

Original Article

Aortic vascular reactivity to angiotension II is modulated by down-regulation of TRPC in renal hypertensive rats

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Abstract: The present study investigated the relationship between Ang II and TRPC channels of aortic vascular smooth muscle tissues from renal hypertensive rats (RHRs). The hypertensive animal model was established by one-sided renal arterial coarctation with a silver clip. Vascular smooth muscle (VSM) tension was measured using isometric force transducers, and the expression of TRPC and Ang type 1 (AT1) in VSM tissues was measured by western blotting. The results indicated that the Ang II-induced aortic VSM peak tension (AVSPT) was significantly reduced and that AT1 expression was enhanced in hypertensive rats. Ang II significantly enhanced aortic smooth muscle tension under external calcium-free conditions, and AVSPT was potentiated when the external calcium-free environment was switched to normal calcium. The external calcium-induced increase in AVSPT was completely blocked by the TRPC channel blockers NiCl₂ or SKF in normal rats. AVSPT was also suppressed by NiCl₂ in RHRs, but the sensitivity of AVSPT to NiCl₂ was significantly decreased. Additionally, the external calcium-induced increase of AVSPT was significantly suppressed, whereas AVSPT was potentiated under external calcium-free conditions in the RHRs. Furthermore, the expression of TRPC1, TRPC3 and TRPC4 was significantly decreased in the RHRs. These results suggest that TRPC down-regulation inhibits the response of VSM to Ang II, which may result in excess buffering of the arterial blood pressure and enhance the early pathogenesis of renal hypertension.

Keywords: Angiotensin, aortic smooth muscle, hypertension, renal hypertensive rats (RHRs), tension, transient receptor potential canonical (TRPC)

Introduction

Hypertension is a significant public health issue for the adult population and a critical risk factor for future adverse cardiovascular events that significantly contribute to the burdens of the cardiovascular and kidney systems [1]. The classic renin-angiotensin system (RAS) is a complex system composed of numerous peptides, enzymes and receptors that are involved in blood pressure regulation and fluid homeostasis. Angiotensin II signaling via the G protein-coupled angiotensin type 1 receptor (AT1R) promotes vasoconstriction and sympathetic tone and increases vasopressin release and aldosterone secretion. All of these effects ultimately increase the effective fluid volume and elevate the blood pressure. Of the many vasoactive agonists that have been implicated in

vascular hyperresponsiveness in hypertension, Ang II appears to play one of the most important roles. However, the signal transduction systems responsible for the enhanced Ang II-elicited excitation-contraction coupling in hypertension are not completely understood, and the signaling processes involved in the pre-hypertensive phase may differ from those in the established hypertension phase [2].

One hypothesis concerning the mechanism underlying hypertension is that vascular smooth muscle cells have altered calcium (Ca²⁺) handling, resulting in the inability to regulate intracellular Ca²⁺ levels [3]. The increased tone observed in spontaneously hypertensive rats (SHR) may be the result of elevated Ca²⁺ levels, and there is also evidence of higher stored Ca²⁺ in SHRs [4-6]. Two sources can elevate the

intracellular calcium concentration. First, the intracellular endoplasmic reticulum (ER) or the sarcoplasmic reticulum (SR) is a source of Ca^{2+} . Ca^{2+} is released from the ER through the stimulation of G-protein-coupled receptors that activate phospholipase (PL) C to generate inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG). Ca^{2+} release from the SR is mediated by the ryanodine receptor, which functions as the Ca^{2+} release channel. Second, the extracellular milieu is a ready source of Ca^{2+} , and several ion channels allow Ca^{2+} to enter the cell from the extracellular medium. In excitable cells, including cardiomyocytes and vascular smooth muscle cells (VSMCs), voltage-dependent calcium channels mediate a Ca^{2+} current to depolarize the cell membrane above a threshold potential during an action potential. Then, TRPC proteins assemble to form ion channels that enable an influx of calcium and sodium ions into the cell [7]. There is evidence that these channels contribute to the signaling events that enable myogenic and agonist-dependent vasodilator and vasoconstrictor effects [8, 9]. The roles of the channels when blood vessels are exposed to and respond to non-physiological stresses may be more important [10-12]. However, at present it is not clear how the VSMCs alter calcium handling to result in the inability to regulate intracellular Ca^{2+} levels, thereby contributing to the early pathogenesis of renal hypertension.

Because Ang II and the TRPC channels are both involved in handling intracellular calcium and regulating the vascular smooth muscle tone, the present study was designed to investigate the relationship between Ang II and the TRPC protein channels and their contributions to pathogenesis in renal hypertensive rats (RHRs).

Materials and methods

Ethics statement

The animal care and experimental protocols conformed to the Animal Management Rules of China (Documentation 55, 2001, Ministry of Health, China), and the investigation was approved by the Animal Research Committee of Shanghai Jiaotong University. All surgery was performed under isoflurane or pentobarbitalum natrium to minimize suffering.

