37°C in PSS containing 5 mg/mL papain, 2 mg/mL albumin bovine V, and 1 mg/mL dithiothreitol. Single cells were obtained by gentle trituration with a wide-bore glass pipette, stored at 4°C, and used within 6 hours.

Intracellular Calcium Transient

The intracellular calcium transient of vascular smooth muscle cells (VSMCs) was measured using Ca^{2+} indicator fura2-acetoxymethyl ester (fura2-AM, life technologies, Eugene, Oregon, USA). VSMCs were loaded in Ca^{2+}-free PSS solution with fura2-AM (2 mmol/L) for 30 minutes at room temperature. Then VSMCs were washed to remove the excess fura2-AM. $[Ca^{2+}]_i$ levels (in nmol/L) were calculated qualitatively by fluorescence ratio of fura-2AM at 340 and 380 nm wavelength ($Ratio_{f340/380}$). Caffeine-induced calcium transients were monitored and recorded continuously.

Electrophysiological Measurements

Whole-Cell Calcium Current Recording

The suspension of cerebral arterial VSMCs was added into the bath tank of the microscope (Leica, Wetzlar, Germany). VSMCs adhered to the wall, and those with slender morphology and good refractivity were chosen. The isolated smooth cells were continuously superfused with a bath solution of (mmol/L) 20 $BaCl_2$, 10 EGTA, 10 glucose, 1.0 $MgCl_2$, 124 choline-Cl (pH 7.3 with TEA-OH). The pipette (3 to 5 MΩ) solution consisted of (mmol/L) 130 cesium glutamate, 1.5 $MgCl_2$, 10 HEPES, 10 EGTA, 10 glucose, 3 Na_2ATP, 0.1 Na_2GTP, and 0.5 MgGTP (pH 7.3 with CsOH). 20 mmol/L $BaCl_2$ was used as a charge carrier to limit current rundown. Whole-cell Ca^{2+} channel currents were recorded in conventional whole-cell configuration voltage-clamp mode using an Axon Multiclamp 700B with Clampex 10.1 and normalized to cell capacitance as picoampere per picofarad. Voltage-dependent Ca^{2+} channel currents densities were assessed using standard pulse protocols and a patch-clamp station.[14,15] AII (10^{-5} mol/L) was added in measuring changes of calcium channel currents.

Activation and Inactivation Curves

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 −60 to +70 mV with 10-mV increments (T-Ca^{2+} currents were eliminated by using a holding potential of −60 mV). The activation data were fit to the Boltzmann distribution. $G/G_{max}=1-\{1+\exp[(V-V_{1/2})/k]\}^{-1}$, where $V_{1/2}$ is the voltage of half-maximal activation, G is the peak conductance at test voltage V, G_{max} is the maximum conductance, and k is the slope factor. To estimate the rate of Ca^{2+} channel inactivation, the voltage dependency of I_{Ba} inactivation was determined using a double-pulse protocol with a 1000-ms conditioning voltage step to potentials between −60 and +60 mV with 10-mV increments. This was followed by a 200-ms test pulse to +20 mV to evaluate inactivation of L-Ca^{2+} currents. The inactivation data were fit with a Boltzmann equation of the form $I/I_{max}=1-\{1+\exp[(V-V_{1/2})/k]\}^{-1}$, where I/I_{max} is the relative current amplitude compared with the maximum current amplitude, k is the slope factor, and $V_{1/2}$ is the voltage at which there is half-maximal inactivation. Data were collected after the whole-cell configuration had been obtained and current amplitude stabilized. Only cells with an input resistance >2 GΩ without substantial rundown were analyzed.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from offspring cerebral arteries with TaKaRa MiniBEST Universal RNA Extraction Kit or RNAiso Reagent (TaKaRa, Shiga, Japan). RNA was reverse transcribed with PrimeScript™ II by using First Strand cDNA Synthesis Kit (TaKaRa). The reference sequence of studied genes was acquired from the UCSC Genome Browser. Relative gene primer sequences (Sangon Biotech, Shanghai, China) are shown in Table 1. The quantitative polymerase chain reaction was performed on a Bio-Rad MyiQ2 Thermal Cycler QPCR machine (Bio-Rad, Hercules, CA) with a SYBR Premix Ex Taq™ mix (TaKaRa). Data were normalized against actin as internal control and calibrated with a normal control cDNA. The relative expression ratio was calculated with the $2^{-\Delta\Delta Ct}$ method.

Data Analysis and Statistics

Data are presented as mean±SEM. Two-way ANOVA analysis followed by Bonferroni post hoc test or t test, when appropriate, was used to determine the statistical significance among groups ($P<0.05$). N presented the number of pregnant rats. If the offspring used included more than 1 from the same mother, their data were averaged, and then the average number was treated as a single sample for analysis. Statistical analyses were conducted using GraphPad Prism 5.

