Alveolar Epithelial Cells Involved in Pulmonary Vascular Remodeling and Constriction of Hypoxic Pulmonary Hypertension

Yanxia Wang
Fourth Military Medical University: Air Force Medical University
Xiaoming Li
Xi'an Peihua University
Wen Niu
Fourth Military Medical University: Air Force Medical University
Jian Chen
Fourth Military Medical University: Air Force Medical University
Bo Zhang
Fourth Military Medical University: Air Force Medical University
Xiumin Zhang
Fourth Military Medical University: Air Force Medical University
Yingmei Wang
Fourth Military Medical University: Air Force Medical University
Shaokang Dang
Tangdu Hospital Fourth Military Medical University: Air Force Medical University Tangdu Hospital
Zhichao Li (lizhic@fmmu.edu.cn)
Fourth Military Medical University

Research

Keywords: alveolar epithelial cells, hypoxic pulmonary hypertension, pulmonary vascular remodeling and constriction, reactive oxygen species

Posted Date: December 18th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-129956/v1

Abstract

Background: Hypoxic pulmonary hypertension (HPH) is a common type of pulmonary hypertension. Alveolar epithelial cells (AECs) are the first to perceive hypoxia of alveolar, however, the role of AECs in HPH remain unclear. HPH is characterized by pulmonary vascular remodeling and constriction. The present study was to whether AECs was involved in pulmonary vascular remodeling and constriction.

Methods: Rat HPH models were built and pulmonary artery smooth cells (PASMCs) and AECs were treatment with hypoxia. Hemodynamic and morphological indicators were measured in samples from rat HPH models. Superoxide dismutase 2 (SOD2), catalase (CAT) and reactive oxygen species (ROS) were detected in AECs or AECs culture medium. To find out the effect of AECs on pulmonary vascular remodeling and constriction, AECs and PASMCs were co-cultured under hypoxia, PASMCs and isolated pulmonary artery (PA) were treatment with AECs hypoxic culture medium. To explore the mechanism of AECs on pulmonary vascular remodeling and constriction, ROS inhibitor N-acetylcysteine (NAC) was used.

Results: In vivo, hypoxia resulted in elevation in pulmonary vascular remodeling and pressure, but had no effect on non-pulmonary vascular. In vitro, hypoxia caused an imbalance of superoxide dismutase 2 (SOD2) and catalase (CAT) and an increase of reactive oxygen species (ROS) in AECs, as well as an increase of hydrogen peroxide (H_2O_2) in AECs culture medium. Also, AECs led to pulmonary artery smooth cells (PASMCs) proliferation under hypoxia by co-culture or substituting culture medium. AECs hypoxic culture medium enhanced the constriction of isolated pulmonary artery (PA). Further, these responses were abrogated by ROS inhibitor N-acetylcysteine (NAC).

Conclusion: The findings of present study demonstrated that AECs involved in pulmonary vascular remodeling and constriction under hypoxia by secreting H_2O_2 to the pulmonary microenvironment.

Background

Pulmonary hypertension is a progressive disorder and characterized by pulmonary vascular remodeling and vasoconstriction [1–3], which ultimately leads to right ventricular hypertrophy and right heart failure with morbidity and mortality [4–6]. Hypoxic pulmonary hypertension (HPH) is highly prevalent in advanced chronic obstructive pulmonary disease and high altitude hypoxia [7]. It is well known that when alveolar hypoxia occurs, only pulmonary artery pressure increases, but no systemic circulation pressure increases. Vascular internality plays an important role, but its peripheral microenvironment should also be an important factor. AECs are primarily components of alveolar wall and involve in the formation of the pulmonary microenvironment. When hypoxia occurs in alveolar cavity, AECs are the first to perceive and suffer from largest change of oxygen content in the body. Previous studies have constantly reported that pulmonary arteriolar endothelial cells and adventitial fibroblast were involved in HPH [8–11]. However, little is known whether AECs are involved in the development of HPH. The purpose of this experiment is to observe the role and mechanism of AECs in the development of HPH.

Reactive oxygen species (ROS) including hydroxyl radical ($HO^{\bullet}$), singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2) are by-products of electron transfer processes [12–14]. Accumulation of $O_2^{\bullet-}$ and H_2O_2 is prevented by the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in the cell normal metabolism[15, 16]. The balance between production and degradation rates determines the steady-state concentrations of ROS in each intracellular compartment. H_2O_2 is a stable and easily diffused out of the cell[14]. It has been documented in increase cell proliferation[17], cell-cycle arrest[18]and apoptosis[19]and vascular smooth muscle constriction[20].

The multiple responses of the lung to oxygen are a physiological paradigm of the variety of cellular responses to ROS in vitro. In the lung, AECs are the first to perceive changes of oxygen concentration in the alveolar cavity, and many other investigators recently provided extensive evidence that hypoxia results in a significant increase in ROS concentration in AECs [21], which inevitably lead to changes of the microenvironment around the pulmonary artery. A recent study demonstrated cell proliferation could only be achieved by exposing the cells to a constant flux of H_2O_2 generated by the G/GO system [22]. Therefore, AECs, as the cells around the pulmonary capillaries, can affect the pulmonary vascular remodeling and constriction by constant release of H_2O_2.

This study provides novel insights into the pathogenesis of HPH. It is considered that the earliest source of pulmonary microenvironment changes under hypoxia is AECs. In this experiment, AECs were used to change microenvironment of PASMCs, which proved that AECs were involved in pulmonary vascular remodeling and constriction under hypoxia and also to be related to the H_2O_2 derived from AECs.

Methods

Animals

Male Sprague-Dawley rats (200–250 g) were purchased from the animal center of the Fourth Military Medical University (Xi'an, China). All experiments were approved by Animal Care and Use Committee of the Fourth Military Medical University and complied with the Declaration of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85 – 23, revised 1985). To obtain pulmonary hypertension rats, rats were housed intermittently in a hypobaric hypoxia chamber depressurized to 380 mmHg (10% oxygen) and taken hypobaric hypoxia challenge of 8 h/d for 4 weeks. Age-matched rats were housed in room air (21% oxygen) accordingly.

Hemodynamic and Morphological Investigation

Right ventricular pressure and hematoxylin and eosin staining of lung and renal tissues were performed according to my published article [23]. After 4 weeks of hypoxia, the animals were anesthetized with 20% ethylurethanm (5 ml/ kg). Then, a soft silica gel catheter linked to a Power lab system (AD Instruments, Colorado Springs, CO, Australia) was inserted into the right jugular vein. The right ventricle peak systolic pressure (RVSP) waveforms were showed on the monitor when the catheter arrived in the right ventricle

chamber. Meanwhile, the mean carotid artery pressure (mCAP) was recorded via a special catheter inserted into the carotid artery. After the weight of right ventricle (RV) and left ventricle plus septum (LV + S) were obtained, the ratio of RV/ (LV + S) was calculated as an index of RV hypertrophy. The right lung and kidney were placed in neutral buffer (pH 7.4) containing 10% formalin for 72 hours. The lung and kidney tissue were embedded in paraffin and sectioned into 5 μm thick sections, then processed hematoxylin and eosin staining. Microscopic evaluation showed structure remodeling of the pulmonary artery. Total 60 of pulmonary artery, bronchial artery and renal artery in approximate round shape were obtained from each group. Their external diameters are50-180 μm and the average size of artery was 78 μm. The outside diameter and inside diameter of pulmonary artery were measured by an image-processing program (Image-Pro Plus, Version 5.1, Media Cybernetics, Rockville, MD, USA). The medial wall thickness, the cross-sectional area of medial wall, and the total cross-sectional vessel area were obtained. Pulmonary vascular structure remodeling was assessed by percent medial wall thickness (MT %) and percent medial wall area (MA %) two indices: MT% = 100 × (medial wall thickness) / (vessel semi-diameter); MA% = 100 × (cross sectional medial wall area) / (total cross-sectional vessel area). All the morphological analysis was conducted via a double-blind method. The following should be explained in our experiment: pulmonary artery is a pulmonary circulatory vessel located in the pulmonary microenvironment, bronchial artery is a systemic circulatory vessel located in the pulmonary microenvironment, and renal artery is a systemic circulatory vessel not located in the pulmonary microenvironment.

Cell culture

AASMCs were used for systemic circulating vascular smooth muscle cells. Rat primary PASMCs and AASMCs were obtained by tissue explants culturing method according to my published articles [18]. Pulmonary artery (PA) and aortic artery(AA) were isolated from male Sprague-Dawley rats (200–250 g) and dissected into small pieces after the adventitial layers were removed, then placed in a overturned culture flask containing Dulbecco Eagle's minimum essential Medium (DMEM, HyClone, Logan, UT) with 15% fetal bovine serum(FBS, CellMax, Beijing, China)at 37 °C in 21% oxygen condition. The flask was carefully turned over after 4 hours. PASMCs and AASMCs crawled out from the tissue in about a week. Cells were used between passages 3 to 6. Smooth muscle cell identity was verified by positive staining for smooth muscle a-actin (mouse monoclonal antibody, Sigma-Aldrich, St. Louis, MO, USA) at each passage.

Alveolar epithelial cells (AECs) in this study included rat primary ATII cells and RLE-6TN cells. Rat primary ATII cells were isolated from male SpragueDawley rats (180–200 g) as previously described [24]. Pooled cells from 2 rats were prepared as follows. Rats were injected intraperitoneally with 20% ethylurethanm (4 ml/kg) and intravenously 4000U/kg heparin sodium. Intubation of pulmonary artery and tracheal were performed. Rats were exsanguinated by cutting the abdominal aorta under the aseptic condition. 50 ml Solution II (in mM: 140 NaCl, 5 KCl, 10 Hepes, 2$CaCl_2$, 2.5 PBS (pH 7.4), 1.3 $MgSO_4$) was perfuse via the pulmonary artery to clear the vascular space of blood. The lungs were removed from the thorax and lavaged with 8 times solution I (10 ml/time) (in mM: 140NaCl, 5 KCl, 10 Hepes, 6 D-glucose, 2.5 PBS (pH

7.4),0.2 EDTA) and 2 times solution II (10 ml/time).Lungs were then filled 2 times with trypsin solution (10 ml/time) prepared in solution II and incubated in incubator for 10 min at 37℃ every time. The lungs were cut into pieces in the bottle with 4 ml DNAse I and 5 ml FBS. The lung tissues were then sequentially filtered through 125 μm and 75 μm stainless mesh. The filtrate was centrifuged at 1000r/min for 8 min. The cell pellet was resuspended in DMEM at 37℃.The cell suspension was plated at a density of 2×10^6 cells/ml in 25 cm^2 bacteriologic plastic dishes coated rat IgG to remove macrophages and incubated at 37℃in 5% CO_2 incubator for 1 h. The unattached cells in suspension were removed and centrifuged at 1000 r/min for 8 min. The cell pellet was plated at a density of 5×10^5 cells/cm^2 in 6-well culture dishes with DMEM, 15% FBS, 100 U/ml penicillin and 100 U/ml streptomycin and incubated at 37℃under 5% CO_2 incubator. The cell purity after 24 h was assessed by a characteristic fluorescence with phosphine3 Ras previously described [24]. RLE-6TN were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in DMEM supplemented with 15% FBS, 100 IU/ml penicillin and 100 mg/ml streptomycin.

The preparation of AECs culture medium

AECs were seeded in 24-well culture dishes at 5×10^3 cells per well under normoxic (21% O_2) or hypoxic conditions (5% O_2). Its culture medium was collected for 24 h to subsequent experiments.

Cell treatment

The effect of hypoxia on the proliferation of PASMCs and AASMCs was investigated under different oxygen concentrations. PASMCs or AASMCs were seeded in 96-well culture dishes at 3×10^4cells per well under normoxic (21% O_2) or hypoxic conditions (10% O_2 and 5% O_2).

The effect of AECs on the proliferation of PASMCs and AASMCs was investigated using 24- well transwell insert co-culture model (0.4 μm pore). PASMCs or AASMCs were seeded in 24-well culture dishes at a density of 3×10^4cells per well, ATII or RLE-6TN were seeded in transwell inserts at a density of 1×10^4 cells per insert. The cells were maintained under normoxia (21% $O_{2)}$ or hypoxia conditions (5% O_2). The cells were grouped as follows, PASMCs: (1) normoxia (2) hypoxia (3) normoxia, co-culture with ATII or RLE-6TN (4) hypoxia, co-culture with ATII or RLE-6TN (5) hypoxia, co-culture with ATII or RLE-6TN + NAC (10 mmol/L, a nonspecific ROS inhibitor, Sigma-Aldrich, St. Louis, MO); AASMCs: (1) normoxia (2) hypoxia (3) normoxia, co-culture with ATII or RLE-6TN (4) hypoxia, co-culture with ATII or RLE-6TN (5) hypoxia, co-culture with ATII or RLE-6TN + NAC (10 mmol/L).

To further confirm that AECs participated in the proliferation of PASMCs or AASMCs,the culture medium of PASMCs and AASMCs were replaced by AECs culture medium under normoxic or hypoxic conditions every 12 hours. The cells were grouped as follows, PASMCs: (1) normoxia (2) normoxia + ATII or RLE-6TN normoxic culture medium (3) normoxia + ATII or RLE-6TN hypoxic culture medium(4) hypoxia (5) hypoxia + ATII or RLE-6TN normoxic culture medium (6) hypoxia + ATII or RLE-6TN hypoxic culture medium; AASMCs: (1) normoxia (2) normoxia + ATII or RLE-6TN normoxic culture medium (3) normoxia + ATII or

RLE-6TN hypoxic culture medium(4) hypoxia (5) hypoxia + ATII or RLE-6TN normoxic culture medium (6) hypoxia + ATII or RLE-6TN hypoxic culture medium.

To determine the effects of H_2O_2 on the proliferation of PASMCs or AASMCs, concentration- response was constructed by cumulative addition of exogenous H_2O_2 (5-1000 μM). The H_2O_2 concentration of the largest proliferation of PASMCs or AASMCs was selected and then treated with NAC to observe the change of proliferation.