Animal model and tissue preparation

The experiments were performed on male Sprague Dawley rats (SLAC laboratory animal

center, Shanghai, China) weighing 250-300 g. The rats were kept under conditions of constant temperature and illumination, with a 12 h light/dark cycle and free access to food and water.

The animals were anesthetized with isoflurane. The left renal artery was separated from the vein near the junction with the aorta, taking care not to traumatize the vein; then, a 0.02 mm silver clip was placed on the renal artery. After ligation, the incision was carefully closed by suturing the muscle layer with 4-0 silk. After six to eight days, the systolic blood pressure began to rise. Control rats in the normotensive groups were subjected to sham operations.

Two weeks after surgery, the rats were anesthetized with isoflurane. The thoracic aorta was quickly removed and placed in aerated (95% O_2 and 5% CO_2) Krebs composed of NaCl 118.5 mM, KCl 4.5 mM, MgCl_2 1.2 mM, NaHCO_3 23.8 mM, KH_2PO_4 1.2 mM, glucose 11.0 mM and CaCl_2 2.4 mM. The thoracic aorta was isolated and cleaned of adhering fat and connective tissue and then washed with iced Krebs solution. Each artery was cut into two 2- to 3-mm-wide rings in an 8 mL organ bath containing warmed (37°C) and oxygenated (95% O_2 and 5% CO_2) Krebs solution. Measurements of the artery rings' isometric contractions were recorded with an isometric force transducer (RM6240C; Chengdu Instrument Factory, China) connected to an amplifier. The artery ring was equilibrated for 1 hour with 0.1-0.2 g of basal tension. The aortic preparations were allowed to equilibrate for 30 min under the resting tension of 3 g prior to the addition of the experimental drugs.

Western blot analysis

Protein samples were extracted from fresh artery smooth muscle as recommended by the RIPA buffer (Beyotime, China). Briefly, dispense 300 μl ice cold 1X RIPA Buffer with protease/phosphatase inhibitors over the cell layer, then immediately dislodge the cells with a cell scraper. This cell suspension is transferred into a 1.5 ml microcentrifuge tube. Tap the tube several times to lyse membranes. Leave the cell suspension on ice for 15 min. Centrifuge the cell lysate in a cooled microcentrifuge at full speed for 15 min to partition supernatant and pellet. Collect the supernatant fraction, which con-

Table 1. Changes in blood pressure

BP (mm Hg)	Preoperative		Post-operation (28 d)	
	SBP	DBP	SBP	DBP
NR	122.07 ± 0.54	81.28 ± 0.32	121.31 ± 0.37	82.36 ± 0.41
RHR	121.85 ± 0.43	78.1 ± 0.49	205.59 ± 1.84*#	129.38 ± 2.9*#

* $P < 0.05$ compared with control normotensive rats; # $P < 0.05$ compared with preoperative rats. Blood pressure (BP), systolic blood pressure (SBP), diastolic blood pressure (DBP), normotensive (NR), mean ± SD.

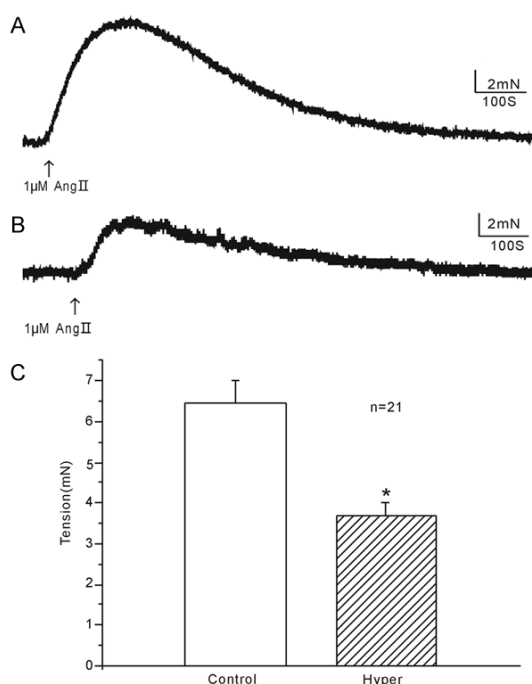


Figure 1. Effect of Ang II on vascular smooth muscle tissues in vitro. A: Sham group vascular smooth muscle response. B: Renal artery vascular smooth muscle constriction response group. C: Comparison of the response of vascular smooth muscle isolated from the normal and hypertensive groups to Ang II. Data showed the means ± SD, $n = 21$, * $P < 0.05$.

tains extracted membrane and cytosolic proteins. Dispense this supernatant into another 1.5 ml microcentrifuge tube that is placed in ice. Equivalent amounts of protein (normally 40 μ g per lane) were separated by 10% SDS-PAGE gel and transferred to an Immobilon-p PVDF membrane with 0.45 mm pores (Millipore, USA). After blocking with 5% non-fat milk, the membranes were incubated with primary antibodies specific for AT1 (Santa Cruz Technologies, USA, 1:200), TRPC1 (Alomone, Israel, 1:400), and overnight at 4°C. Detection was achieved using horseradish peroxidase-conjugated secondary antibodies.