Results

Body Weight, Brain Weight, and Vessel Diameter

Prenatal hypoxia decreased fetal body weight significantly (control 3.783±0.059 g, PH 3.030±0.107 g, N=8, $P<0.0001$). Brain weight in the PH (0.163±0.004 g) was smaller than in the control (0.178±0.003 g) (N=8, $P<0.05$). There was no significant difference in body and brain weight between the 2 offspring groups. The MCA diameter was 270 to 288 μm at 40 mm Hg pressure with no significant

Table 1. Primer Sequences

Gene	Forward Primer (5′–3′)	Reverse Primer (5′–3′)	NCBI Reference Sequence
Actin	CCGCCCTAGGCACCAGGGTG	GGCTGGGGTGTTGAAGGTCTCAAA	NM_031144.3
AT1A	GCGCTCATCAGACTGTAGATAATGAC	AATCCATCCAGCTCCTGACTCTTC	NM_030985.4
AT1B	CTAGTGACTATGACACCATTGTTCC	TATTCAGGCAAGCTGTTCTGTGGTAC	NM_031009.2
AT2R	GCTCACACAAACCGGCAGATAAGC	GTCAGCCACAGCCAGATTGAAGATG	NM_012494.3
Cav1.2α1C	CTTCAAACGTGGCCACAGAC	GCCCGAATCATTGTGACTCC	NM_012517.2
Cav3.2α1H	CTGAGAGA GGCTCAG CATGAT	CTGTCCAGGA AGCATCGGTT	NM_153814.2
Cavβ2	ATGGCCATCTCATTCGAGG	ATGCTGTAGCCTCATGTTCTCTAG	NM_053851.1
Cavβ3	CTGGATGAGAACCAGCTGGAC	TCATCCGAGGGCATCAAACTG	NM_012828.2
Ip3r1	CCTGTTGACCTAGACAGCCA	AGAACATCCACGAGCACAGA	NM_001270597.1
Ip3r2	GCAACAACTACCGGATCGTC	AGGAAGGTGTGGGCTAAGTC	NM_031046.3
Ip3r3	CTGACAGAGGAGACCAAGCA	GAACACTGCCAGGTTGAAGG	NM_013138.1
RyR2	CACTCCTCTATGGACACGCC	CAAAGGCCAGTTTGTCGGTG	NM_001191043.1
Serca1	AGTGATGGAGAACTCGTTCAGTG	AGAACGCCATCGTGAGGAG	NM_058213.1
Serca 2	GTTCGAAGTCTGCCTTCTGTG	TGCATAGGTTGATCCAGTTATGG	NM_001110139.2

NCBI indicates National Center for Biotechnology Information.

difference in diameter between the 2 groups, and neither did the thickness of the vessel wall differ (Table 2).

Prenatal Hypoxia Increased AII-Mediated Vasoconstriction and Decreased Endothelial Relaxation in the MCA

There was no significant difference in KCl-induced maximal contraction (Figure 1A). Figure 1A showed that concentration-response curves of AII-induced MCA contractions were greater in the PH (control 42.93±6.373, PH 72.00±7.022, **P*<0.05). Figure 1B showed that L-Name, an endothelial NO synthase inhibitor, increased AII-mediated constriction significantly in the control but not the PH group. In the PH group, losartan decreased AII-induced contraction significantly more than it did in the control (Figure 1C). PD123,319, an AT2 receptor inhibitor, potentiated AII-mediated constriction in the control but not in the PH group (Figure 1D). These results showed AII-increased constriction was due to an increase of AT1R-mediated constriction and to decreased AT2R/eNOS-mediated relaxation in PH group.

Table 2. Vessel Diameter and Wall Thickness of the Middle Cerebral Arteries in Offspring (n=N)

	C	PH
Vessel diameter, μm	288.279±5.004	270.158±8.456
Wall thickness, μm	21.742±2.912	27.257±4.246
N	8	9

C indicates control; PH, prenatal hypoxia.

Functional Voltage-Dependent Calcium Channels Were Potentiated by PH

AII-mediated constriction curves with nifedipine or mibefradil overlapped in both the control group and the PH group (Figure 2B), suggesting that T-type calcium channels played little role in AII-altered constriction in the MCA. Nifedipine inhibited the AII-induced vasoconstrictions, and the inhibition was significantly greater in the PH group (Figure 2A), suggesting that L-type calcium channels may play a greater role in the regulation of MCA tone. We also investigated whole-cell calcium currents in VSMCs from offspring MCA (Figure 3). The real-time recordings (Figure 3A) showed that amplitude of calcium currents was greater in the PH group. PH caused the voltage-step activation curve to shift to the left, although the inactivation curve was unchanged (Figure 3A). This demonstrated that PH increased activities of L-type calcium channels. However, AII significantly increased calcium channel currents in the control (Figure 3B), but this phenomenon was not seen in the cells from the PH group (Figure 3C). AII-mediated activation and inactivation curves in the VSMCs of the MCA were the same between the control and the PH groups (Figure 3B and 3C). These data suggested that AII-potentiated tension in the PH group was not due to an increase of calcium channels.