MTT Assay

All cells were quiesced in serum-free medium for 24 hours after growing to subconfluence and then cultured with 5% fetal bovine serum for 48 hours under normoxic or hypoxic conditions. Subsequently, MTT (5 mg/mL) was added into the plates (80μL/well in 24-well plates or 20μL/well in 96-well plates) and incubated for another 4 hours. Dimethyl sulfoxide was added into each well, and all plates were shaken for 10 minutes in a shaker. The optical density (OD) values were collected using a spectrophotometer (PowerWave XS, BioTekInc, Winooski, VT).

Cell counting

To better investigate the effects of hypoxia on cell proliferation, direct cell counting was performed. Cells were seeded 6×10^4 cells in 6-well plates and cultured overnight. The cells were then incubated in serum-free for 24 h. Cells were cultured with normoxic (21% O_2) or hypoxic conditions (10% O_2 and 5% O_2), and after 48 h they were washed with phosphate buffered solution, harvested by mild trypsinization, and counted with a hematocytometer.

qRT-PCR

qRT-PCR was performed with SYBR PrimeScript™ RT-PCR kit (TakaRa, Dalian, China). The total RNA of cells was extracted using Trizol (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was synthesized from RNA.GAPDH was used as an internal control. Primer sequences were designed using the Primer Premier 5.0 software (PREMIER Biosoft International, CA, USA) and synthesized by the DNA Bio Tec (Shanghai, China).Primers sequences used were asfollows:forward:5’-TGGGAAACAACACCCCTATTTT-3’and reverse: 5’-CGAAGATACCACCAGTCGTAGTTG-3’for CAT; forward: CTGTGGCTGAGCTGTTGTAA and reverse: ACAGCGTCCAAGCAATTCAA for SOD_2; forward: 5’-CTATCGGCAATGAG CGGTTC-3’and reverse: 5’–GATCTTGATCTTCATGGTGCTAGG-3’for GAPDH.

Measurement of ROS

PASMCs or AASMCs in N48, H48, H48 + NAC (10 mmol/L) groups were stained with an oxidant-sensitive fluorescence dye DCFH-DA (10 μmol/L, Nanjing Jian Cheng Bioengineering Institute, Nanjing, China). Subsequently, the intracellular total ROS were detected through fluorescence microscopy (Leica, Heidelberg, Germany) and flow cytometry.

Measurement of H_2O_2

The content of H_2O_2 in AECs and its culture medium were detected using a commercially available Hydrogen Peroxide Assay Kit (Beyotime Inc, Jiangsu, China) according to the recommended protocols. The concentrations of H_2O_2 in different groups were finally normalized to the corresponding protein concentrations.

Measurement of SOD

The content and activity of SOD in AECs was detected using a commercially Tatal Superoxide Dismutase Assay Kit with WST-8 (Beyotime Inc, Jiangsu, China) according to the recommended protocols. The content of SOD in different groups was finally normalized to the corresponding protein concentrations. The activity of SOD is calculated by the formula.

Isolated pulmonary artery ring preparation

Pulmonary artery and aortic artery were obtained according to published articles[25].Rats was anesthetized with 20% ethylurethanm (4 ml/kg). Lung and heart were removed and immersed into cold oxygenated Krebs-Henseleit solution (in mM: NaCl 127, KCl 4.7, $NaHCO_3$ 17, $MgSO_4$ 1.17, KH_2PO_4 1.18, $CaCl_2$ 2.5, D-glucose 5.5) after median sternotomy was performed. Under a dissecting microscope, the third-division (external diameter < 300 μm) pulmonary artery and aorta were isolated carefully and cleared of connective tissue and cut into 3-mm-length rings. Endothelium was removed by gently rubbing the lumen with swab in rings. Pulmonary ring and aorta ring were suspended respectively on stainless steel hook connected to force transducers (AD Instruments, Colorado Springs, CO) for isometric force recording in individual water jacketed organ chamber containing modified Krebs-Henseleit solutions parged continuously with 95% O_2/5% CO_2 at 37 °C. Force displacement was recorded with Power Lab (AD Instruments) eight-channel data acquisition system (sampling rate 100/s).

Experimental protocols

The pulmonary artery ring and aortic artery ring were stretched to a predetermined optimal passive tension of 500 mg and 1000 mg respectively and equilibrated for 60 min with three washouts at 20-min intervals. Then reproducibility of contractile responses to 1 μM phenylephrine (> 300 mg) was established. The rings were rinsed with Krebs-Henseleit solution and the tension returned to baseline. To determine the effects of H_2O_2 on the constriction of pulmonary artery and aortic artery, concentration-response curve was constructed by cumulative addition of exogenous H_2O_2 (5-1000 μM) at 10-min interval on ring. The H_2O_2 concentration of the largest contractile force of artery was selected and then treated with NAC after 10 min to observe the change of contractile force. To further confirm that AECs participated in the constriction of pulmonary artery and aortic artery, pulmonary artery ring and aortic ring from HPH and normoxic rats had treatment with AECs culture medium and NAC at 10-min intervals to observe the change of contractile force. The medium with 5% fetal bovine serum was used as a negative control. In the experiments involving NAC and H_2O_2, darkened conditions were employed where kymograph chambers were wrapped in foil with overhead lights switched off.For the vasoconstriction experiment, 1 μmol/L PE was used. Vascular ring contraction to maximum was labeled as 100%, the

contraction data for each group was expressed as a percentage of the maximum contraction caused by 1 μmol/L PE.

Statistical analysis

Using SPSS 20.0 software to perform statistical analysis of the data, each group of data is represented by an average ± standard deviation (mean ± SD).And statistical analysis was assessed by analysis of variance (one-way ANOVA) for multiple group comparisons and pairing T test for two group comparisons. A significant difference was accepted as significant if $P < 0.05$.

Results

Hypoxia induced pulmonary artery pressure elevation, pulmonary and bronchial artery remodeling, but did not mCAP and renal artery remodeling

The RVSP was measured by catheterization via jugular vein to right ventricle, which substitutes for the pulmonary pressure. Four weeks after exposure to hypoxia, RVSP (Fig. 1A) and RV/ (LV + S) % (Fig. 1C) were significantly elevated in hypoxic group compared with normoxic group, while hypoxia did not influence mCAP considerably (Fig. 1B). MT% and MA% of PA and bronchial artery (BA) were significantly elevated in hypoxic group compared with normoxic group (Fig. 2A and 2B), while hypoxia did not influence MT% and MA% of renal artery (RA) considerably (Fig. 2A and 2B). In vitro, Hypoxia promoted the proliferation of PASMCs (Fig. 3A and 3B) and did not affect the proliferation of AASMCs (Fig. 3C and 3D) compared with normoxic group. These results suggested that the different reactions of pulmonary artery and systemic artery to hypoxia may be related to the microenvironment in which they are located.

Co-culture with AECs promoted the proliferation of PASMCs or AASMCs under hypoxia

To explore the effect of AEC on PASMCs or AASMCs in vitro, the level of proliferation of PASMCs or AASMCs were detected by MTT. Under normoxia, co-culture with ATII had no significant effect on the proliferation of PASMCs or AASMCs compared with without ATII group (Fig. 4A and 4B), and co-culture with RLE-6TN inhibited the proliferation of PASMCs or AASMCs compared with without RLE-6TN group (Fig. 4C and 4D). Under hypoxia, co-culture with ATII or RLE-6TN promoted significantly the proliferation of PASMCs or AASMCs compared with without ATII (Fig. 4A and 4B) or RLE-6TN group (Fig. 4C and 4D).These data indicated that AECs play an important role in the proliferation of vascular smooth muscle cells in hypoxia.

AECs hypoxic culture medium promoted the proliferation of PASMCs or AASMCs

To investigate the involvement of AECs conditioned medium in the proliferation of PASMCs or AASMCs, the culture medium of PASMCs or AASMCs were replaced by ACE conditioned culture medium to observe their proliferation. Under normoxia, the normoxic culture medium of ATII had no significant effect on the proliferation of PASMCs or AASMCs compared with without ATII culture medium group (Fig. 5A and 5B), and the normoxic culture medium of RLE-6TN inhibited the proliferation of PASMCs or AASMCs compared with without RLE-6TN culture medium group(Fig. 5C and 5D), while ATII or RLE-6TN hypoxic culture medium promoted significantly the proliferation of PASMCs or AASMCs compared with without ATII or RLE-6TN culture medium group(Fig. 5A, B, C, D). Under hypoxia, the normoxic culture medium of ATII or RLE-6TN had no significant effect on the proliferation of PASMCs or AASMCs compared with without ATII or RLE-6TN culture medium group (Fig. 5A, B, C, D), while ATII or RLE-6TN hypoxic culture medium promoted significantly the proliferation of PASMCs or AASMCs compared with without ATII or RLE-6TN culture medium group (Fig. 5A, B, C, D). These data indicated that AECs hypoxic culture medium promoted the proliferation of PASMCs and AASMCs and also proved that the microenvironment induced by AECs in hypoxia play an important role in the proliferation of vascular smooth muscle cells.

AECs hypoxic culture medium promoted the constriction of PA and AA

To explore whether AECs culture medium can regulate pulmonary or aortic artery ring, we further examined the effects of AECs culture medium on PA or AA from normoxic and HPH rats. For normoxic rats, the normoxic culture medium from ATII or RLE-6TN had no significant effect on the constriction of PA (Fig. 6A and 6B) and AA (Fig. 7A and 7B) compared with the culture medium group, while the hypoxic culture medium from ATII or RLE-6TN promoted the constriction of PA (Fig. 6A and 6B) and AA (Fig. 7A and 7B) compared with the culture medium or the normoxic culture medium group. For HPH rats, the normoxic culture medium from ATII or RLE-6TN had no significant effect on the constriction of AA (Fig. 9A and 9B) compared with the culture medium group, and the normoxic culture medium from ATII had also no significant effect on the constriction PA (Fig. 8A) compared with culture medium group, but the normoxic culture medium from RLE-6TN promoted the constriction of PA (Fig. 8B), and the hypoxic culture medium from ATII or RLE-6TN promoted the constriction of PA (Fig. 8A and 8B) and AA (Fig. 9A and 9B) compared with the culture medium or the normoxic culture medium group. These results showed that AECs hypoxic culture medium could induce the constriction of PA and AA and also proved that the microenvironment induced by AECs in hypoxia play an important role in the vasoconstriction.

Effect of hypoxia on ROS in AECs

To explore the possible mechanism involved the effect of AECs on the proliferation of PASMCs or AASMCs, total intracellular ROS was detected through DCFH-DA and the content of H_2O_2 in AECs and its culture medium were determined by commercial kit. Hypoxia increased the production of ROS and H_2O_2 in AECs and AECs culture medium (Fig. 10C, D, and E). Meanwhile, we examined mRNA level of SOD2 and CAT by QT-PCR, and detected the total content and activity of SOD in AECs by commercial kits. Hypoxia increased the mRNA of SOD_2 (Fig. 11A) and the total content and activity of SOD (Fig. 10A and 10B),

while hypoxia have no significant effect on the mRNA of CAT (Fig. 11B). These results showed that the increase of ROS and H_2O_2 in AECs was caused by imbalance of SOD2 and CAT in hypoxia.

Effect of exogenous H_2O_2 on the proliferation of PASMCs or AASMCs

Some studies have reported that the effects of different concentrations of H_2O_2 on cell proliferation were different. We used exogenous 0-1000 μM H_2O_2 to observe how H_2O_2 affects the proliferation of PASMCs or AASMCs under normoxia. Results showed that 0–50 μM H_2O_2 led to a dose-dependent increase in the proliferation of PASMCs, and then the proliferation of PASMCs is gradually inhibited by 100–1000 μM H_2O_2 (Fig. 12A).Similar to the effects of H_2O_2 on PASMCs, 0-100 μM H_2O_2 led to a dose-dependent increase in the proliferation of AASMCs, and then the proliferation of AASMCs is gradually inhibited by 200–1000 μM H_2O_2 (Fig. 12B). To observe the effect of NAC on PASMCs or AASMCs proliferation, we choose 50 μM and 100 μM H_2O_2, which were the most obvious concentration that affected the proliferation of PASMCs and AASMCs respectively. Results showed that NAC (5 mM and 10 mM) effectively inhibited the proliferation of PASMCs or AASMCs, of which the effect of 10 mM NAC was most obvious (Fig. 12C and 12D).

Effect of exogenous H_2O_2 on the constriction of PA or AA

To explore whether H_2O_2 regulate PA or AA ring, we used exogenous H_2O_2 (5-1000 μM) to observe how H_2O_2 affected the constriction of PA or AA from normal rats. Results showed that 5-400 μM H_2O_2 led to a dose-dependent increase in the constriction of PA, and then the constriction of PA is gradually inhibited by 600–1000 μM H_2O_2 (Fig. 13A). Similar to the effects of H_2O_2 on PA, 5-600 μM H_2O_2 led to a dose-dependent increase in the constriction of AA, and then the constriction of AA is gradually inhibited by 800–1000 μM H_2O_2 (Fig. 13B). To observe the effect of NAC on PA or AA constriction, we choose 400μM and 600 μM H_2O_2, which were the most obvious concentration that affected constriction of PA and AA respectively. Results showed that 10 mM NAC effectively inhibited the constriction of PA or AA (Fig. 14A and 14B).

NAC effectively inhibited the proliferation of PASMCs or AASMCs co-cultured with AECs

The concentration of H_2O_2 in AECs cell culture medium for 24 h in hypoxia was detected in the range of promoting cell proliferation (Fig. 10E). NAC intervention was performed in the co-culture medium. The proliferation of PASMCs (Fig. 15A and 15B) and AASMCs (Fig. 15C and15D) was effectively inhibited by10mM NAC. This experiment suggested that H_2O_2 from ATII or RLE-6TN was involved in the proliferation of PASMCs or AASMCs.

NAC effectively inhibited the constriction of PA or AA

The concentration of H_2O_2 in AECs culture medium for 24 h in hypoxia was detected in the range of promoting constriction. NAC intervention was performed in the hypoxic culture medium from ATII or RLE-6TN, the constriction of PA (Fig. 16A and 17A) or AA (Fig. 16B and 17B) was effectively inhibited by 10 mM NAC. This experiment suggested that H_2O_2 from ATII or RLE-6TN was involved in the constriction of PA or AA.