Antibodies and drugs

The following antibodies and drugs were used: isoflurane from Yipin pharmaceutical company (China), Ang II from Tocris (Tocris Bioscience, Bristol, UK); and Nicardipine, SKF-96365 and NiCl₂ from Sigma (Sigma-Aldrich, St. Louis, MO, USA). AT1 antibody (sc-1173) was purchased from Santa Cruz Biotechnology (Santa Cruz, USA), and Anti-TRPC1 (ACC-010), Anti-TRPC3 (ACC-016), Anti-TRPC4 (ACC-018) and Anti-TRPC6 (ACC-017) from Alomone Labs Company. The secondary antibody was horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (sc-2005) also purchased from Santa Cruz Biotechnology (Santa Cruz, USA).

Statistical analysis

The data were expressed as the mean ± SD calculated using Origin 7.5 software. Data from at least seven independent experiments for each condition were evaluated using one-way analysis of variance followed by a post-hoc Bonferroni test. The Student's paired t-test was used to evaluate paired data sets. A P value lower than 0.05 was considered statistically significant.

Results

An investigation of the links between vascular Ca²⁺ signaling and blood pressure regulation under control and hypertensive conditions needs to include an examination of the relationship between Ang II and the TRPC channels, which are related proteins in vascular smooth muscle tissues. To investigate the relationship between Ang II-induced aortic vascular smooth muscle tension (AVST) and the TRPC channels and the pathogenesis of hypertension, we examined the response of aortic smooth muscle to Ang II and TRPC blockers and measured the expression of AT1 and TRPCs in RHR.

Renal hypertensive rats

The systolic blood pressure in the animals that underwent ligation of the left renal artery started to increase on days 3 and 4 and reached its maximum on days 6-8 after the ligation of the renal artery (**Table 1**).

TRPC down-regulation and renal hypertension

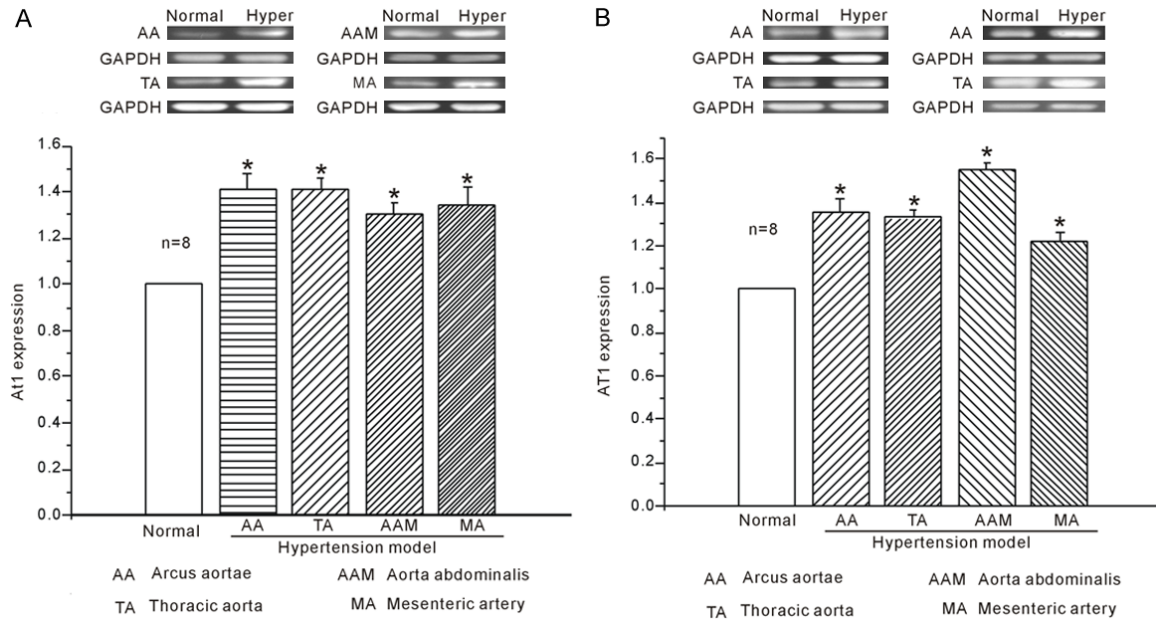


Figure 2. AT1R expression in RHR aortic smooth muscle. A: AT1 expression levels in the arcus aortae (AA), thoracic aorta (TA), aorta abdominalis (AAM), mesenteric artery (MA), and vascular smooth muscle in the renal hypertensive rat group. The western blotting bands were detected with an anti-AT1 antibody to evaluate the AT1 expression levels. GAPDH was used as the internal control to normalize for differences in loading. Data showed the means \pm SD, $n = 8$, $*P < 0.05$ versus the control group. B: AT1 expression levels in the arcus aortae (AA), thoracic aorta (TA), aorta abdominalis (AAM), and mesenteric artery (MA) vascular smooth muscles in SHR rats. The western blotting bands were detected with an anti-AT1 antibody to evaluate the AT1 expressions levels. GAPDH was used as the internal control to normalize for differences in loading. Data showed the means \pm SD, $n = 8$, $*P < 0.05$ versus the WKY group.