IP_3- or Ryanodine Receptor-Gated Calcium Channels

The receptor-operated calcium channels include IP_3 receptor (IP3R)-operated and ryanodine receptor (RyR)-operated calcium channels in the sarcoendoplasmic reticulum. 2APB, an

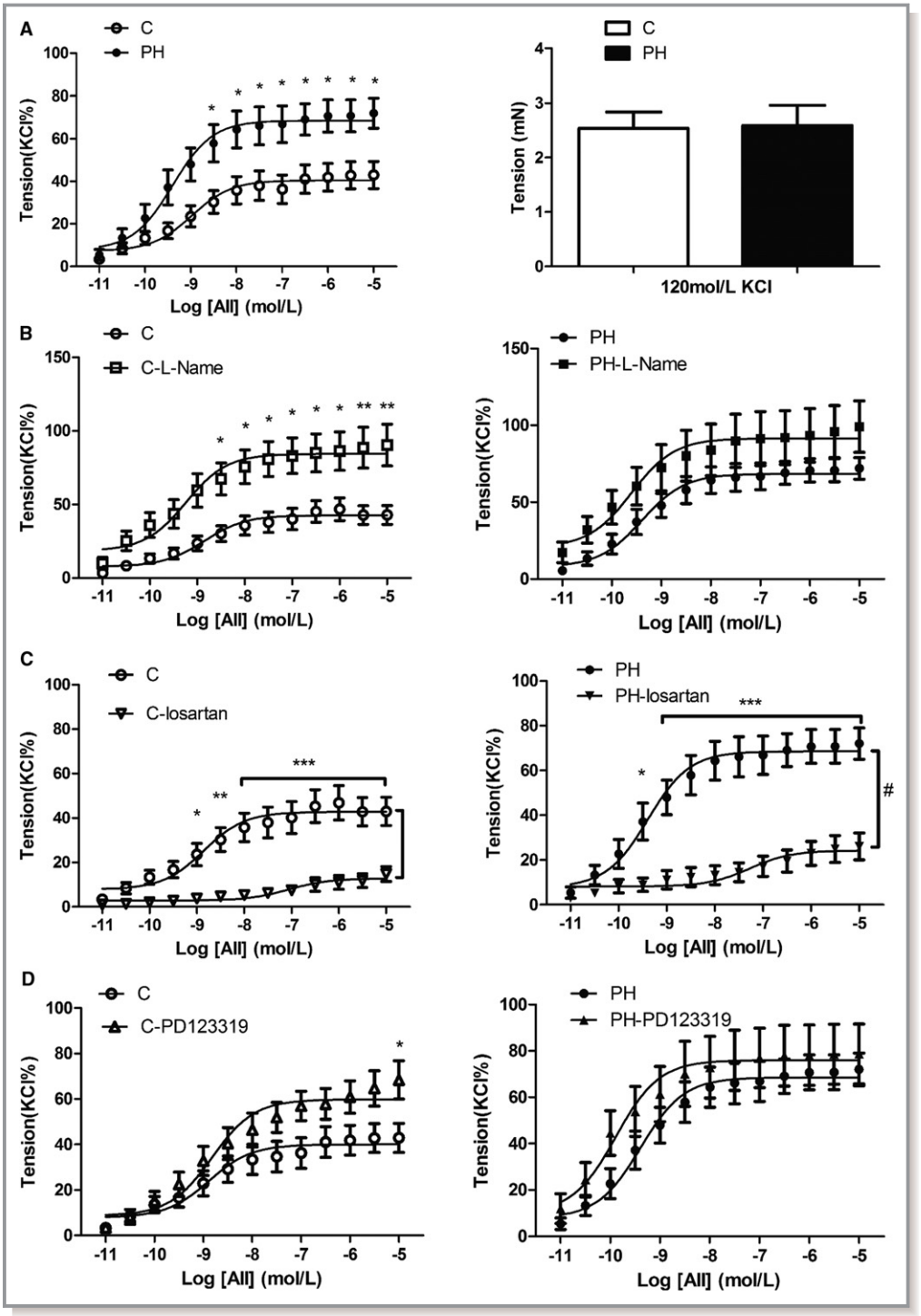

Figure 1. Angiotensin II (AII)-induced contraction and endothelial nitric oxide synthase (eNOS)-related relaxation in the offspring middle cerebral arteries (MCA). A, Cumulative concentration of AII induced response curves in MCA. Potassium chloride (KCl) mediated constriction in both groups. B through D, showed dose–response curves of AII-induced contraction with or without L-Name (N^G-nitro-L-arginine), losartan, and PD123,319, respectively. **P*<0.05, ***P*<0.01, ****P*<0.001, $^{\#}$*P*<0.05, control vs PH. N=10, n=10 to 12 in each group. C indicates control; PH, prenatal-hypoxia group.

inhibitor of IP3R-operated calcium channels, restrained the AII-induced constriction in the control at 10^{-5} mol/L. In the PH group, such inhibiting effects were significantly stronger (Figure 4A). AII-mediated vasoconstriction was reduced by ryanodine, a RyR-gated calcium channel inhibitor, with a right shift in the PH group (Figure 4B). Caffeine (10 mmol/L)