Discussion

In this study, we first showed that AECs not only caused the proliferation of PASMCs, but also induced proliferation of AASMCs under hypoxia, and the hypoxic culture medium of AECs promoted both constriction of PA or AA in vitro. At the same time, we also confirmed that it was related to H_2O_2 derived from AECs which was induced by the imbalance between SOD2 and CAT in AECs. NAC, the inhibitor of ROS, markedly ameliorated the proliferation of PASMCs or AASMCs and the constriction of PA or AA. The present study first demonstrated that the difference in pressure variation between pulmonary artery and aortic artery in HPH rats was related to pulmonary microenvironment in which AECs involved, and the effect of AECs on the remodeling and constriction of pulmonary vessel was through H_2O_2.

In 1969, researches have confirmed that only alveolar hypoxia caused hypoxic pulmonary vasoconstriction, and hypoxemia did not [26, 27]. Though bronchial artery belongs to systemic circulatory vessel, it is also exposed to pulmonary microenvironment and has undergone remodeling. In the case of ventilatory dysfunction and low oxygen in the plateau, AECs is the first to perceive hypoxia. Therefore, we thought that AECs should play an important role in HPH. In our study, we constructed the model of HPH to observe hemodynamic and morphological of PA, BA and RA. The results showed that hypoxia led to RVSP, RV/ (LV + S) %, MT% and MA% of PA and BA significantly elevate, while did not mCAP and MT% and MA% of RA. In vitro, co-culture with AECs and treatment with AECs hypoxia culture medium significantly promoted not only PASMCs proliferation and PA constriction, but also AASMCs proliferation and AA constriction. Therefore, in vivo and in vitrodata suggested that AECs played a critical role on HPH, and in vitrodata shown thatsystemic circulatory vessel placed in the microenvironment where AECs exists also undergone remodeling and constriction.

It is interesting to consider the regulation of key antioxidant enzymes such as SODand CATin evaluating the role of redox-regulating signaling in pulmonary vascular diseases [28]. SOD catalyze the rapid dismutation of superoxide (O2•-) to hydrogen peroxide (H_2O_2) and molecular oxygen, however, CAT catalyzes the two-stage conversion of H_2O_2 to water and oxygen[28].H_2O_2 is a stable and easily diffused out of the cell[14]. SOD has three isoforms, which are located in the cytosol (SOD1), mitochondria (SOD2) or extracellular compartment (SOD3)[29]. Accumulating evidence suggested that impaired activity of SOD2 and SOD3contributed to pulmonary hypertension, and the level and activity of SOD1 has not been found significantly perturbed in human pulmonary hypertension[30, 31]. Our data suggested that the mRNA level of SOD2 and the content and activity of total SOD were increase in AECs under hypoxia, while the mRNA level of CAT have no significant change. And the content of ROS and H_2O_2 in AECs and H_2O_2

in AECs culture medium also were increase under hypoxia. Hence, in vitro data suggested that H_2O_2 derived from AECs affected pulmonary microenvironment.

Recently, an increasing number of studies showed that H_2O_2 can regulate a variety of cellular functions, including proliferation, differentiation, and more generally gene expression[32, 33]. However, quantitative data on the basal concentrations of H_2O_2 and the thresholds for cell proliferation are limited, and it is also reported that treatment with a continuous extracellular source of H_2O_2 increases cell proliferation [22, 34]. The present study provided the range of concentrations of exogenous H_2O_2 for PASMCs and AASMCs proliferation and that hypoxia increased intracellular and extracellular H_2O_2 of AECs. Moreover, the extracellular H_2O_2 concentration of AECs was within the range of H_2O_2 concentrations for PASMCs and AASMCs proliferation. Co-culture with AECs under hypoxic or the intermittent replacement of AEC hypoxic culture medium which were consistent with the conditions of continuous extracellular source of H_2O_2 induced PASMCs or AASMCs proliferation. In other words, the method described above effected the proliferation of PASMCs or AASMCs by changing the microenvironment of PASMCs and AASMCs. In addition, NAC intervention reduced the proliferation PASMCs and AASMCs co-cultured with AECs under hypoxia, which further indicated extracellular H_2O_2 derived from AECs play important role in PASMCs and AASMCs proliferation under hypoxia. Jin N [35]reported that exposure to H_2O_2 cause smooth muscle constrictions and dysfunction in isolated pulmonary artery. The present study also provided the range of exogenous H_2O_2 concentrations for PA or AA constriction. Moreover, the extracellular H_2O_2 concentration of AECs in hypoxia was within the range of H_2O_2 concentrations for PA and AA constriction. AECs hypoxic culture medium induced PA and AA constrictions, and NAC intervention could reduce this effect. It's indicated hypoxic culture mediumH_2O_2derived from AECs played important role in PA and AA constriction. Taken together, these in vitro data indicated AECs involved in remodeling and constriction of pulmonary vessel through releasingH_2O_2under hypoxia. Meanwhile, it also showed that systemic circulatory vessel also undergo the same change as pulmonary vessel in the AECs-induced microenvironment by H_2O_2under hypoxia.

Conclusion

The proliferation of PASMCs and constrictions of PA are essential for the development of HPH. Our study had shown that the pulmonary microenvironment was beneficial to HPH and AECs played a crucial part in construct an pulmonary microenvironment. The continuous extracellular source of ROS is necessary to induce PASMCs proliferation and PA constriction. H2O2 derived from AECs influenced the pulmonary microenvironment and also was involved in pulmonary vascular remodeling and constriction in HPH.

Abbreviations

HPH: Hypoxic pulmonary hypertension; AECs: alveolar epithelial cells; PA: pulmonary artery; AA: aortic artery; PASMCs :pulmonary artery smooth cells; AASMCs :aortic artery smooth cells; SOD2:superoxide

dismutase 2; CAT: catalase; ROS {reactive oxygen species, (); hydrogen peroxide, (H2O2); N-acetylcysteine, (NAC);

Declarations

Disclosure statement

The authors declare that they have no conflicts of interest

Authors' contributions

Yanxia Wang and Xiaoming Li contributed to the experimental design and overall experimentation, Wen Niu, Jian Chen, Bo Zhang, Xiumin Zhang and Yingmei Wang conceptualized the project and data analysis, Zhichao Li and Shaokang Dang contributed to the experimental design, funding, and writing of the manuscript.

Funding

This present study was funded by the National Nature Science Foundation of China (grant nos. 31670328, 81800046, 81471816 and 81571839).

Availability of data and materials

We would like to share part of our data, because some of our date will be used in future research.

Ethics approval and consent to participate

The study was approved and supervised by the Medical Research Ethics Committee of the Fourth Military Medical University. All participants fully understood the information files. Informed consent was obtained from all participants. All experiments were performed in accordance with the relevant guidelines and regulations. The animal protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), the Fourth Military Medical University, China

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

References

1. Barbera JA, Blanco I: Pulmonary hypertension in patients with chronic obstructive pulmonary disease: advances in pathophysiology and management. Drugs 2009, 69:1153-1171.

2. Penaloza D, Arias-Stella J: The heart and pulmonary circulation at high altitudes: healthy highlanders and chronic mountain sickness. Circulation 2007, 115:1132-1146.
3. Wilkins MR, Ghofrani HA, Weissmann N, Aldashev A, Zhao L: Pathophysiology and treatment of high-altitude pulmonary vascular disease. Circulation 2015, 131:582-590.
4. Yan D, Li G, Zhang Y, Liu Y: Angiotensin-converting enzyme 2 activation suppresses pulmonary vascular remodeling by inducing apoptosis through the Hippo signaling pathway in rats with pulmonary arterial hypertension. Clin Exp Hypertens 2019, 41:589-598.
5. Liu M, Wang Y, Zheng L, Zheng W, Dong K, Chen S, Zhang B, Li Z: Fasudil reversed MCT-induced and chronic hypoxia-induced pulmonary hypertension by attenuating oxidative stress and inhibiting the expression of Trx1 and HIF-1alpha. Respir Physiol Neurobiol 2014, 201:38-46.
6. Zakynthinos E, Daniil Z, Papanikolaou J, Makris D: Pulmonary hypertension in COPD: pathophysiology and therapeutic targets. Curr Drug Targets 2011, 12:501-513.
7. Wang LN, Yu WC, Du CH, Tong L, Cheng ZZ: Hypoxia is involved in hypoxic pulmonary hypertension through inhibiting the activation of FGF2 by miR-203. Eur Rev Med Pharmacol Sci 2018, 22:8866-8876.
8. Makino A, Firth AL, Yuan JX: Endothelial and smooth muscle cell ion channels in pulmonary vasoconstriction and vascular remodeling. Compr Physiol 2011, 1:1555-1602.
9. Zhu P, Huang L, Ge X, Yan F, Wu R, Ao Q: Transdifferentiation of pulmonary arteriolar endothelial cells into smooth muscle-like cells regulated by myocardin involved in hypoxia-induced pulmonary vascular remodelling. Int J Exp Pathol 2006, 87:463-474.
10. Luo Y, Dong HY, Zhang B, Feng Z, Liu Y, Gao YQ, Dong MQ, Li ZC: miR-29a-3p attenuates hypoxic pulmonary hypertension by inhibiting pulmonary adventitial fibroblast activation. Hypertension 2015, 65:414-420.
11. Gore B, Izikki M, Mercier O, Dewachter L, Fadel E, Humbert M, Dartevelle P, Simonneau G, Naeije R, Lebrin F, Eddahibi S: Key role of the endothelial TGF-beta/ALK1/endoglin signaling pathway in humans and rodents pulmonary hypertension. PLoS One 2014, 9:e100310.
12. Smith KA, Schumacker PT: Sensors and signals: the role of reactive oxygen species in hypoxic pulmonary vasoconstriction. J Physiol 2019, 597:1033-1043.
13. Bonnet S, Boucherat O: The ROS controversy in hypoxic pulmonary hypertension revisited. Eur Respir J 2018, 51.
14. Xu Z, Rothstein SJ: ROS-Induced anthocyanin production provides feedback protection by scavenging ROS and maintaining photosynthetic capacity in Arabidopsis. Plant Signal Behav 2018, 13:e1451708.
15. Zuo L, Chuang CC, Clark AD, Garrison DE, Kuhlman JL, Sypert DC: Reactive Oxygen Species in COPD-Related Vascular Remodeling. Adv Exp Med Biol 2017, 967:399-411.
16. Jaitovich A, Jourd'heuil D: A Brief Overview of Nitric Oxide and Reactive Oxygen Species Signaling in Hypoxia-Induced Pulmonary Hypertension. Adv Exp Med Biol 2017, 967:71-81.

17. Roberto D, Micucci P, Sebastian T, Graciela F, Anesini C: Antioxidant activity of limonene on normal murine lymphocytes: relation to H2O2 modulation and cell proliferation. Basic Clin Pharmacol Toxicol 2010, 106:38-44.
18. Hwang JW, Kim EK, Lee SJ, Kim YS, Moon SH, Jeon BT, Sung SH, Kim ET, Park PJ: Antioxidant activity and protective effect of anthocyanin oligomers on H(2)O(2)-triggered G2/M arrest in retinal cells. J Agric Food Chem 2012, 60:4282-4288.
19. Lo YC, Lin YC, Huang YF, Hsieh CP, Wu CC, Chang IL, Chen CL, Cheng CH, Chen HY: Carnosol-Induced ROS Inhibits Cell Viability of Human Osteosarcoma by Apoptosis and Autophagy. Am J Chin Med 2017, 45:1761-1772.
20. Costa RM, Filgueira FP, Tostes RC, Carvalho MH, Akamine EH, Lobato NS: H2O2 generated from mitochondrial electron transport chain in thoracic perivascular adipose tissue is crucial for modulation of vascular smooth muscle contraction. Vascul Pharmacol 2016, 84:28-37.
21. Li Z, Fang F, Xu F: Effects of different states of oxidative stress on fetal rat alveolar type II epithelial cells in vitro and ROSinduced changes in Wnt signaling pathway expression. Mol Med Rep 2013, 7:1528-1532.
22. Sigaud S, Evelson P, Gonzalez-Flecha B: H2O2-induced proliferation of primary alveolar epithelial cells is mediated by MAP kinases. Antioxid Redox Signal 2005, 7:6-13.
23. Wang YX, Liu ML, Zhang B, Fu EQ, Li ZC: Fasudil alleviated hypoxia-induced pulmonary hypertension by stabilizing the expression of angiotensin-(1-7) in rats. Eur Rev Med Pharmacol Sci 2016, 20:3304-3312.
24. Takano M, Nagahiro M, Yumoto R: Transport Mechanism of Nicotine in Primary Cultured Alveolar Epithelial Cells. J Pharm Sci 2016, 105:982-988.
25. Wang J, Dong MQ, Liu ML, Xu DQ, Luo Y, Zhang B, Liu LL, Xu M, Zhao PT, Gao YQ, Li ZC: Tanshinone IIA modulates pulmonary vascular response to agonist and hypoxia primarily via inhibiting Ca2+ influx and release in normal and hypoxic pulmonary hypertension rats. Eur J Pharmacol 2010, 640:129-138.
26. Hauge A: Hypoxia and pulmonary vascular resistance. The relative effects of pulmonary arterial and alveolar PO2. Acta Physiol Scand 1969, 76:121-130.
27. Lourenco AP, Fontoura D, Henriques-Coelho T, Leite-Moreira AF: Current pathophysiological concepts and management of pulmonary hypertension. Int J Cardiol 2012, 155:350-361.
28. Song T, Zheng YM, Wang YX: Cross Talk Between Mitochondrial Reactive Oxygen Species and Sarcoplasmic Reticulum Calcium in Pulmonary Arterial Smooth Muscle Cells. Adv Exp Med Biol 2017, 967:289-298.
29. McCord JM, Fridovich I: Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969, 244:6049-6055.
30. Masri FA, Comhair SA, Dostanic-Larson I, Kaneko FT, Dweik RA, Arroliga AC, Erzurum SC: Deficiency of lung antioxidants in idiopathic pulmonary arterial hypertension. Clin Transl Sci 2008, 1:99-106.