The response of aortic smooth muscle to Ang II in RHRs

To investigate the response of vascular smooth muscle from RHRs to Ang II, the tension of thoracic aortic smooth muscle rings was examined. A total of $1 \mu\text{M/L}$ of Ang II significantly enhanced smooth muscle tension, but the effect of Ang II on the tension was obviously suppressed in the RHRs (**Figure 1A**). The Ang II-induced peak tensions were 0.64 ± 0.06 mN and 0.37 ± 0.03 mN in the normal rats and RHRs, respectively (**Figure 1B**, $n = 21$, $P < 0.05$).

The expression of AT1 in aortic smooth muscle from RHRs

Ang II binds with AT1R and stimulates IP3-mediated calcium stores to release calcium and induce smooth muscle contractions. To determine whether AT1 expression was down-regulated in hypertensive rats, the expression of AT1 in aortic and mesenteric smooth muscle tissues from RHRs was measured by western blotting. The AT1R expression levels in the aor-

tic and mesenteric smooth muscle tissues were significantly increased in the hypertensive rats (**Figure 2**, $n = 8$, $P < 0.05$). The results suggest that Ang II/AT1R signaling was up regulated and the response of aortic smooth muscle to Ang II was significantly weakened in the RHRs.

The effect of the TRPC blocker on AVSPT in normal rats

Ang II/AT1R signaling stimulates the release of calcium from intracellular stores and increases intracellular free calcium concentrations. The intracellular calcium stores depend on the influx of extracellular calcium via the voltage-dependent calcium channel and TRPC channels. To investigate the contradictory effect of Ang II on vascular smooth muscle tension in RHRs, the relationship between Ang II and the TRPC channel was examined. Ang II enhanced smooth muscle tension under the external calcium-free condition. The peak tension was potentiated by the switch from no external calcium to the presence of normal external calcium. However, NiCl_2 and SKF (TRPC channel blockers) completely abolished the effect of

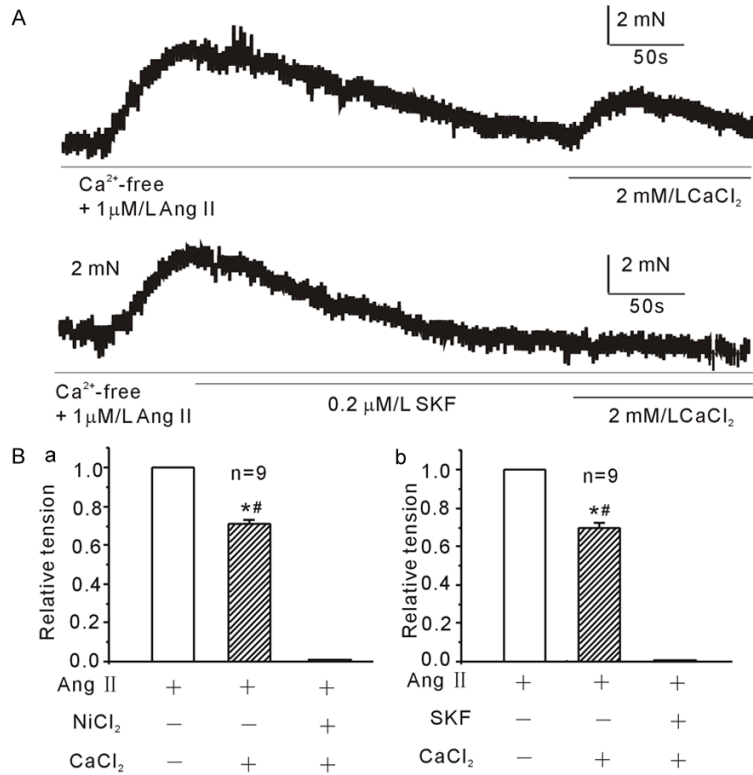


Figure 3. The effect of the TRPC blocker on AVSPT in normal rats. A: Blockers influence on vascular smooth muscle contraction; B: Comparison of the effects of blockers on vascular smooth muscle contractions. a: NiCl₂ effect. b: SKF effect. Data showed the means \pm SD, $n = 9$, $*P < 0.05$.

external calcium on the peak tension (**Figure 3A**). In the external calcium-free condition, the Ang II-induced peak tension of the control was suppressed to 0.27 ± 0.03 and 0.11 ± 0.03 by NiCl₂ and SKF, respectively, whereas the external calcium-induced tension was increased from 0.27 ± 0.03 mN and 0.11 ± 0.03 mN to 0.71 ± 0.02 mN and 0.70 ± 0.02 mN by NiCl₂ and SKF, respectively (**Figure 3B**, $n = 9$, $P < 0.05$). The results suggested that both the intracellular calcium store and external calcium were involved in Ang II-induced smooth muscle tension and that the external calcium entered the cytoplasm in response to Ang II via TRPC channels.