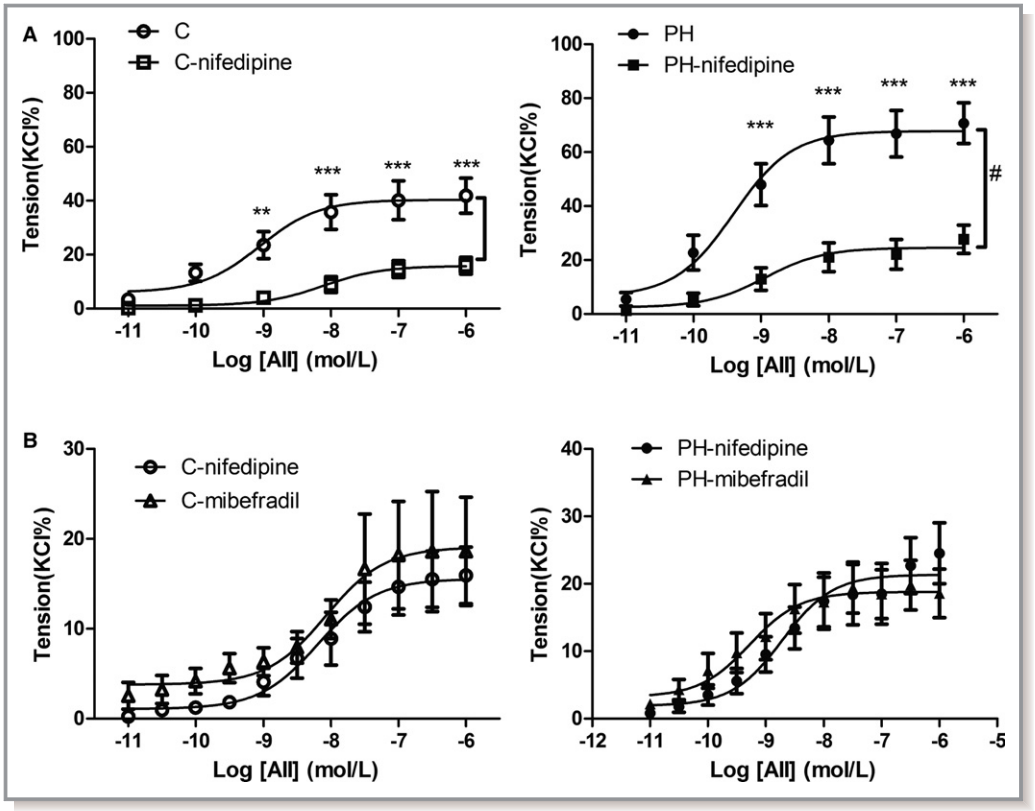

Figure 2. Membrane voltage-dependent calcium channels of middle cerebral arteries. A, Cumulative angiotensin II (AII)-mediated response curves in the presence or absence of nifedipine. B, The comparison of cumulative AII-mediated response curves in the presence of nifedipine and mibefradil between C and PH groups. N=12, n=12 to 14 in each group, $^{**}P<0.01$, $^{***}P<0.001$. $^{\#}P<0.05$, control vs PH. C indicates control; PH, prenatal-hypoxia group.

induced stronger cerebrovascular tone and higher calcium transient in VSMCs in the PH group (Figure 4D). These data indicated that prenatal hypoxia increased activities of IP_3 and ryanodine receptors as well as sarcoendoplasmic reticulum store capacity.

The Sarcoendoplasmic Reticulum Ca^{2+}-ATPase Was Increased in the PH Group

Thapsigargin depleted IP_3-independent sarcoendoplasmic reticulum calcium activities and inhibited the sarcoendoplasmic reticulum Ca^{2+}-ATPase. Figure 4C showed AII-mediated constrictions were depressed by thapsigargin, and the inhibited effect was potentiated significantly in the PH group, demonstrating that the increased Ca^{2+}-ATPase activities and intracellular calcium were removed in a faster manner in PH group.

The Relative mRNA Expression

Angiotensin II receptors (AT1A, AT1B and AT2R) were increased significantly in the PH group (Figure 5A). Voltage-dependent calcium channels, especially L-type (Cav1.2α1C) and T-type (Cav3.2α1H), were enhanced after exposure to prenatal hypoxia. There was no significant difference in the subtype Cavβ2 and β3 between the 2 groups (Figure 5B). Receptor-gated calcium channels and Serca1/2 were also detected, and RyR2 was increased in the PH group (Figure 5C).

Discussion and Summary

The present study demonstrated (Figure 6) the following: (1) chronic prenatal hypoxia elevated AII-induced vasoconstriction in the MCA of the male offspring, mainly mediated by AT1 receptors; (2) L-type calcium channel activities were potentiated in the offspring exposed to prenatal hypoxia, whereas T-type calcium channels showed little influence on the AII-increased constriction in the MCA. AII-increased cerebrovascular tone in the PH was not due to voltage-dependent calcium channels; (3) IP_3 receptor– and ryanodine receptor–gated calcium channels played roles in the regulation of cerebrovascular intensity and sensitivity in the PH offspring, with an increase of sarcoendoplasmic reticulum calcium store capacity.