31. Nozik-Grayck E, Woods C, Stearman RS, Venkataraman S, Ferguson BS, Swain K, Bowler RP, Geraci MW, Ihida-Stansbury K, Stenmark KR, et al: Histone deacetylation contributes to low extracellular superoxide dismutase expression in human idiopathic pulmonary arterial hypertension. Am J Physiol Lung Cell Mol Physiol 2016, 311:L124-134.
32. Hirobe T, Shibata T, Sato K: Human fibroblasts treated with hydrogen peroxide stimulate human melanoblast proliferation and melanocyte differentiation, but inhibit melanocyte proliferation in serum-free co-culture system. J Dermatol Sci 2016, 84:282-295.
33. Park WH: Exogenous H2O2 induces growth inhibition and cell death of human pulmonary artery smooth muscle cells via glutathione depletion. Mol Med Rep 2016, 14:936-942.
34. Porter KM, Kang BY, Adesina SE, Murphy TC, Hart CM, Sutliff RL: Chronic hypoxia promotes pulmonary artery endothelial cell proliferation through H2O2-induced 5-lipoxygenase. PLoS One 2014, 9:e98532.
35. Jin N, Rhoades RA: Activation of tyrosine kinases in H2O2-induced contraction in pulmonary artery. Am J Physiol 1997, 272:H2686-2692.

Figures

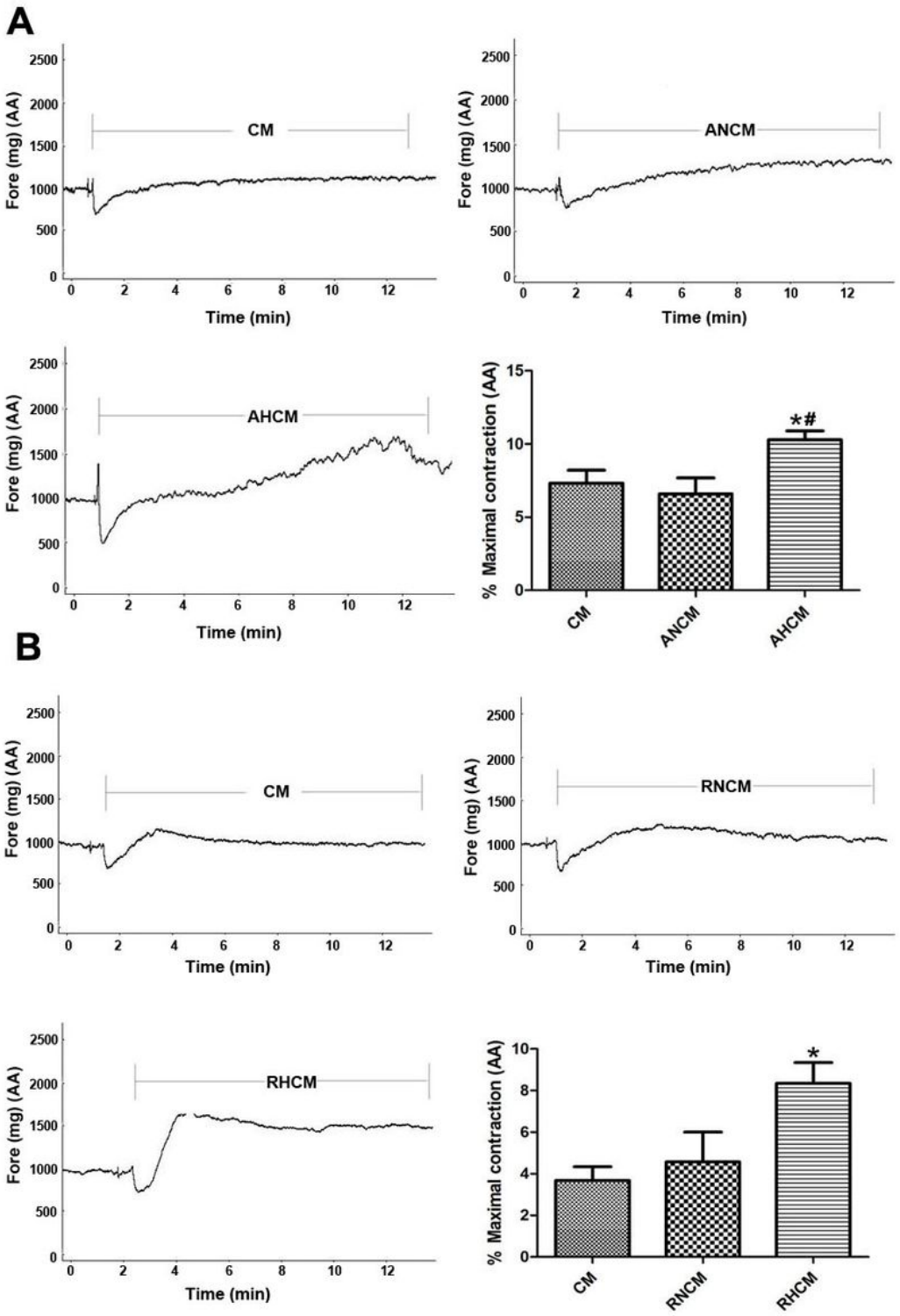

Figure 1

ATII and RLE-6TN hypoxic culture medium promoted the constriction of AA from normoxic rats (A) The effect of ATII normoxic or hypoxic culture medium on the constriction of AA from normoxic rats (B) The effect of RLE-6TN normoxic or hypoxic culture medium on the constriction of AA from normoxic rats.CM= culture medium, ANCM=ATII normoxic culture medium, AHCM= ATII hypoxic culture medium, RNCM=RLE-

6TN normoxic culture medium, RHCM=RLE-6TN hypoxic culture medium. n=5, Data are means ± S.D.*P< 0.05vs.CM.

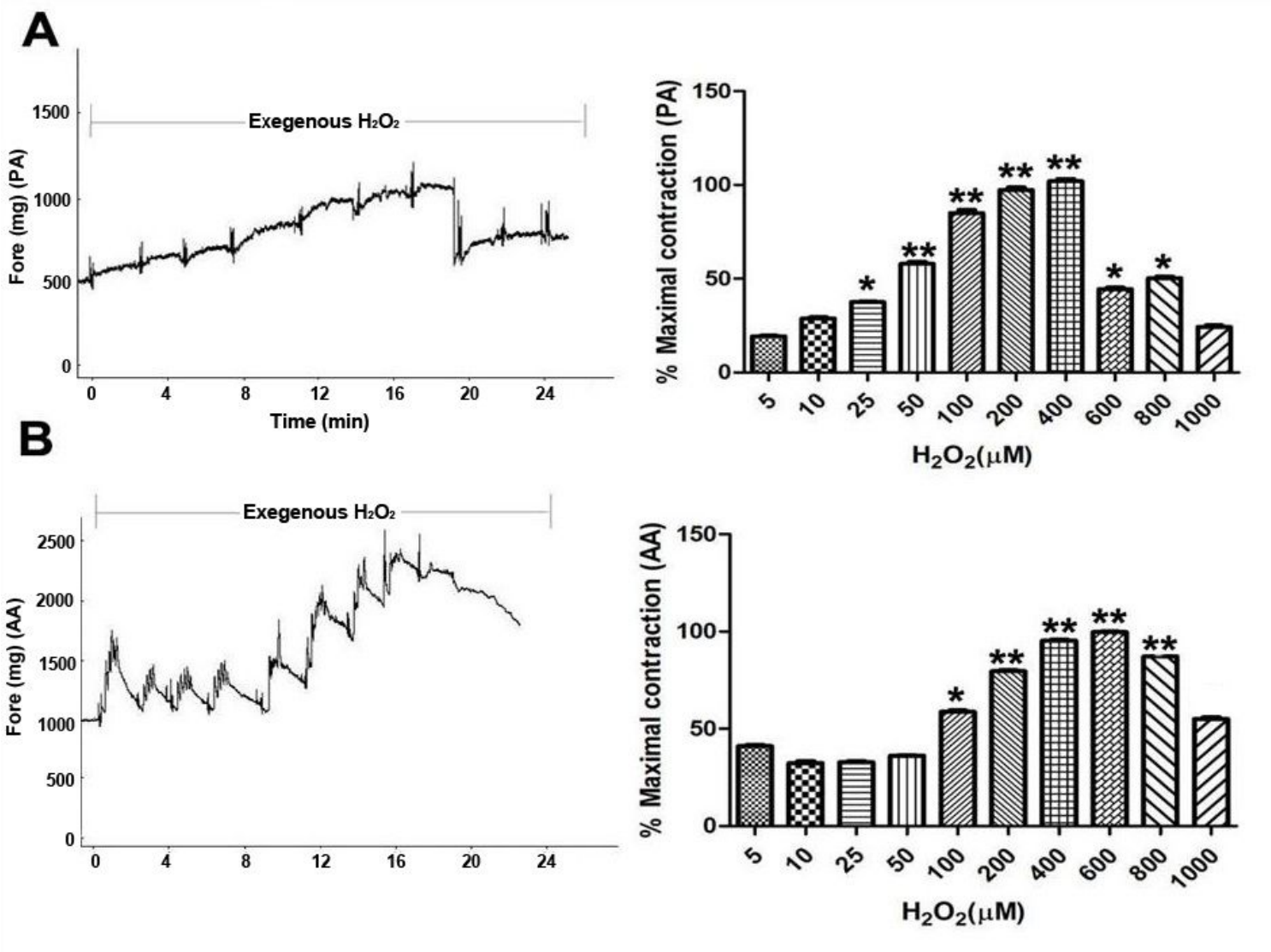

Figure 1

Effect of exogenous H2O2 on the constriction of PA or AA. (A) 5-400 μM H2O2 led to a dose-dependent increase in the constriction of PA, and the constriction of PA was gradually inhibited by H2O2 from 600 μM to1000 μM. (B) 5-600 μM H2O2 led to a dose-dependent increase in the constriction of AA, and the constriction of AA was gradually inhibited by H2O2 from 800 μM to 1000 μM. n=5, Data are means ± S.D.*P< 0.05 or **P< 0.01vs.5μM H2O2.

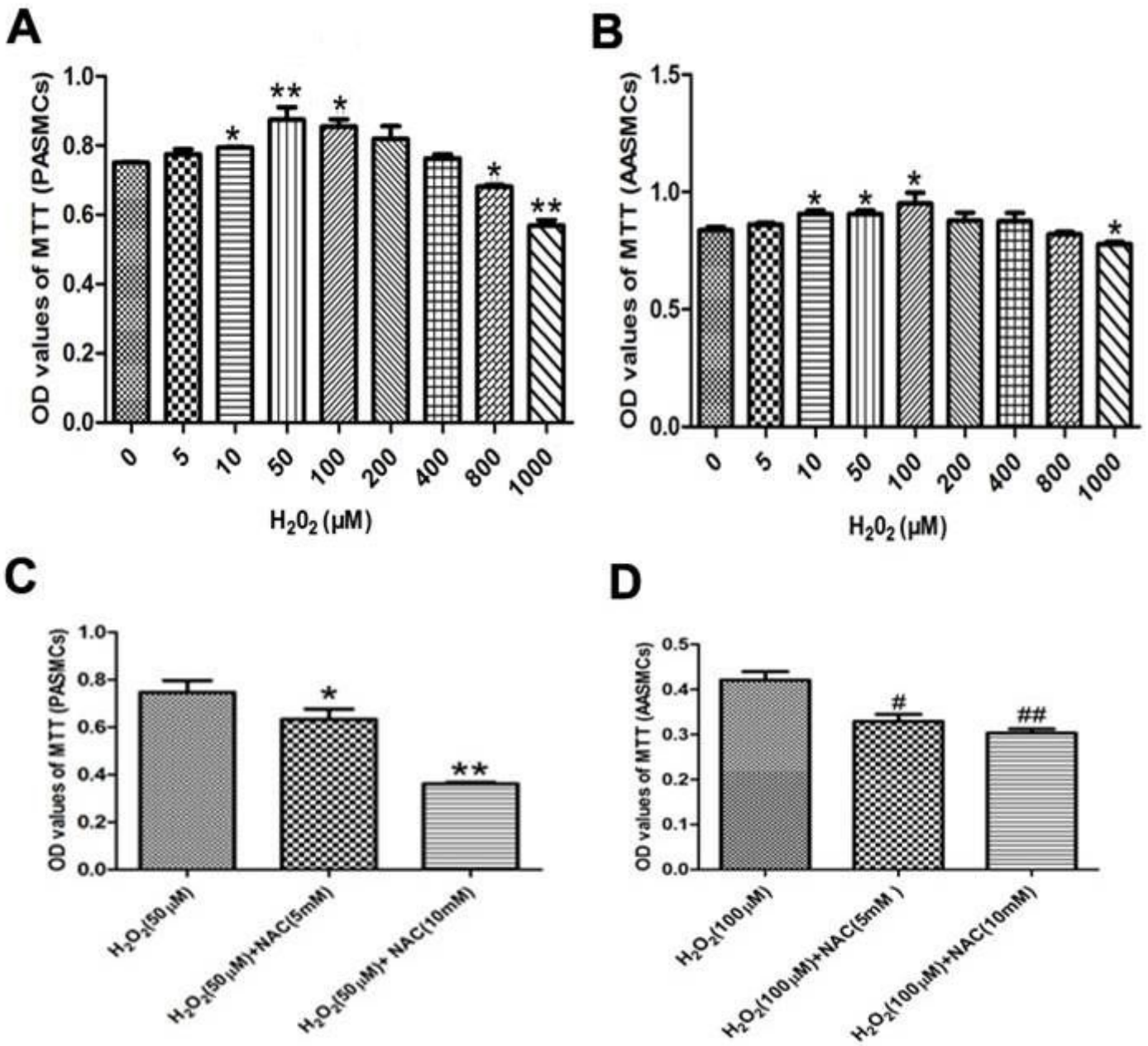

Figure 1

Effect of exogenous H2O2 andNAC intervention on the proliferation of PASMCs or AASMCs. (A) 0-50 μM H2O2 led to a dose-dependent increase in the proliferation of PASMCs, and the proliferation of PASMCs was gradually inhibited by H2O2from 100 μM to 1000 μM (B) 0-100 μM H2O2 led to a dose-dependent increase in the proliferation of AASMCs, the proliferation of AASMCs was gradually inhibited by H2O2 from 200μM to 1000 μM. (C) 5mM and 10mM NAC effectively inhibited the proliferation of PASMCs (D) 5mM and 10mM NAC effectively inhibited the proliferation of AASMCs. n=8, Data are means ± S.D.，A, B， **P< 0.01 or *P< 0.05vs.0 μM H2O2，C, D，**P< 0.01 or *P< 0.05vs.50 μM H2O2, ##P< 0.01 or #P< 0.05vs.100 μM H2O2.