The effect of TRPC channel blockers on AVSPT in RHRs

Because the TRPC channels were involved in Ang II-induced smooth muscle tension, the effect of TRPC channel blockers on Ang II-induced smooth muscle tension was observed in RHRs. The Ang II-induced smooth muscle tension peak was significantly inhibited by NiCl₂, which is a non-specific blocker of TRPC

channels. The tension was completely abolished by the addition of the L-type calcium channel blocker nifedipine in both normal rats and RHRs (**Figure 4A**). RHRs were more sensitive to the inhibitory effect of NiCl₂ on the Ang II-induced peak tension compared to the normal rats; the relative peak tension from the controls decreased to 0.52 ± 0.06 mN, 0.44 ± 0.05 mN, and 0.28 ± 0.02 mN and 0.77 ± 0.02 mN, 0.67 ± 0.03 mN, and 0.44 ± 0.02 mN at 100 s, 200 s and 300 s after the administration of NiCl₂ in the normal rats and RHRs, respectively (**Figure 4B**, $n = 9$, $P < 0.05$).

To determine the fractions of the intracellular calcium store and extracellular calcium involved in the Ang II-induced smooth muscle tension under the normal and hypertensive conditions, Ang II-induced smooth muscle peak tension was examined under external normal calcium and calcium-free conditions. Ang II significantly enhanced aortic smooth muscle peak tension under the calcium-free condition, but this effect was potentiated in the RHRs (**Figure 5A**). Under the external normal calcium condition, the Ang II-induced maximal tension was more sensitively suppressed by the TRPC blocker NiCl₂ (**Figure 5A**). The peak relative tensions were 0.46 ± 0.03 and 0.66 ± 0.04 in the normal rats and RHRs, respectively (**Figure 5Ba**, $n = 12$, $P < 0.05$). However, external calcium-induced AVSPT was potentiated in the normal rats; the related peak tensions were 2.27 ± 0.16 and 1.57 ± 0.09 in the normal rats and RHRs, respectively (**Figure 5Bb**, $n = 12$, $P < 0.05$). These results suggest that the intracellular calcium store fraction involved in the Ang II-induced tension was increased in the RHRs, while the extracellular calcium fraction was decreased.

The expression of TRPC channels in aortic smooth muscle tissues from RHRs

Because the fraction of extracellular calcium sensitive to NiCl₂ or SKF in AVSPT was signifi-

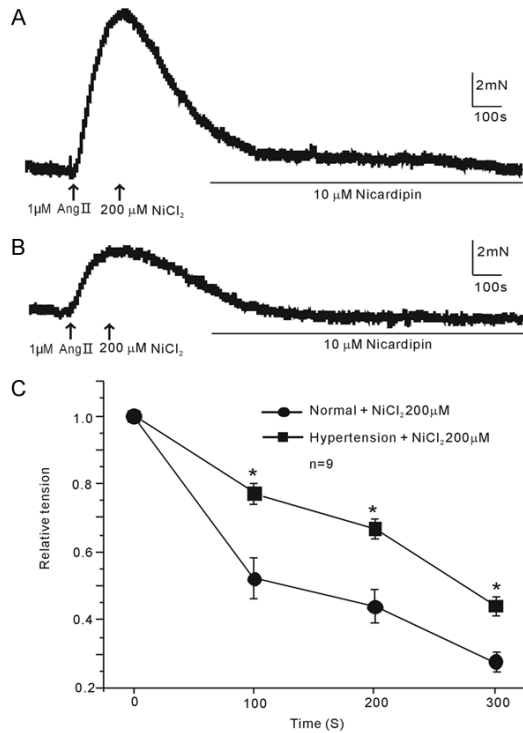


Figure 4. The effect of TRPC channel blockers on AVSPT in RHR. Ang II significantly increased smooth muscle tension in hypertensive rats (A) and normal rats. (B) The effect was abolished by NiCl₂ and nicardipine. (C) Summary of the effects of NiCl₂ on aortic vascular smooth muscle tissues after treatment with Ang II.

cantly decreased in the RHRs, the TRPC channel expression in vascular smooth muscle tissue was measured by western blotting. The TRPC1, TRPC3, TRPC4 and TRPC6 proteins were expressed in the thoracic aorta and mesenteric artery, whereas the expression of TRPC1, TRPC3, and TRPC4 was significantly decreased in the RHRs (Figure 6, $n = 7$, $P < 0.05$). Finally, the expression of TRPC2 and TRPC5 was very low or not detected in the thoracic aorta and mesenteric artery.