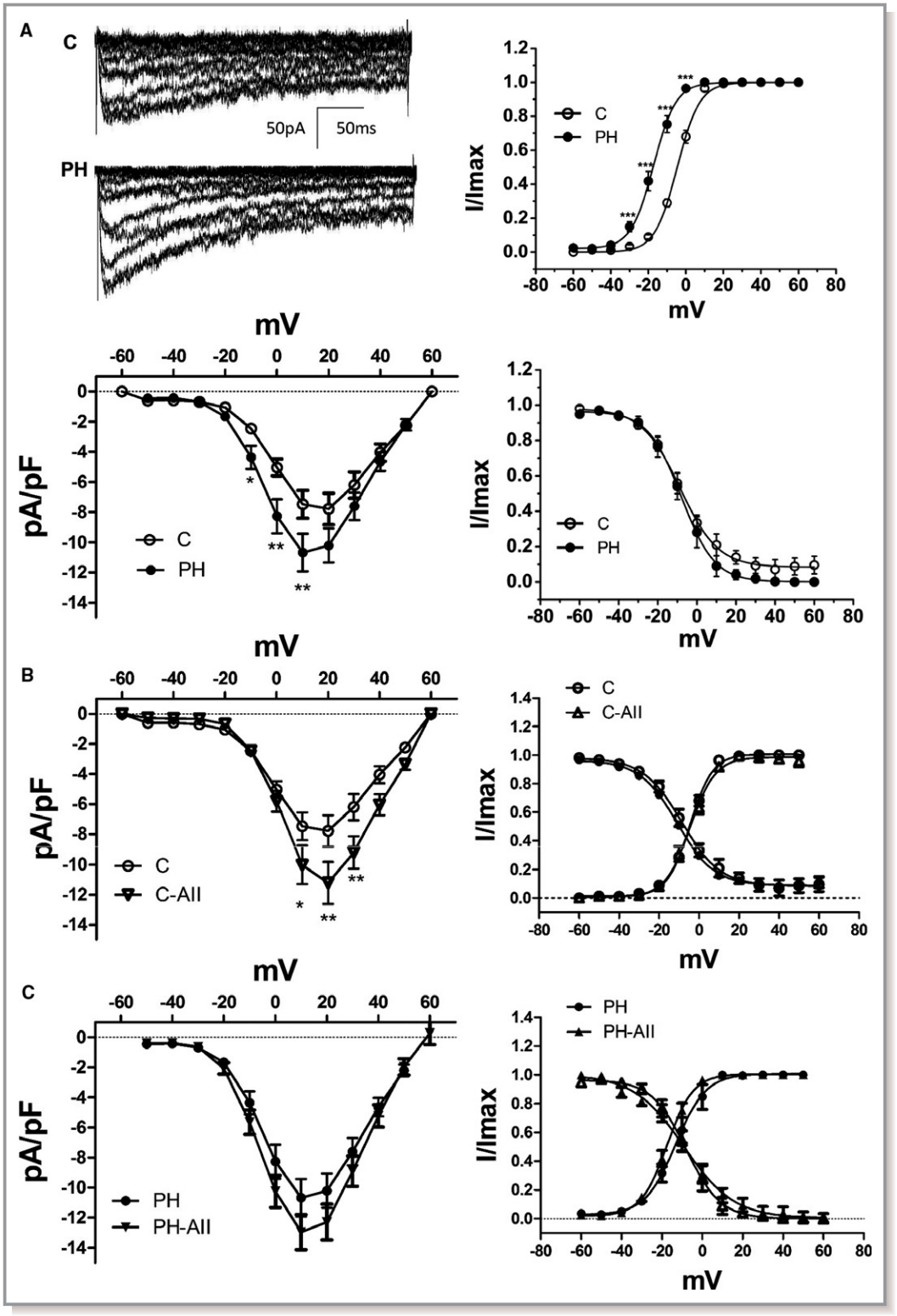

Figure 3. Whole-cell calcium channel currents in vascular smooth muscle cells. The first real-time images represented the whole-cell calcium channel currents of both groups. A, Whole-cell calcium current density, activation, and inactivation curves at depolarizing voltage steps. B and C, AII potentiated the amplitude of calcium current density in the control but not in the PH group. Meanwhile, the activation and inactivation curves were not affected by AII. N=6, n=6, 2 to 4 cells for each offspring, **P*<0.05, ***P*<0.01, ****P*<0.001. AII indicates angiotensin II; C, control; PH, prenatal-hypoxia group.

