Nitric Oxide 88 (2019) 1–9

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Angiotensin-(1-7) induced vascular relaxation in spontaneously hypertensive rats

Feng Zhang[a,1], Haiyang Tang[b,1], Shuo Sun[a], Yuru Luo[b], Xingsheng Ren[a], Aidong Chen[a], Yu Xu[a], Peng Li[c], Ying Han[a,*]

[a] Key Laboratory of Targeted Intervention of Cardiovascular Disease, Collaborative Innovation Center for Cardiovascular Disease Translational Medicine, Department of Physiology, Nanjing Medical University, Nanjing, Jiangsu, 211166, China
[b] College of Veterinary Medicine, Northwest A&F University, Yangling, 712100, China
[c] Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, 210029, China

ARTICLE INFO

Keywords:
Hypertension
Angiotensin-(1-7)
Vascular tension
Nitric oxide
cGMP-PKG signaling pathway

ABSTRACT

Enhanced vasoconstriction and decreased vasodilatation due to endothelial dysfunction contribute to the progression of hypertension. Angiotensin (Ang)-(1-7) plays important roles in regulating the cardiovascular activity. The current study aimed to investigate the roles of Ang-(1-7) in modulating blood pressure, vascular tension and its signal pathway in spontaneously hypertensive rats (SHR). The effects of intravenous injection of drugs were determined in rats with anesthesia *in vivo*. Mesenteric artery (MA), coronary artery (CA) and pulmonary artery (PA) were isolated from rats and isometric tension measurements in arteries were performed. Compared with Wistar-Kyoto rats (WKY), the high K^+ induced vasoconstriction was enhanced and acetylcholine-induced vasodilatation were attenuated in the MA, CA and PA in SHR. Intravenous injection of Ang-(1-7) decreased, while A-779 increased mean arterial pressure and abolished the hypotensive effect of Ang-(1-7) in SHR. Ang-(1-7) caused dose-dependent relaxation in MA, CA and PA in SHR, which was inhibited by pretreatment with Mas receptor antagonist A-779, nitric oxide (NO) synthase inhibitor L-NAME, guanylate cyclase inhibitor ODQ and protein kinase G (PKG) inhibitor DT-2. The Mas receptor expression, NO, cGMP and PKG levels of the three above arteries of SHR were lower than that of WKY. Ang-(1-7) increased the NO, cGMP and PKG levels in arteries from SHR, which was blocked by A-779. Activation of the Mas receptor by Ang-(1-7) relaxes the MA, CA, and PA through the NO-cGMP-PKG pathway, which contributes to the decrease of arterial pressure in SHR.

1. Introduction

Micro- and macrovascular complications contribute to mortality and morbidity in patients with hypertension [1]. Numerous studies have shown that endothelial dysfunction of small arteries occurs in patients with essential [2,3] or secondary hypertension [4,5], and in the early stage of various hypertensive models [6]. Normally, endothelial cells release nitric oxide (NO) [7,8], and NO then induces the vascular smooth muscle cells (VSMC) relaxation, through the intracellular cGMP-protein kinase G (PKG) signal pathway [9]. Decreased NO generation induced by endothelial dysfunction of small arteries, and impaired NO-dependent vascular relaxation are implicated in the development and progression of hypertension [7,10] and contribute to further progression of organ damage [11].

Enhanced vasoconstriction and attenuated vasodilatation especially in the mesenteric artery (MA) resulted in increased total peripheral resistance, and sustained high blood pressure in hypertension [12]. Coronary artery (CA) disease is one usual cause of mortality, due to attenuated vasodilatation and sustained coronary arterial contraction in hypertensive patients [13,14]. Endothelial dysfunction induces impaired coronary arterial relaxation, reduces the blood supply to the heart and subsequently causes myocardial ischemia and angina [15]. Although pulmonary arterial endothelial dysfunction in hypertensive patients is not common, there are still occurrences of pulmonary hypertension in hypertensive patients [16,17], and in hypertensive models [18,19]. Attenuated pulmonary arterial relaxation increases

Abbreviations: SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; MA, mesenteric artery; CA, coronary artery; PA, pulmonary artery; Ang-(1-7), angiotensin-(1-7); A-779, D-Alanine-Ang-(1-7); PKG, protein kinase G; NO, nitric oxide

* Corresponding author.
E-mail address: yhancn@njmu.edu.cn (Y. Han).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.niox.2019.03.007
Received 2 February 2019; Received in revised form 10 March 2019; Accepted 11 March 2019
Available online 14 March 2019
1089-8603/

pulmonary vascular resistance and the risk of pulmonary hypertension [20].