Discussion

In hypertension or other cardiovascular diseases, chronic activation of the renin-angiotensin aldosterone system (RAAS) leads to dysfunction of the vasculature, including increased vascular tone, inflammation, fibrosis and thrombosis. Cross-talk between the main mediators of the RAAS, aldosterone and Ang II participates in the development of this vascular dysfunction. Immediate signaling in response to Ang II

leads to a contraction involving the production of IP₃, which acts on the intracellular stores to release calcium and increase the intracellular calcium concentration [13]. Ang II and the TRPC channels are both involved in handling intracellular calcium and regulating the vascular smooth muscle tone. Therefore, the present study was designed to investigate the relationship between Ang II and the TRPCs with the pathogenesis of renal hypertension. The findings of this study serve to illustrate the overarching concept that TRPC channel down regulation can overcome the excessive response of vascular smooth muscle to Ang II, which may serve as a buffer for the arterial blood pressure in the pathogenesis of renal hypertension.

In present, the molecular mechanisms by which angiotensin II signaling cause vascular dysfunction and contribute to hypertension are unclear [14]. Generally, artery narrowing in hypertension can only result from structural remodeling of the artery [15-17] or increased contraction via increased smooth muscle intracellular calcium levels [18] and/or increased calcium sensitivity [19]. For instance, the femoral arteries of living Ang II hypertensive mice had higher intracellular calcium levels and were more constricted, primarily due to elevated sympathetic nerve activity and some increased arterial AT₁R activation. However, evidence of altered artery reactivity or remodeling was not found [20]. In the present study, we observed the response of aortic vascular smooth muscles to Ang II in RHRs for the first time. Our results demonstrated that the Ang II-induced aortic smooth muscle peak tension was significantly suppressed and AT₁ expression was markedly enhanced in the RHRs (Figures 1 and 2). The results suggest that the response of vascular smooth muscle to Ang II may be compensated by a decrease via an unknown mechanism involved in the pathogenesis of renal hypertension but not by the down regulation of AT₁ expression. A previous study demonstrated that the maximal tension exerted on the aortic ring by Ang II was significantly lower in SHR compared to WKY rats [21]. This difference may be linked to the change in Ca²⁺ handling observed in vascular smooth muscle cells (VSMC) from SHR and differences in their excitation-contraction coupling [22]. The intracellular Ca²⁺ concentration and Ca²⁺ storage pools in cultured aortic VSMCs from SHR were increased under non-stimulated conditions. Moreover, Ca²⁺ mobilization

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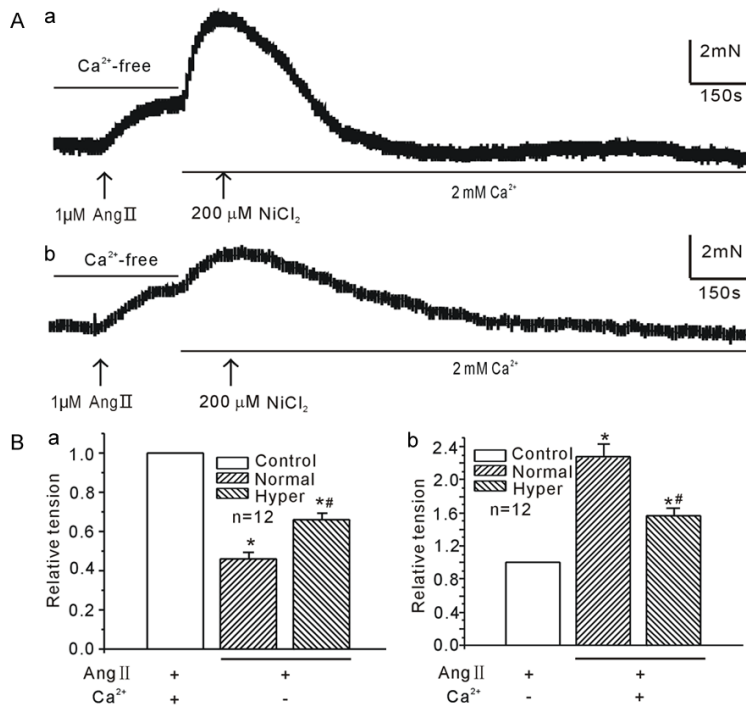


Figure 5. The effects of intracellular calcium stores and extracellular calcium. A: Comparison of intracellular and extracellular Ca²⁺ effects. B: Summary of the effects of Ca²⁺ on aortic vascular smooth muscle tissues after treatment with Ang II. a: Intracellular Ca²⁺ effect. b: Extracellular Ca²⁺ effect. Data showed the means \pm SD, $n = 12$, $*P < 0.05$.

induced by Ang II was also enhanced in SHR [22].