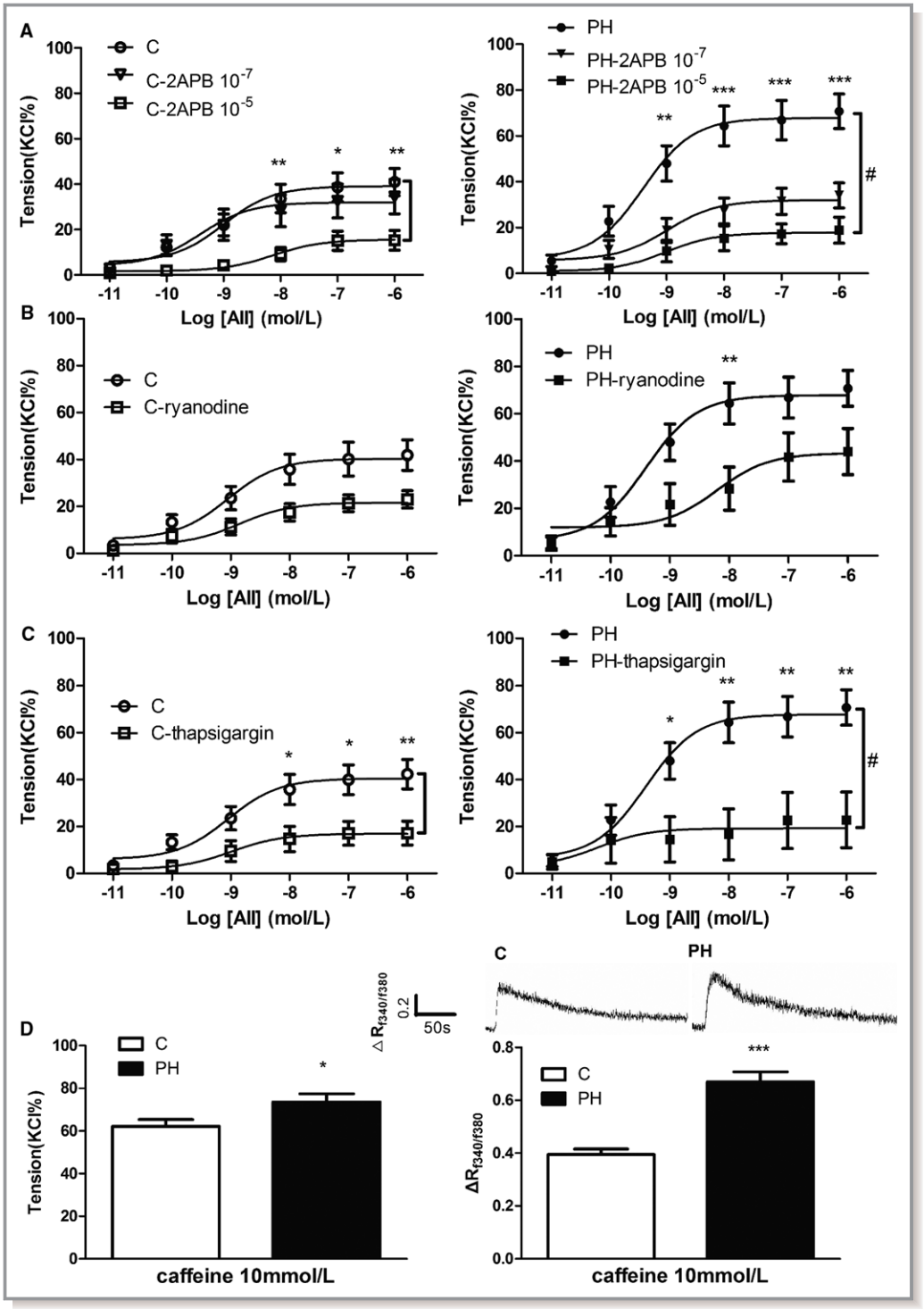

Figure 4. Receptor-gated calcium channels and sarcoendoplasmic reticulum Ca^{2+}-ATPase. A through C, AII-induced contractions in the presence or absence of 2APB (2-aminoethyl diphenylborinate, 10^{-7} mol/L and 10^{-5} mol/L), ryanodine, and thapsigargin in both groups. D, Caffeine caused contraction of middle cerebral arteries and calcium transient of vascular smooth muscle cells in both groups. The insets show the real-time curves of caffeine-induced calcium transients. N=7, n=7 to 13 in each group, **P*<0.05, ***P*<0.01, ****P*<0.001. #*P*<0.05, control vs PH. AII indicates angiotensin II; C, control; PH, prenatal-hypoxia group.

Prenatal hypoxia affected the fetal brain and kidney development as well as learning and memory in the male offspring.[4,16] Hypoxia during pregnancy, like exposure to nicotine and malnutrition, could cause adult diseases in fetal origins.[17-19] In the present study hypoxia caused in utero growth restriction as evidenced by lower birth weight and