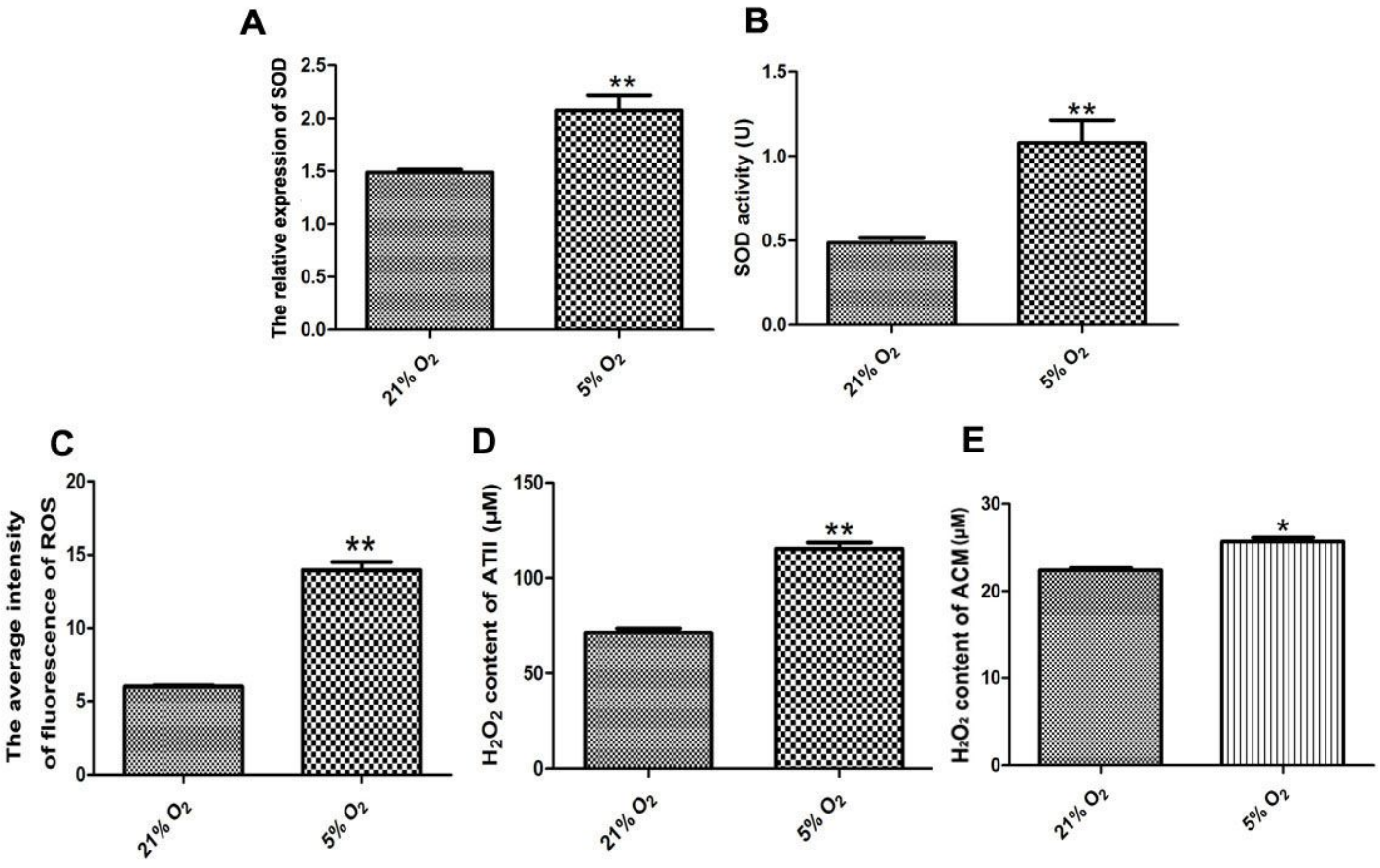

Figure 1

Hypoxia increased total intracellular ROS (A) Hypoxia increased the total content of SOD in ATII (B) Hypoxia increased the activity of SOD in ATII (C) Hypoxia increased the intensity of ROS in ATII (D) Hypoxia increased the content of H2O2 in ATII(E) Hypoxia increased the content of H2O2 in ATII culture medium.

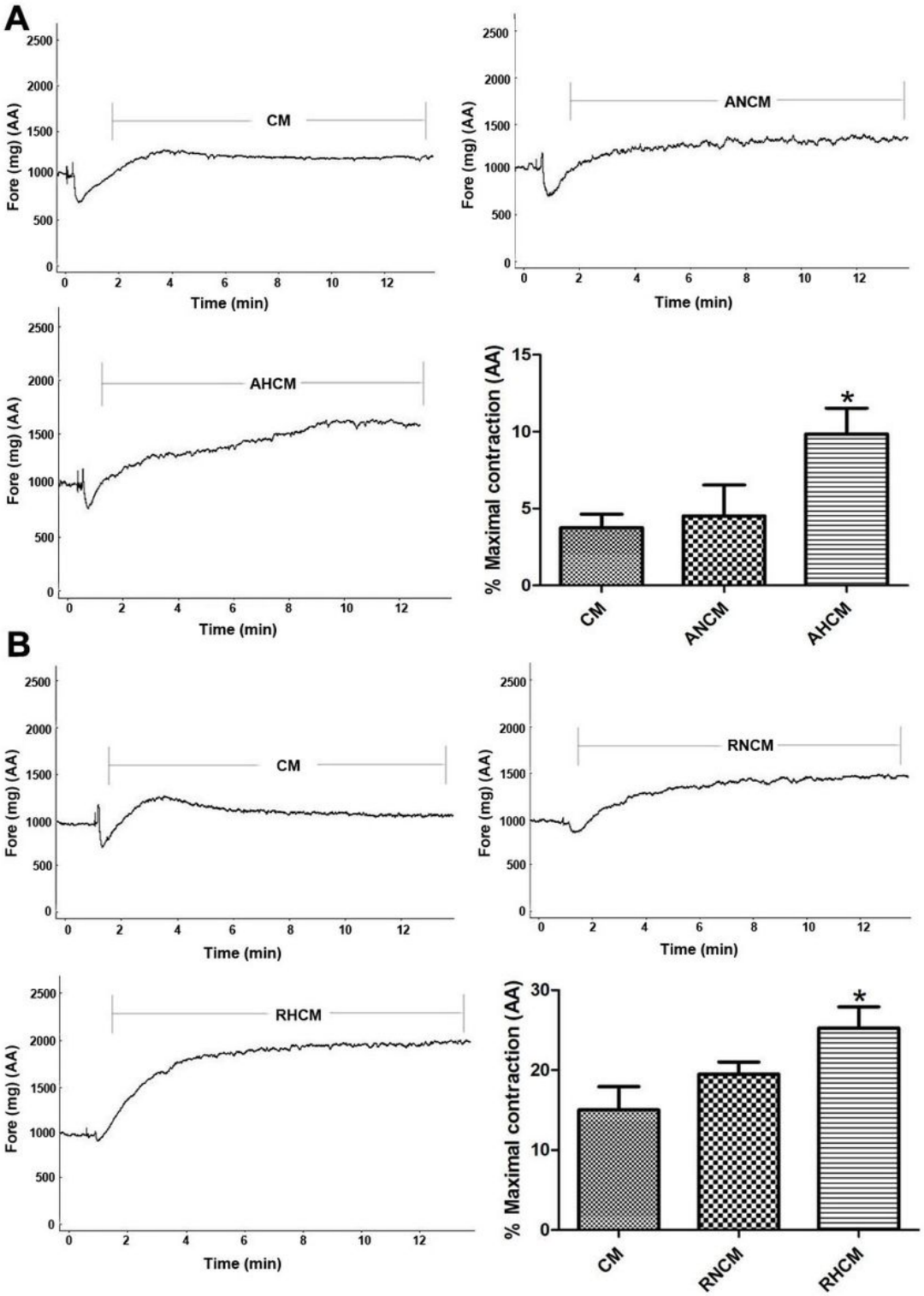

Figure 1

ATII and RLE-6TN hypoxic culture medium promoted the constriction of AA from HPH rats (A) The effect of ATII normoxic or hypoxic culture medium on the constriction of AA from HPH rats (B) The effect of RLE-6TN normoxic or hypoxic culture medium on the constriction of AA from HPH rats.CM=culture medium, ANCM=ATII normoxic culture medium, AHCM=ATII hypoxic culture medium, RNCM=RLE-6TN

normoxic culture medium, RHCM=RLE-6TN hypoxic culture medium. n=5, Data are means ± S.D.*P< 0.05 orvs.CM.

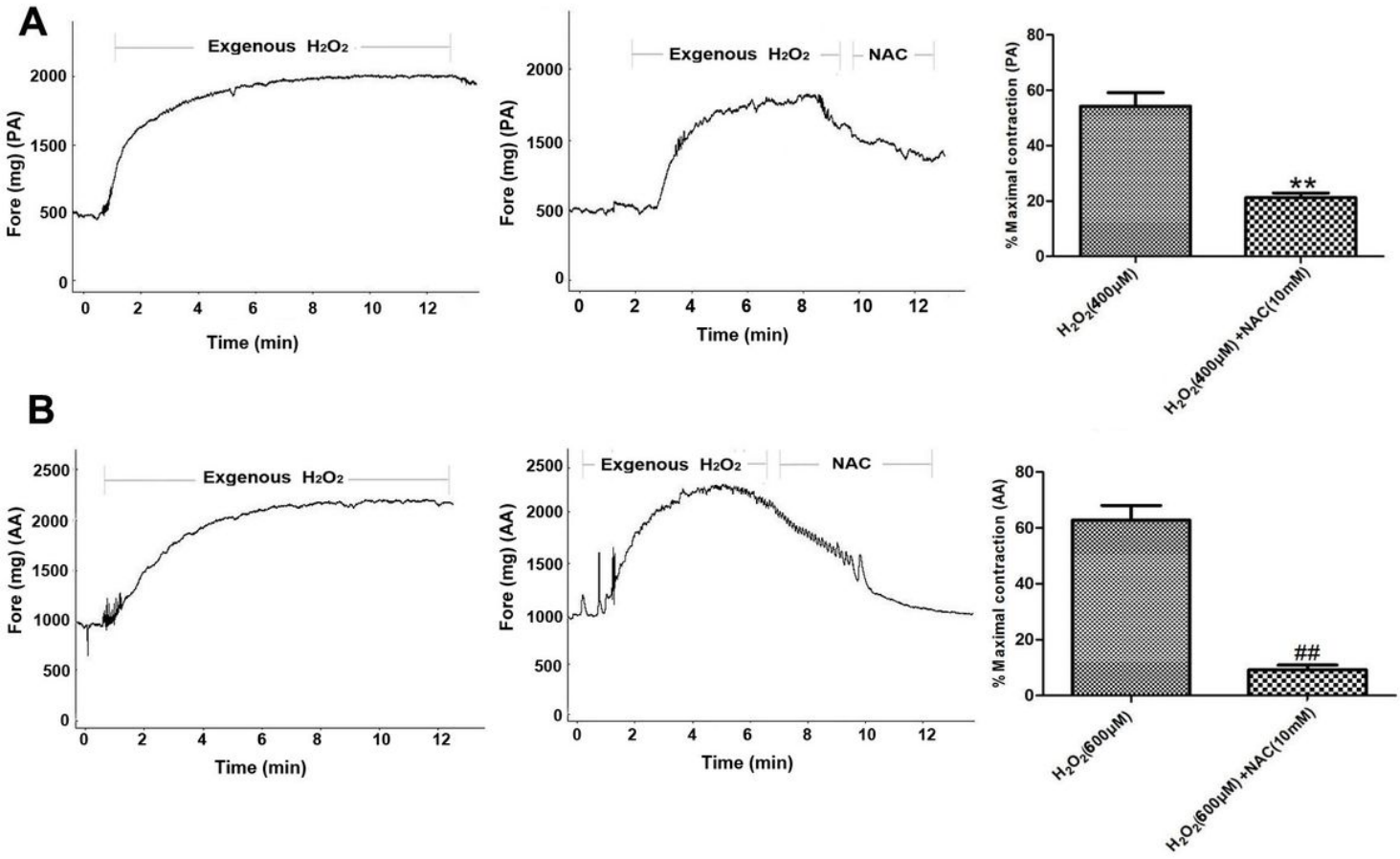

Figure 1

Effect of NAC intervention on the constriction of PA or AA. (A) 10mM NAC effectively inhibited the constriction of PA (B)10mM NAC effectively inhibited the constriction of AA. n=5, Data are means ± S.D. **P< 0.01, ##P< 0.01vs.400 μM H2O2 or 600 μM H2O2.