TRPC genes are expressed in the vasculature to form functional proteins that give rise to Ca²⁺- and Na⁺-permeable channels in the plasma membrane [7]. Generally, TRPCs act as receptor-operated calcium channels (ROCC) or store-operated calcium channels (SOCC) that are activated by diacylglycerol (DAG) or result in store depletion upon stimulation of phospholipase C (PLC)-coupled receptors [23, 24]. Our results showed that AT1R expression was increased but the response of aortic vascular smooth cells to Ang II was depressed. Therefore, we examined the relationship between TRPC and Ang II-induced smooth muscle peak tension. In normal rats, Ang II significantly enhanced aortic smooth muscle tension under the external calcium-free condition, and the TRPC blockers NiCl₂ or SKF completely abolished Ang II-induced smooth muscle tension when the external calcium-free environment was switched to a normal calcium environment (Figure 3). These results indicated that TRPCs were involved in Ang II-induced vascular smooth

muscle tension and the Ang II-stimulated release of calcium from the intracellular stores, which in turn activated the TRPC channels.

Vasomotion, i.e., oscillations of vascular tone due to synchronized oscillations of smooth muscle cell tension, occurs in arteries either spontaneously or in response to pressure, stretching or the application of vasoconstrictor agonists [25-27]. Transient receptor potential canonical (TRPC) cation channels have been reported to play an important role in transplasma membrane calcium influx [28]. Therefore, we examined the sensitivity of aortic vascular smooth muscle tissues to TRPC channel blockers in normal rats and RHRs. The result demonstrated that the TRPC blocker NiCl₂ significantly inhibited Ang II-induced smooth muscle tension in normal rats

and RHRs; however, the response of Ang II-induced smooth muscle tension to the TRPC channel blocker was depressed in the RHRs (Figure 4). The hemodynamic effects of Ang II are mediated through binding to angiotensin AT1 receptors located on the VSMC, leading to Ca²⁺ release from the internal stores and Ca²⁺ influx from the extracellular spaces via TRPC and the L-type calcium channel [29]. Next, the fractions of intracellular and extracellular calcium involved in Ang II-induced aortic vascular smooth muscle tension in normal rats and RHRs were examined. Ang II-induced smooth muscle tension was potentiated under the external calcium-free condition but was significantly suppressed by the switch from the external calcium-free condition to the normal calcium condition in the RHRs (Figure 5). The results demonstrated that Ang II-induced smooth muscle maximal tension exhibited an increased dependence on the intracellular calcium store in the RHRs compared to the normal rats, whereas the fraction of Ang II-induced smooth muscle maximal tension that depended on external calcium was decreased in the RHRs

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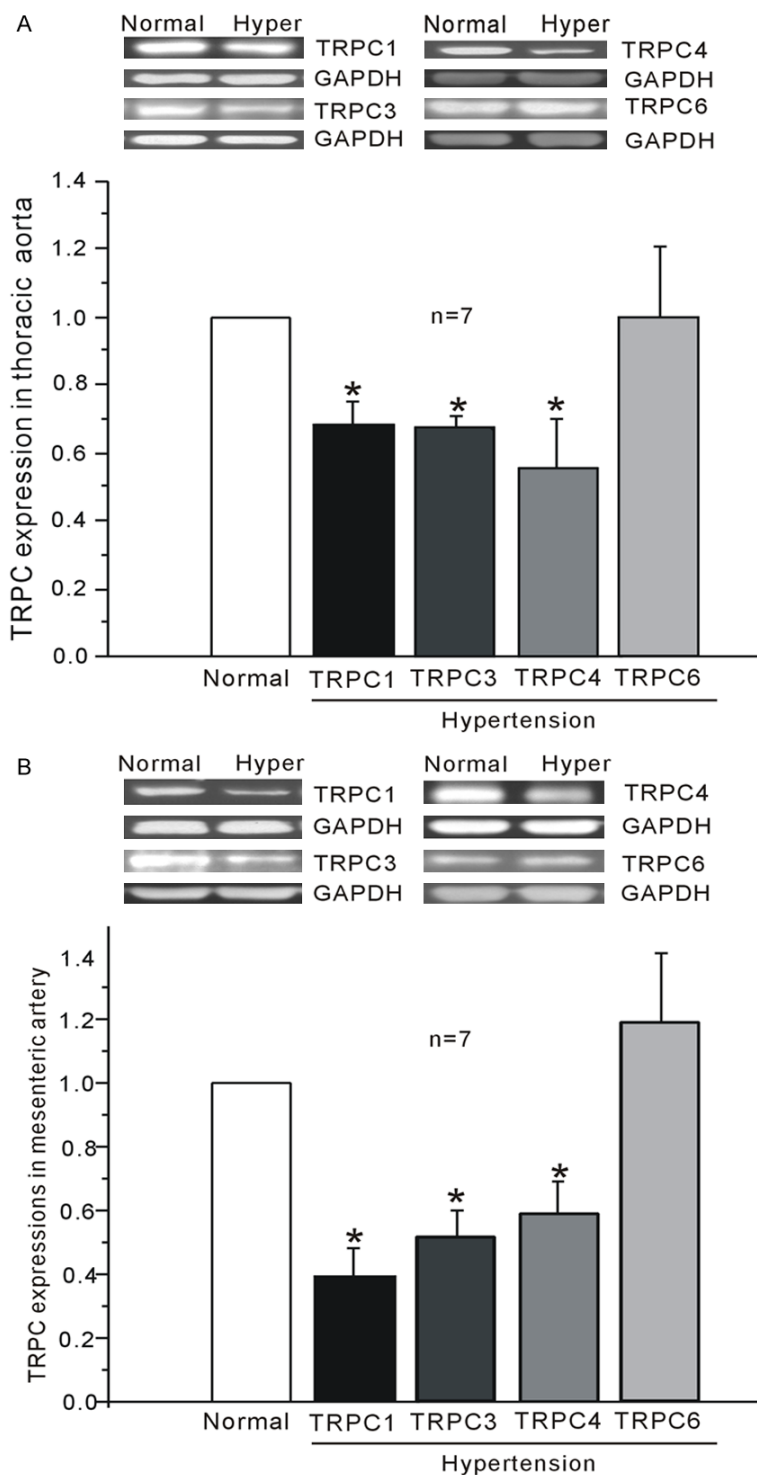