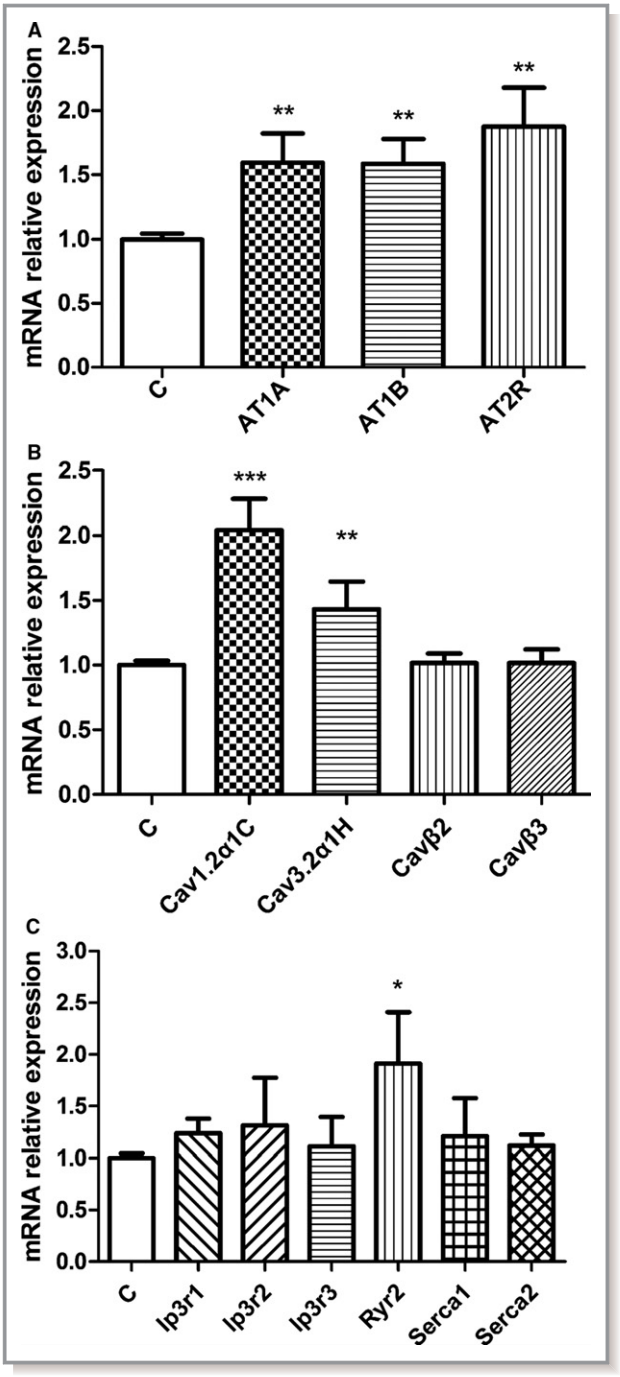

Figure 5. Relative mRNA expression in the cerebral arteries. A, The mRNA expression of angiotensin II receptors, (B) expression of voltage-dependent calcium channel isoforms, and (C) sarcoplasmic reticulum receptors and Ca^{2+}-ATPase expression. In the prenatal-hypoxia group, AT1A, AT1B, AT2R, Cav1.2α1C, Cav3.2α1H, and RyR2 were increased compared with the control; Cavβ2, Cavβ3, Ip3r1-3, and Serca1/2 showed no significant differences between the groups. N=4, **P*<0.05, ***P*<0.01, ****P*<0.001. C indicates control group.

brain weight. Those weight differences were reversed at adult stage in the offspring, suggesting catch-up growth as reported.[20] Our previous study demonstrated that maternal hypoxia in pregnancy could induce vascular dysfunction in renal arteries of the offspring.[5] Other work has also showed that maternal hypoxia caused peripheral vascular damage in offspring rats.[19,21] The MCA is a critical pathway supplying oxygen and nutrition to the brain, and damage to a functional MCA would lead to central nervous diseases.[22] Because vascular wall thickness and diameters could be altered in vascular diseases,[23] we measured them in offspring. Wall thickness and diameter of the MCA did not show significant differences between the 2 groups. However, MCA functions were changed in the hypoxia offspring. The increased MCA contractility as a consequence following PH could increase susceptibility to vasospasm and risks in developing stroke. The present study was the first to demonstrate that AII-mediated cerebral circulation and vessel functions could be altered by prenatal hypoxia in the offspring, which is important for further understanding the development of cerebral vascular dysfunction of fetal origin. Future studies should consider histological analysis on the MCA following prenatal hypoxia.

AT1 and AT2 receptors are major functional units in vascular systems. Our studies showed that the altered AII-mediated vasoconstriction by prenatal hypoxia was mainly due to AT1 receptors. Interestingly, the AT2 receptor inhibitor in the MCA potentiated AII-mediated constriction in the control, not in the PH group. This finding presents 2 novel meanings: first, effects of AT2 receptors on vascular regulations have been uncertain[24]—some works showed vasorelaxation in the mesenteric arteries and uterine arteries,[25,26] whereas others indicated no effects on vascular tension. Our results indicated that AT2 receptors in the central vascular systems may have protective effects in vasorelaxation, and PH-produced MCA dysfunction was not only from abnormal contractility but also caused by AT2 receptor-mediated dilatation. To the best of our knowledge, this was the first demonstration that AT2 receptors play certain roles in vasorelaxation in the MCA of rats. Because AII is critical in the control of blood flow in the MCA, the finding is important for further understanding of AII-mediated central vascular regulations. Second, prenatal hypoxia could cause functional changes in AT2 receptors in the MCA, which deserves further investigation. These findings also raised an immediate question: How did prenatal hypoxia cause alterations in AII-mediated vascular regulations in the MCA? Previous studies had reported that hypoxia during pregnancy was harmful to the endothelium-dependent vasodilatation in pulmonary and mesenteric arteries.[19,21] The present study demonstrated that it damaged endothelial NO synthase in the MCA. AT2 receptor-induced vasodilatation depended on generation of NO via endothelial NO synthase.[27,28] Thus, the weakening of the AT2 receptor-mediated vasodilatation in the MCA of the PH might be due to the damaged endothelial NO synthase (Figure 6).