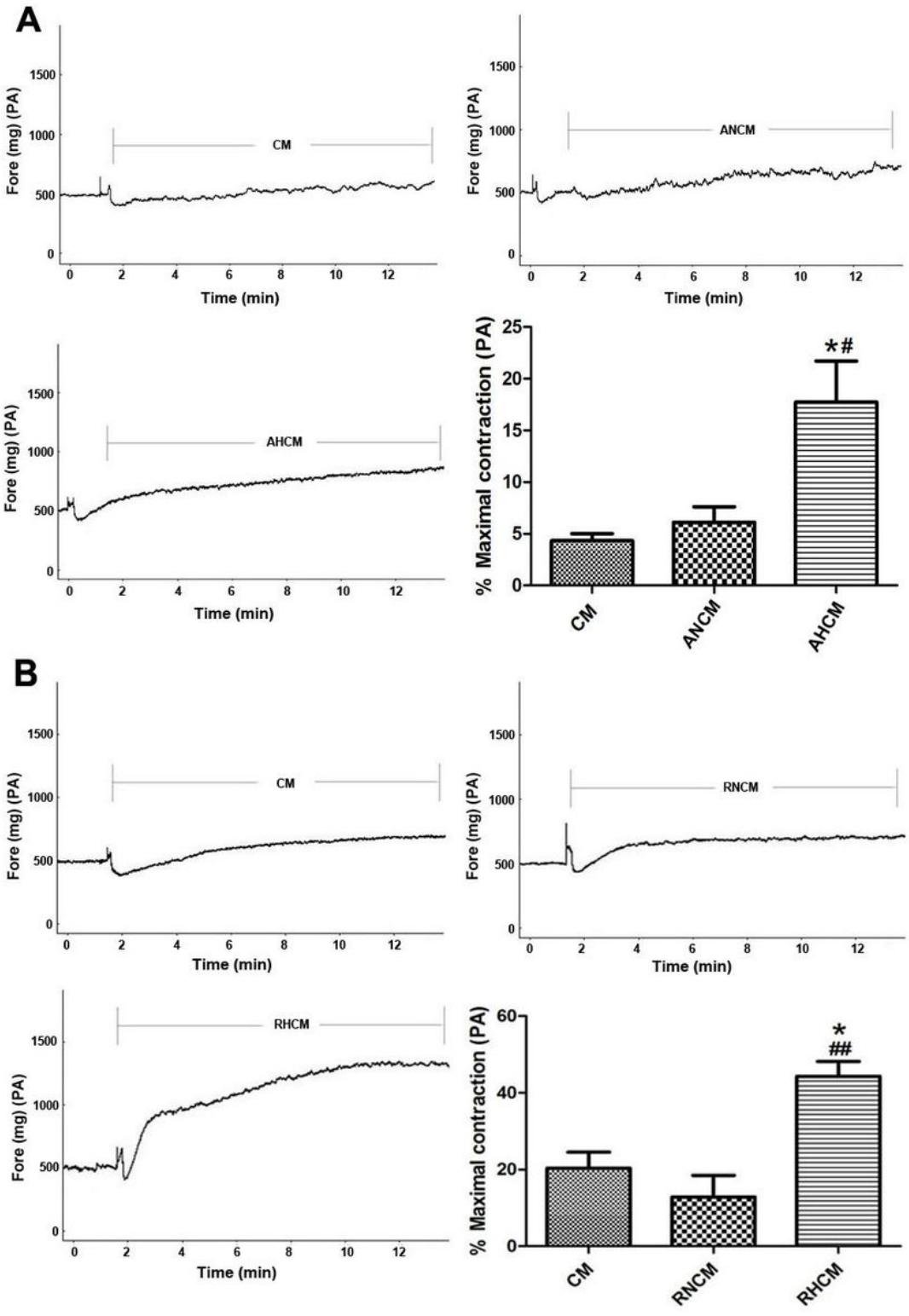

Figure 1

ATII and RLE-6TN hypoxic culture medium promoted the constriction of PA from normoxic rats (A) The effect of ATII normoxic or hypoxic culture medium on the constriction of PA from normoxic rats (B) The effect of RLE-6TN normoxic or hypoxic culture medium on the constriction of PA from normoxic rats.CM=culture medium, ANCM=ATII normoxic culture medium, AHCM= ATII hypoxic culture medium,

RNCM= RLE-6TN normoxic culture medium, RHCM= RLE-6TN hypoxic culture medium. n=5, Data are means ± S.D. *P < 0.05 vs.CM, #P < 0.05 or ##P < 0.01 vs. ANCM or RNCM.

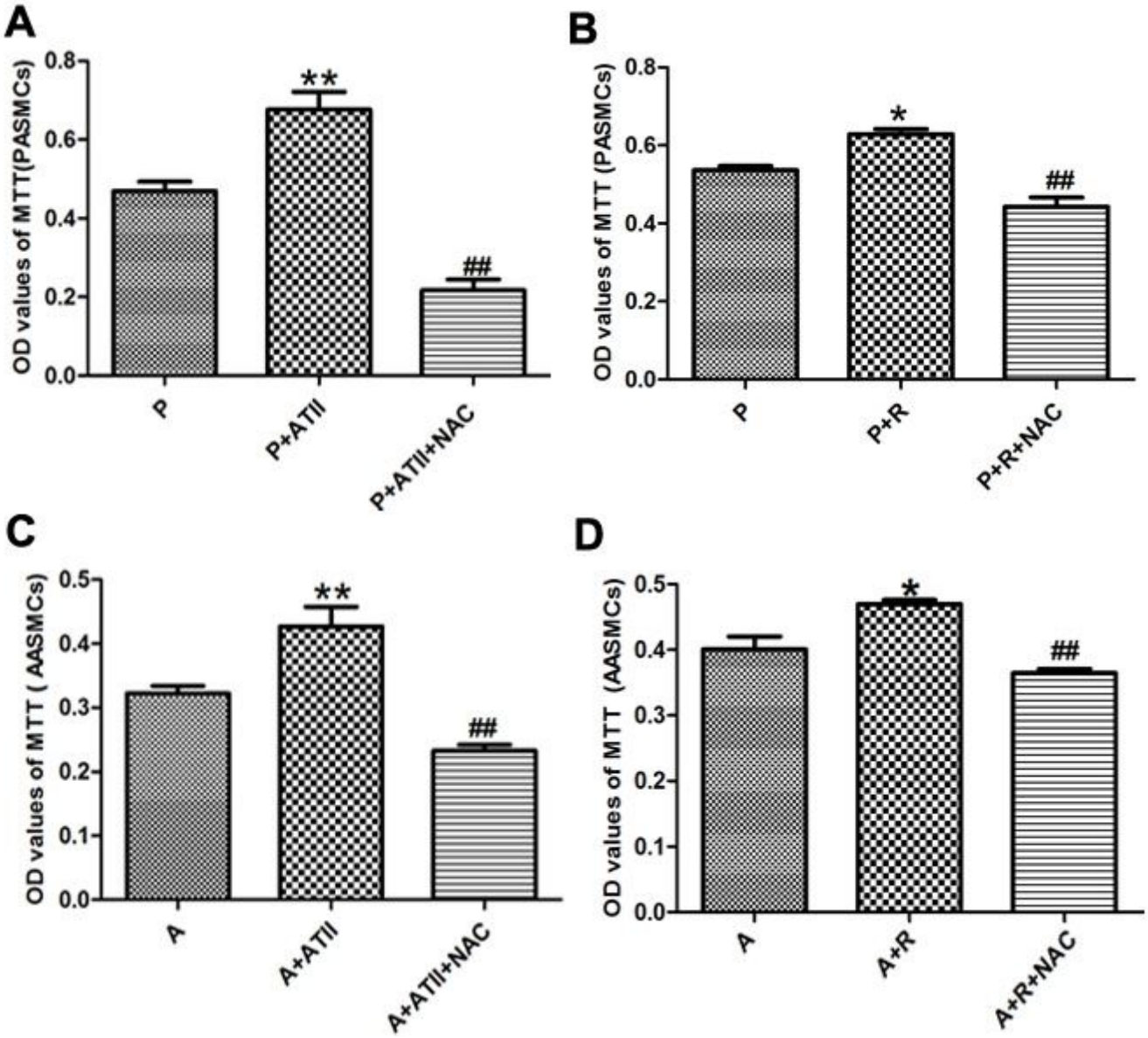

Figure 1

NAC effectively inhibited the proliferation of PASMCs or AASMCs co-cultured with AECs. (A) The proliferation of PASMCs co-cultured with ATII was effectively inhibited by 10 mM NAC (B) The proliferation of PASMCs co-cultured with RLE-6TN was effectively inhibited by 10 mM NAC (C) The proliferation of AASMCs co-cultured with ATII was effectively inhibited by 10 mM NAC (D) The proliferation of AASMCs co-cultured with RLE-6TN was effectively inhibited by 10 mM NAC. P=PASMCs A=AASMCs. n=8, Data are means ± S.D. **P< 0.01,*P< 0.05 vs. P or A, ##P< 0.01, #P< 0.05 vs. P+ATII or P+R, A+ATII or A+R.

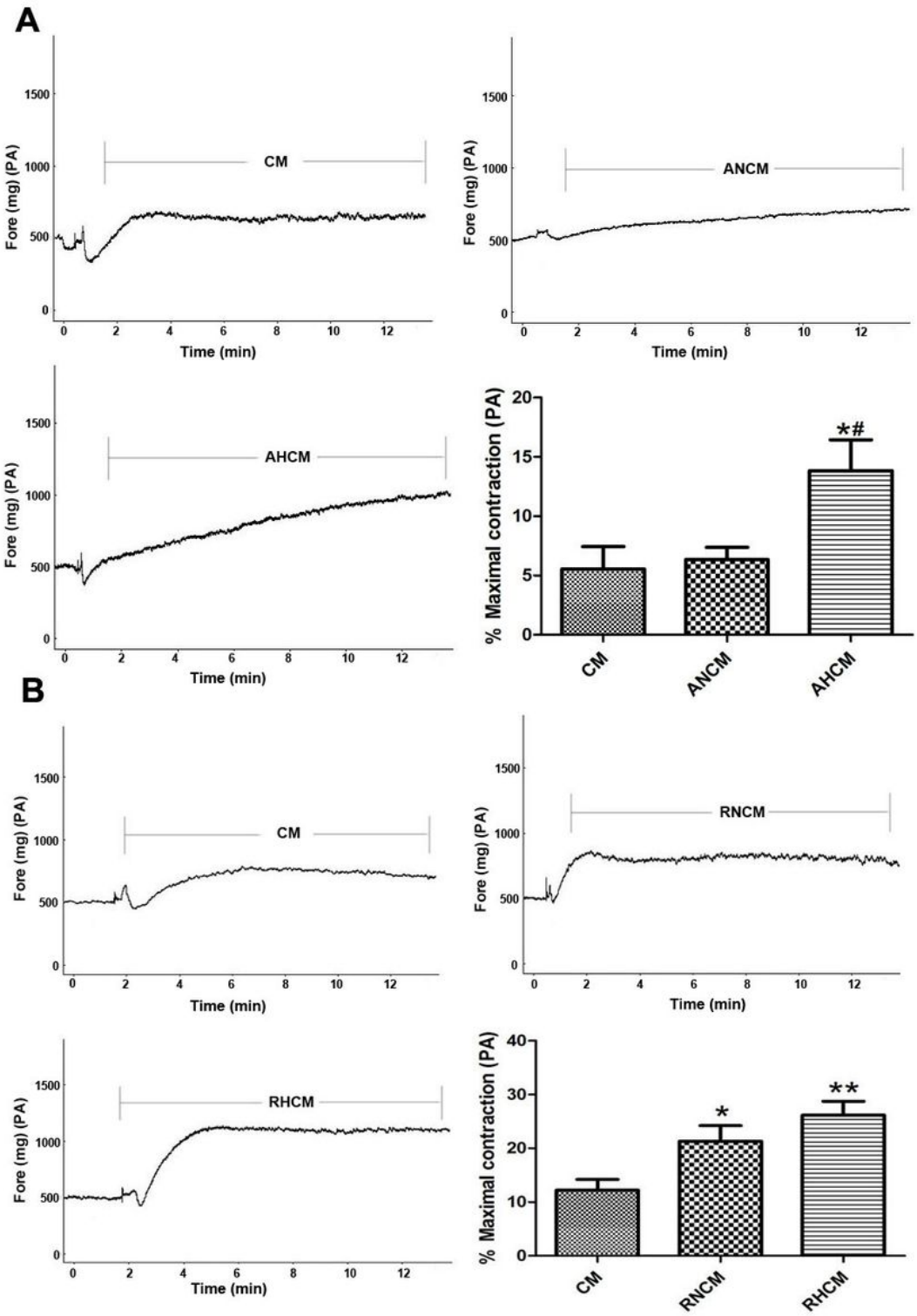

Figure 1

ATII and RLE-6TN hypoxic culture medium promoted the constriction of PA from HPH rats (A) The effect of ATII normoxic or hypoxic culture medium on the constriction of PA from HPH rats (B) The effect of RLE-6TN normoxic or hypoxic culture medium on the constriction of PA from HPH rats.CM=culture medium, ANCM=ATII normoxic culture medium, AHCM=ATII hypoxic culture medium, RNCM=RLE-6TN

normoxic culture medium, RHCM=RLE-6TN hypoxic culture medium. n=5, Data are means ± S.D.*P< 0.05 or **P< 0.01vs.CM, #P< 0.05 vs. ANCM.

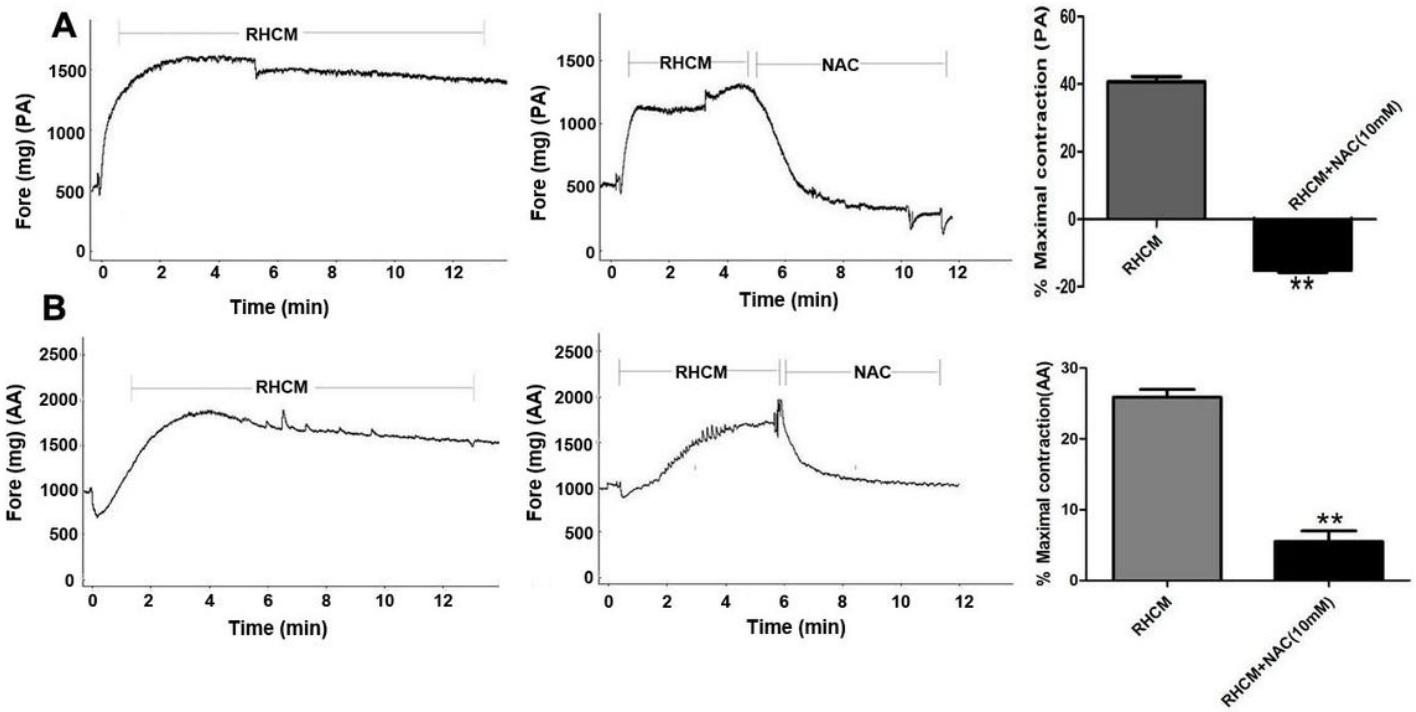

Figure 1

NAC effectively inhibited the constriction of PA or AA induced by RLE-6TN hypoxic culture medium.(A) The constriction of PA induced by RLE-6TN hypoxic culture medium was effectively inhibited by 10 mM NAC (B) The constriction of AA induced by RLE-6TN hypoxic culture medium was effectively inhibited by 10 mM NAC. RHCM=RLE-6TN hypoxic culture medium. n=5, Data are means ± S.D. **P< 0.01 vs. RHCM.