Figure 6. The expression of TRPC channels in RHR aortic smooth muscle tissues. **A:** TRPC expression in the thoracic aorta (TA) smooth muscle groups. The western blotting bands were detected with an anti-TRPC antibody to evaluate the TRPC expression levels. GAPDH was used as the internal control to normalize for differences in loading. Data showed the means \pm SD, $n = 7$, $*P < 0.05$ versus the control group. **B:** TRPC expression in the mesenteric artery (MA) smooth muscle groups. The western blotting bands were detected with an anti-TRPC antibody to evaluate the TRPC expression levels. GAPDH was used as the internal control to normalize for differences in loading. Data showed the means \pm SD, $n = 7$, $*P < 0.05$ versus the control group.

compared to the normal rats. These results suggested that the TRPC function of vascular smooth muscle tissues was down-regulated in the RHRs, possibly to compensate for the reduced sensitivity of the vascular smooth muscle to Ang II. Moreover, the aortic maximal tension induced by Ang II in the external calcium-free condition was potentiated in the RHRs. The increased tone was also observed in spontaneously hypertensive rats (SHR) and may explain the elevated Ca^{2+} levels. There was also evidence of higher stored Ca^{2+} in SHRs [30-32].

There is evidence that transient receptor potential (TRP) channels are involved in the regulation of vascular tone and the pathogenesis of hypertension [28]. An increase in TRP canonical type 3 (TRPC3) expression has been described in several animal models of hypertension and in patients with essential hypertension. TRPC3 protein expression was increased in SHRs compared to normotensive Wistar-Kyoto rats [33, 34]. These findings suggest that store-operated Ca^{2+} dynamics may be altered in hypertensive states. Many observations [35-37] imply that constant Ca^{2+} entry, which can raise the cytoplasmic or nuclear Ca^{2+} concentration and refill Ca^{2+} in depleted SRs, is necessary for VSMC proliferation. VSMC proliferation is one of the causes of vascular remodeling and is associated with the development of hypertension, atherosclerosis, and restenosis after balloon angioplasty [38], but its early signals are not completely understood. In the present study, the expression of TRPC1,

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TRPC3, and TRPC4 was significantly decreased in RHR aortic and mesenteric vascular smooth muscle tissues. Our results suggest that TRPC expression is decreased during the early state of RHR to reduce the sensitivity of vascular smooth muscle to Ang II. Thus, we can speculate that TRPC expression will be enhanced during the vascular remodeling state of RHR.

In conclusion, the immediate signaling of vascular smooth muscle in response to Ang II led to a contraction involving the production of IP₃, which acted on intracellular stores to release calcium and increase the intracellular calcium concentrations. Conversely, the depletion of intracellular calcium stores stimulated TRPC channels in the cytosolic membrane to induce extracellular calcium entry. During the early stage of RHR, the response of aortic smooth muscle to Ang II induced a compensatory reduction via the down-regulation of TRPC1, TRPC3 and TRPC4, which might be involved in excess buffering of the arterial blood pressure and therefore the enhancement of the early pathogenesis of renal hypertension.

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Disclosure of conflict of interest

None.

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