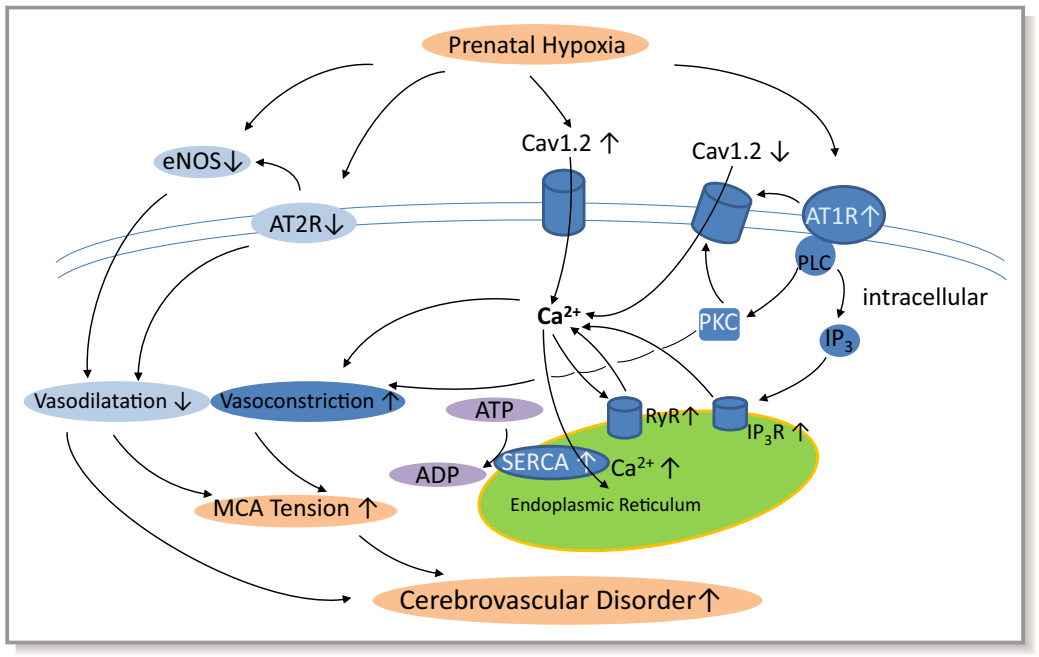

Figure 6. This image shows how prenatal hypoxia affects vascular functions in the middle cerebral arteries (MCA), which may lead to increased risks of stroke. AT1R, AT2R indicate AT1 and 2 receptors; Cav1.2, L-type calcium channels; eNOS, endothelial nitric oxide synthase; IP3r, IP_3 receptor; PKC, protein kinase C; PLC, phospholipase C; RyR, ryanodine receptor; Serca, sarcoendoplasmic reticulum Ca^{2+}-ATPase.

Ion channels, especially calcium channels, play critical roles in vascular tone. The present study focused on voltage-dependent and receptor-gated calcium channels. The mRNA expression of L- and T-type calcium channels was demonstrated in the cerebral arteries.[29,30] Hypoxia elevated the expression and activities of L- and T-type calcium channels in the pulmonary arteries.[31] The present study showed that L-type, but not T-type, calcium channels should be important in the changed vascular tone in the MCA. In isolated MCA smooth muscle cells, patch-clamp experiments revealed that baseline activities of L-type calcium channels were altered by prenatal hypoxia (Figure 6). However, AII significantly increased calcium currents in the VSMCs of the controls, not in the PH, and the activation as well as inactivation curves were unchanged with or without AII. These data suggested that activities of L-type calcium channels at the baseline were changed by prenatal hypoxia but were not significantly involved in the AII-increased vasoconstrictions in the MCA. This interesting finding was further supported by the increased mRNA expression of Cav1.2α1C and Cav3.2α1H, but not Cavβ2 and Cavβ3, in the MCA of the PH offspring. To explain why the L-type channel blocker nifedipine could manipulate AII-increased vasoconstrictions, we consider that nifedipine-inhibited vasoconstriction by AII was not mainly due to voltage-dependent calcium channels but occurred via other pathways such as protein kinase C routes (Figure 6), which deserves further investigation.

Intracellular calcium contributes critically to vasoconstrictions. Besides calcium channels on cellular membrane, the present study paid special attention to receptor-gated calcium channels on the sarcoendoplasmic reticulum. The calcium release from the sarcoendoplasmic reticulum not only depends on specific receptors but also relies on calcium store capacity and calcium ATPase.[32,33] In the present study, activities of IP_3 receptors were increased by prenatal hypoxia. The ryanodine receptor sensitivity was enhanced in PH offspring, and this was also supported by the increased mRNA expression of RyR2. Moreover, caffeine, at 10 mmol/L, which could deplete sarcoendoplasmic reticulum calcium store,[34] induced stronger constriction in the MCA and a higher calcium transient in the smooth muscle cells of the PH compared with the control. These suggested that chronic PH increased the calcium store capacity in the MCA. Thapsigargin could inhibit Ca^{2+}-ATPase in the sarcoendoplasmic reticulum.[35] Our experiments showed that the capability of Serca was strengthened by PH so that overload intracellular calcium could be transported back to the sarcoendoplasmic reticulum. Because protein kinase signaling pathways also play roles in regulation of cerebral vascular tone,[36,37] they also are worthy of further investigations.

Limitation

The approaches used in this study revealed that PH affected MCA functions via certain receptors and ion channels/pumps.

Other approaches are needed to explain how hypoxia influenced the MCA receptor or those channels/pumps.

Conclusions

Chronic PH could induce cerebrovascular dysfunction in the male offspring, which may increase risks in the development of stroke and other brain diseases. The underlying mechanisms in the altered MCA functions included the changed activities of AII receptors, the calcium channels on the membrane, and calcium pumps on the sarcoendoplasmic reticulum, as well as the altered endothelial NO synthase. Notably, many clinical conditions could cause in utero hypoxia during pregnancy. This study is the first to demonstrate that PH could significantly affect ion channel functions in the MCA of the adult offspring. The data gained contribute new information on the development of cerebrovascular problems in fetal origins and provide new insight into early prevention of these diseases.

Author Contributions

Tang wrote the manuscript. Chen and Tang did vascular experiments and prepared Figures 1, 2, and 4. Li did electrophysiological experiments and prepared Figure 3. Zhou and Liu measured intracellular calcium transients and prepared Figure 4D. Gao and Zhang detected mRNA expression and prepared Figure 5. All authors have reviewed the manuscript.

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Disclosures

The authors declare no conflicts of interest.

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