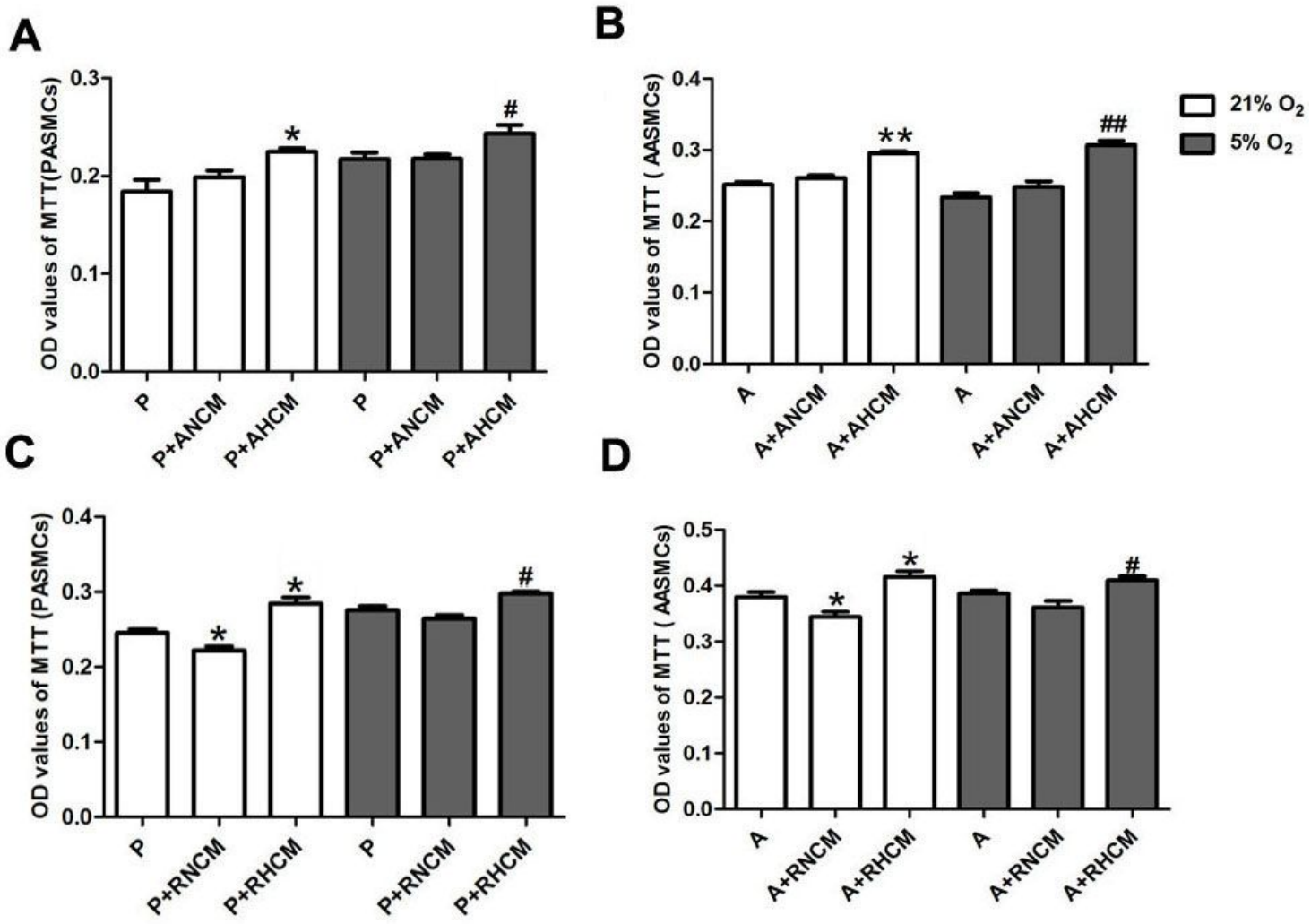

Figure 1

AECs hypoxic culture medium promoted the proliferation of PASMCs or AASMCs (A) PASMCs+ ATII normoxic or hypoxic culture medium (B) AASMCs + ATII normoxic or hypoxic culture medium (C)PASMCs+RLE-6TN normoxic or hypoxic culture medium (D) AASMCs+RLE-6TN normoxic or hypoxic culture medium. ANCM=ATII normoxic culture medium, AHCM=ATII hypoxic culture medium, RNCM=RLE-6TN normoxic culture medium, RHCM=RLE-6TN hypoxic culture medium, P=PASMCs, A=AASMCs. n=8, Data are means ± S.D. #P< 0.05 or ##P< 0.01 vs. 5% O2 PASMCs or AASMCs,*P< 0.05 or ** P< 0.01vs. 21% O2 PASMCs or AASMCs.

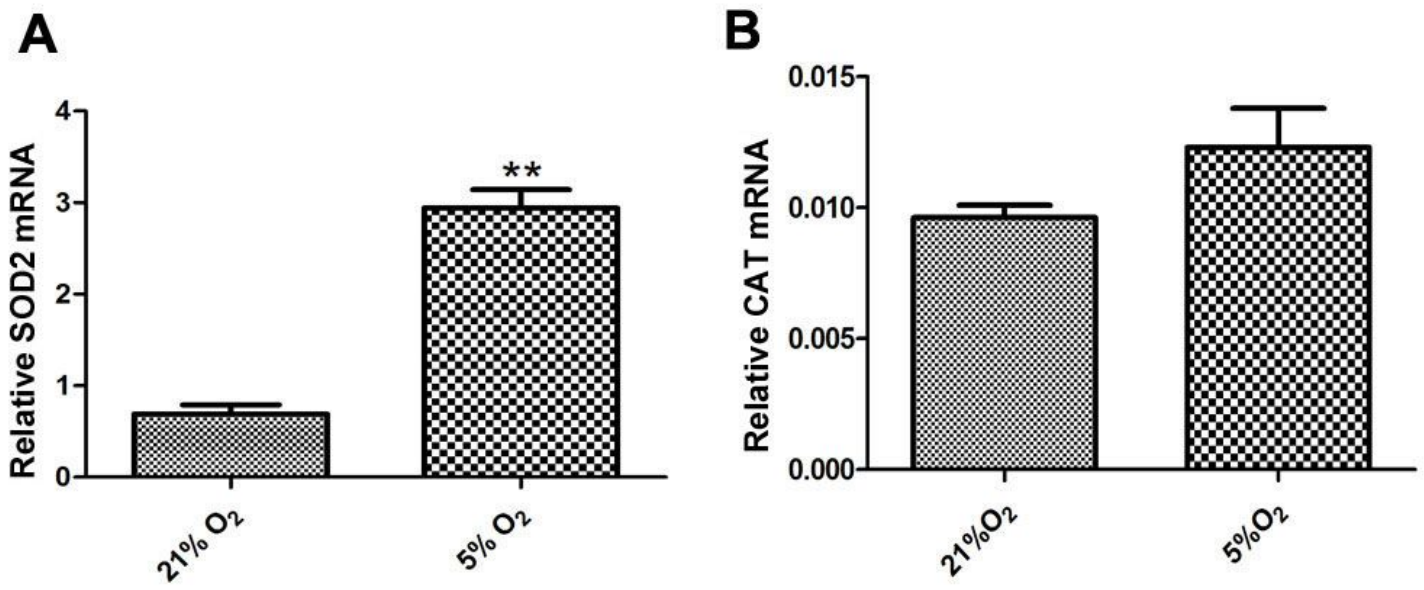

Figure 1

Hypoxia increased SOD2 mRNA level of ATII (A) Hypoxia increased SOD2 mRNA level of ATII. (B) Hypoxia didn't increased CAT mRNA level of ATII n=3, Data are means ± S.D.**P< 0.01 vs.21% O2.

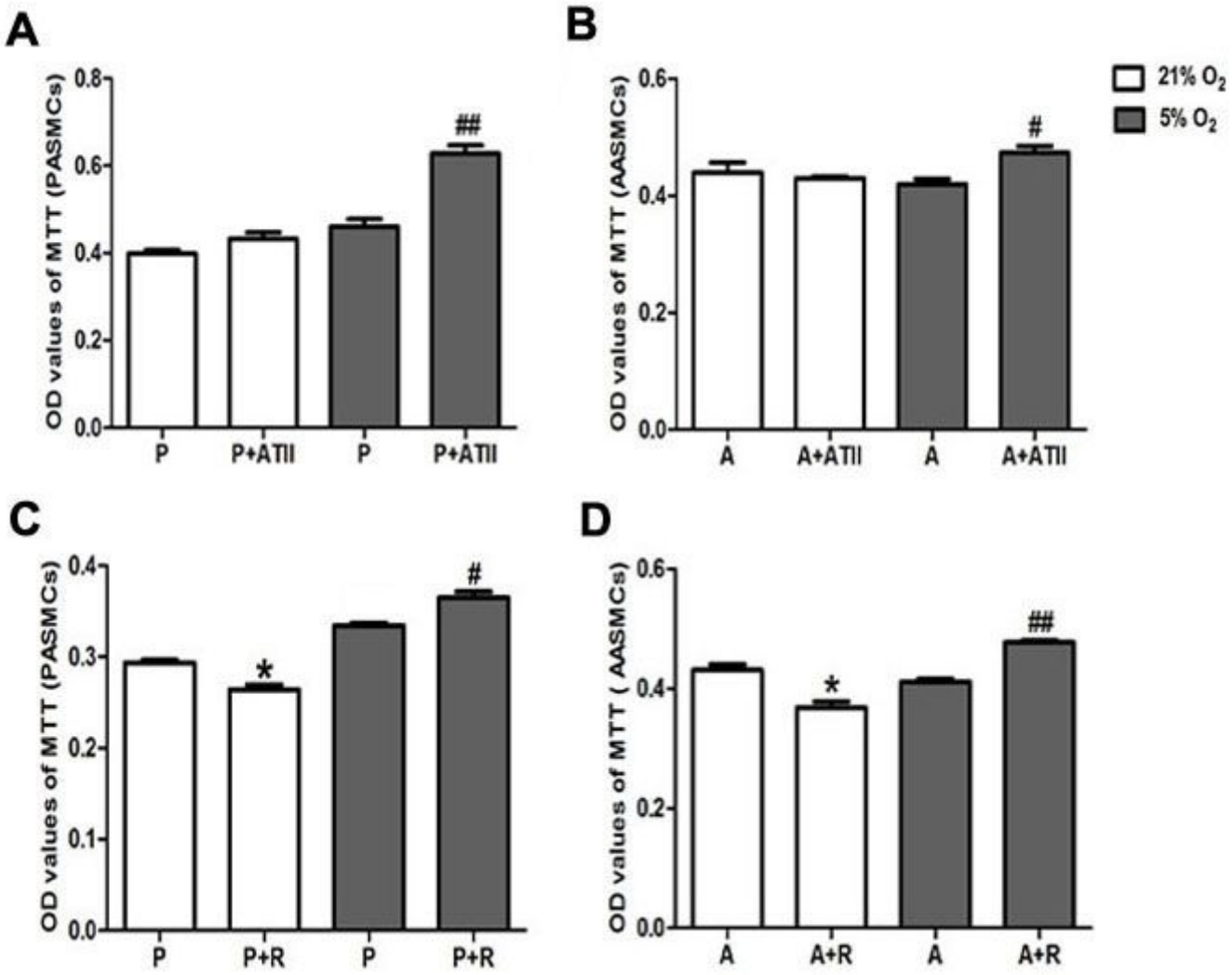

Figure 1

Co-culture with AECs promoted the proliferation of PASMCs or AASMCs under hypoxia. (A) PASMCs co-culture with ATII (B) AASMCs co-culture with ATII (C) PASMCs co-culture with RLE-6TN (D)AASMCs co-culture with RLE-6TN. P=PASMCs, A=AASMCs, R=RLE-6TN. n=5, Data are means ± S.D.#P< 0.05 or ##P< 0.01 vs.5% O2 PASMCs or AASMCs.* P< 0.05 vs.21% O2 PASMCs or AASMCs.

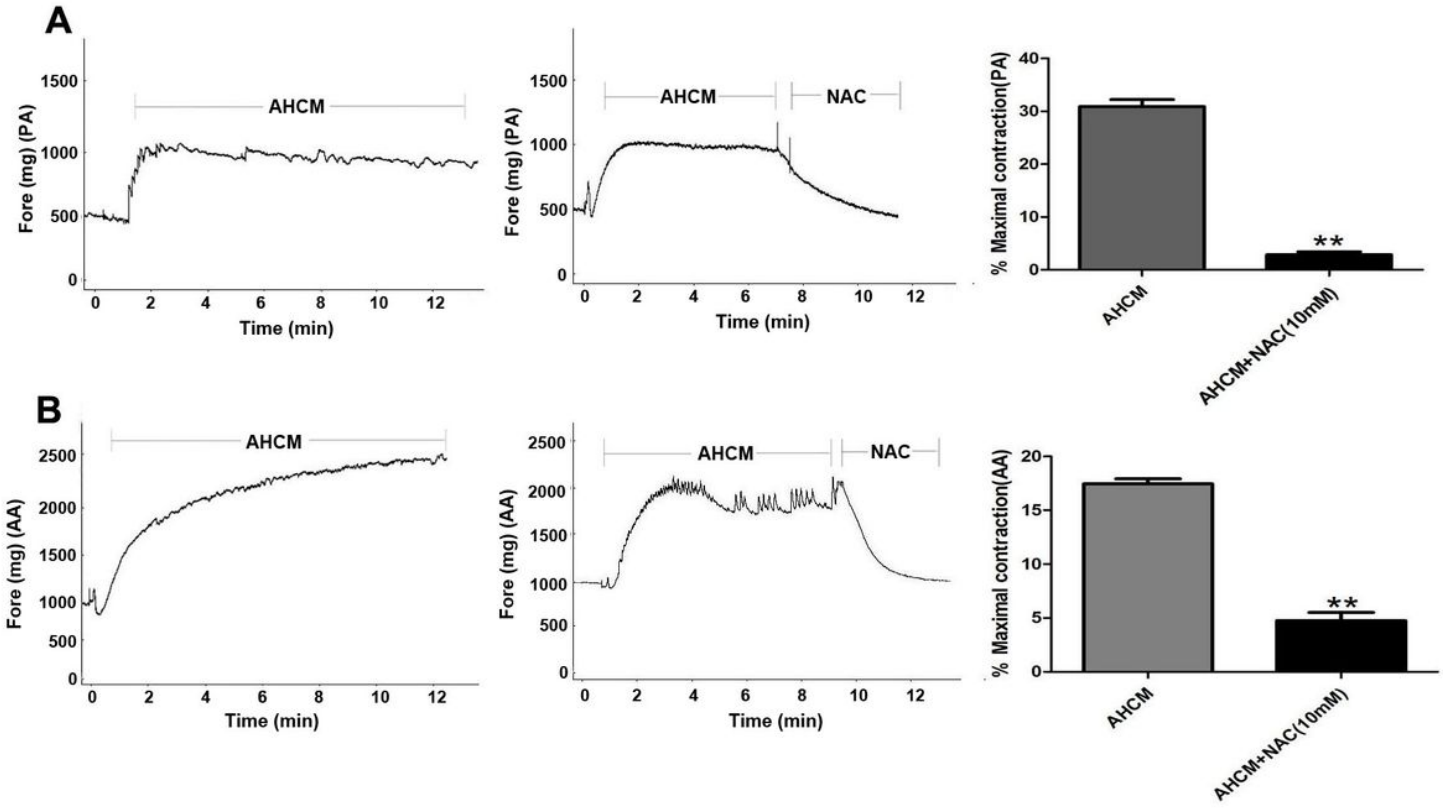

Figure 1

NAC effectively inhibited the constriction of PA or AA induced by ATII hypoxic culture medium.(A) The constriction of PA induced by ATII hypoxic culture medium was effectively inhibited by 10 mM NAC (B) The constriction of AA induced by ATII hypoxic culture medium was effectively inhibited by 10 mM NAC.AHCM=ATII hypoxic culture medium. n=5, Data are means ± S.D. **P< 0.01 vs. AHCM.

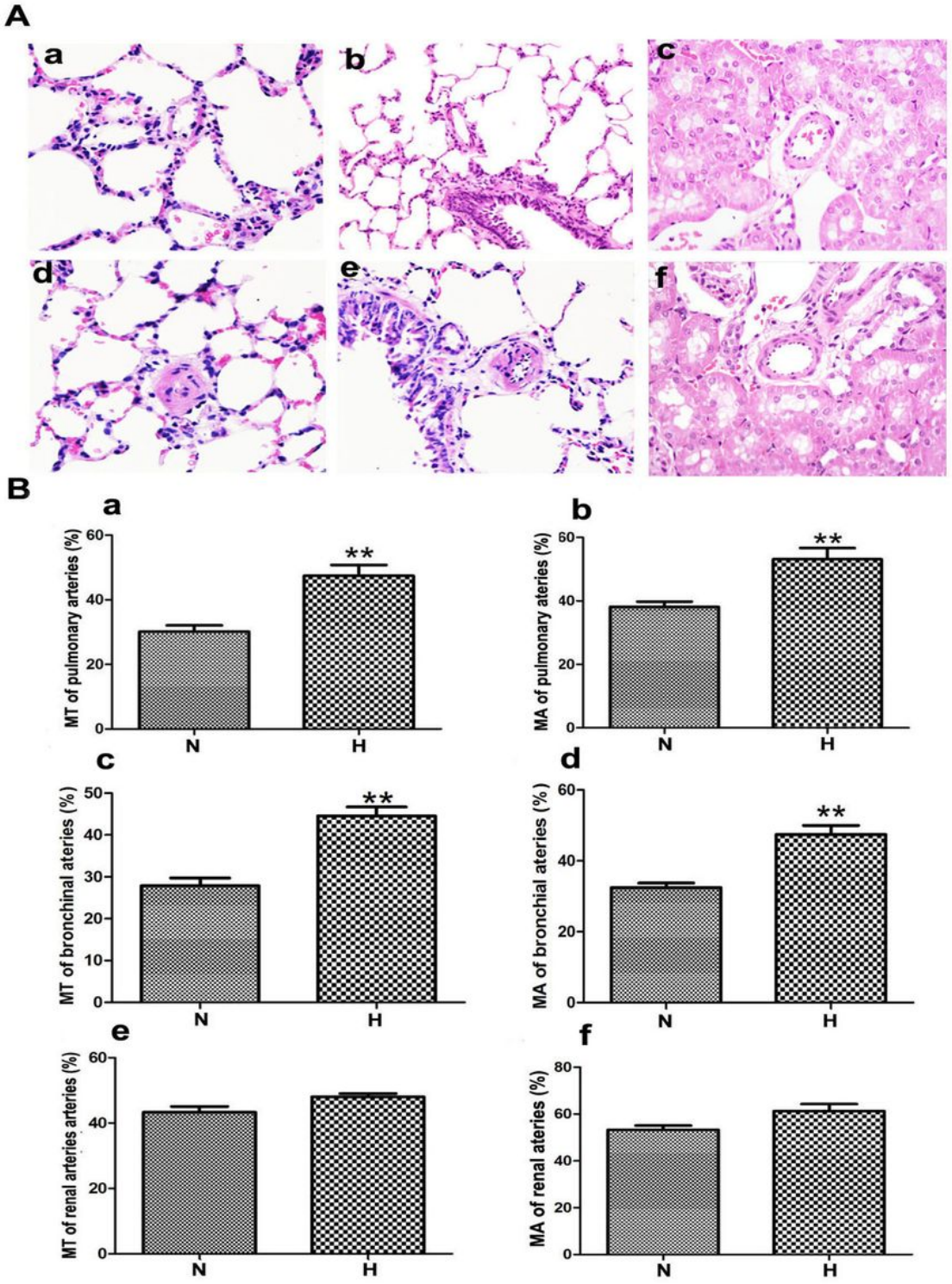

Figure 1

Effects of hypoxia on structure of pulmonary, bronchial and renal artery in rats. (A)Reconstruction of pulmonary artery and bronchial artery were observed by HE staining a,b,c) pulmonary artery, bronchial artery and renal artery in normoxic group d,e,f) pulmonary artery, bronchial artery and renal artery in hypoxic group. (B) MT% and MA% of pulmonary and bronchial artery were significantly elevated in hypoxic group compared with the normoxic group a, b) MT% and MA% of pulmonary artery c, d)MT% and

MA% of bronchial artery e, f) MT% and MA% of renal artery. N= normoxic group H=hypoxic group. n=12, Data are means ± S.D. **P < 0.01 vs normoxic group.

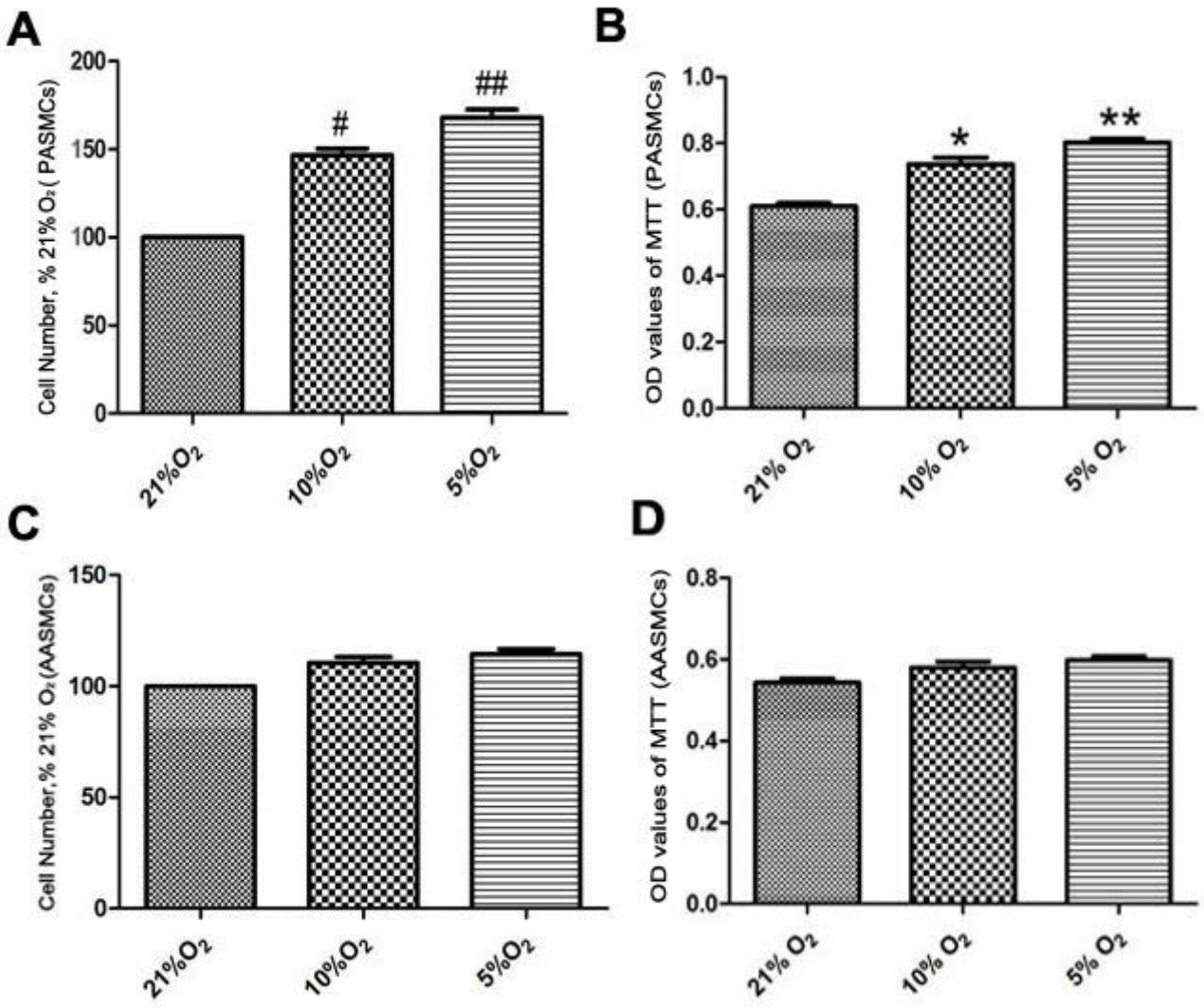

Figure 1

Effects of hypoxia on proliferation of PASMCs and AASMCs. (A and B) Hypoxia promoted the proliferation of PASMCs (C and D) Hypoxia had no obvious effect on the proliferation of AASMCs. n=8, Data are means ± S.D. #P < 0.05, ## P <0.01,*P < 0.05 or **P < 0.01 vs 21% O2.

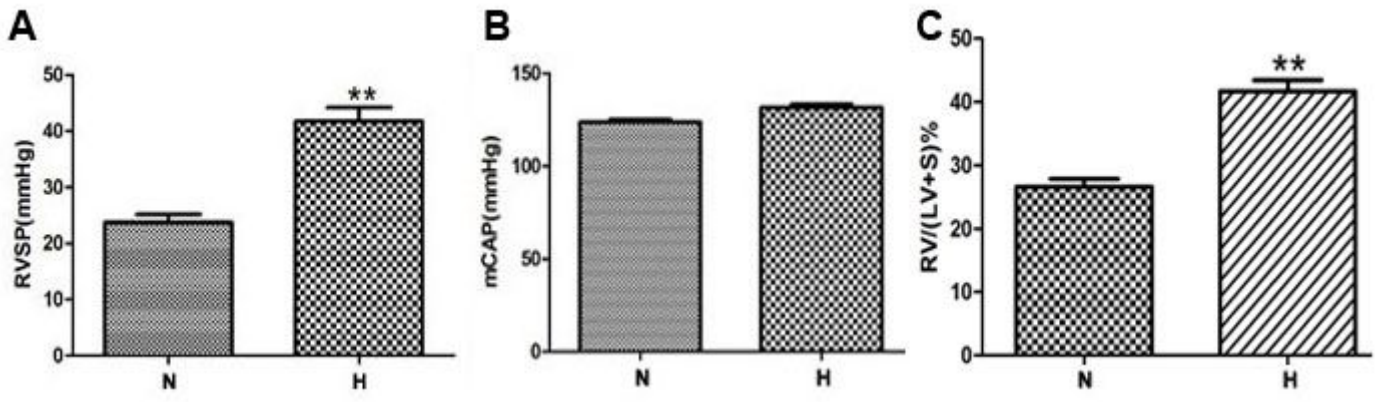

Figure 1

Effects of hypoxia on RVSP, mCAP and right ventricular hypertrophy index in rats. (A) RVSP was significantly elevated in hypoxic group compared with the normoxic group (B) Hypoxia did not influence mCAP considerably. (C) RV/ (LV+S) % was significantly elevated in hypoxic group compared with normoxic group, N= normoxic group H=hypoxic group. n=12, Data are means ± S.D. **P< 0.01 vs normoxic group.