Angiotensin (Ang)-(1-7) is accepted as one important biologically active peptide in the renin-angiotensin system (RAS) family to adjust cardiovascular activity [21,22]. Ang-(1-7) is generated from the hydrolysis of Ang II or Ang I catalyzed by angiotensin converting enzyme 2 (ACE2) [23]. Most of its effects are mediated by the Mas receptor [24]. The Mas receptor expresses abundantly in the vessel endothelial cell and is selectively blocked by its specific antagonist D-Alanine-Ang-(1-7) (A-779) [25–27]. Our recent studies found that microinjection of Ang-(1-7) into either paraventricular nucleus (PVN), or rostral ventrolateral medulla (RVLM) increases arterial blood pressure and sympathetic activity in renovascular hypertensive rats or chronic heart failure rats [28–30]. While some studies show the opposite effect of Ang-(1-7) in peripheral tissues on modulating arterial blood pressure. It has been reported that intravenous injection of Ang-(1-7) decreases blood pressure [31] and induces the mesenteric arterial relaxation in normal rats [32]. Ang-(1-7) prevented Ang I- and Ang II-mediated changes in vascular resistance more potently in SHR-stroke prone than in WKY [33]. Ang-(1-7) improves endothelial function and delays the development of cardiac remodeling and heart failure in rats with myocardial infarction [34]. These findings suggest the probable beneficial effect of Ang-(1-7) on endothelial function and vascular tension. However, whether Ang-(1-7) induces MA, CA, and pulmonary artery (PA) relaxation, and decreases blood pressure in hypertension, is still unclear. Whether NO and the downstream cGMP-PKG signal pathway mediate the Ang-(1-7) effects on arteries is also unclear.

The present study was designed to determine whether Ang-(1-7), by means of the NO-cGMP-PKG signal pathway, decrease blood pressure, and induce MA, CA, and PA relaxation in spontaneously hypertensive rats (SHR).

2. Materials and methods

The experiments were carried out in male adult Wistar-Kyoto rats (WKY) and SHR at an age of 13 weeks old (Vital River Laboratory Animal Technology Co. Ltd, Beijing, China). The procedures were approved by Nanjing Medical University Experimental Animal Care and complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication, 8th edition, 2011). The rats were kept in a temperature-controlled at 22 °C room on a 12 h–12 h light–dark cycle with free access to standard chow and tap water.

2.1. Systolic blood pressure measurements

The tail artery systolic blood pressure was measured in conscious rats with a noninvasive computerized tail-cuff system (NIBP, ADInstruments, Australia). The rats were warmed for 10–20 min at 28 °C before the measurements to allow for detection of tail arterial pulsations and to achieve the steady pulse. The systolic blood pressure was obtained by averaging 10 measurements.

2.2. Mean arterial pressure and heart rate recording

Each rat was intraperitoneally anesthetized with urethane (800 mg/kg) and α-chloralose (40 mg/kg). An appropriate level of anesthesia was maintained by supplemental doses of anesthetic. A rodent ventilator (model 683, Harvard Apparatus Inc., USA) was used for mechanical ventilation with room air. The right carotid artery was cannulated and connected to a pressure transducer (MLT0380, ADInstruments, Australia) to record continuous arterial blood pressure, mean arterial pressure and heart rate.

2.3. Intravenous injection

External jugular vein catheterization was performed for intravenous injection of Ang-(1-7) and A-779. The injection rate was controlled by a dual-channel microdialysis infusion syringe pump (53101V, Stoelting Co., Illinois, USA) and the microinjection volume was 100 μL; an application lasting for 10 min.

2.4. Renal sympathetic nerve activity recording

The left renal sympathetic nerve was isolated after a retroperitoneal incision was made as we previous reported [30]. To eliminate its afferent activity, the renal nerve was cut distally. The nerve was then placed on a pair of silver electrodes and immersed in warm mineral oil. The nerve signals were amplified and recorded by a 4-channel AC/DC differential amplifier (DP-304, Warner Instruments, Hamden, CT, USA) with a high pass filter at 10 Hz and a low pass filter at 3,000 Hz. The renal sympathetic nerve activity was integrated at a time constant of 100 ms. At the end of each experiment, the background noise was determined after sectioning the central end of the nerve and subtracted from the integrated values of the renal sympathetic nerve activity. The raw and integrated renal sympathetic nerve activity, arterial blood pressure, mean arterial pressure and heart rate were simultaneously recorded with a PowerLab data acquisition system (8SP, ADInstruments, Australia). The renal sympathetic nerve activity change was expressed as the percent change from the values before chemical intervention.

2.5. Isometric tension measurements in arteries

Isometric tension was measured to evaluate vascular function as previously described [35]. Third-order coronary arteries, pulmonary arteries or mesenteric arteries were isolated from rats and put into Krebs-Henseleit solution (containing (in mmol/L) 118.0 NaCl, 25.0 $NaHCO_3$, 1.2 NaH_2PO_4, 1.2 $MgSO_4$, 1.8 $CaCl_2$, 4.7 KCl and 11.1 glucose and equilibrated with carbogen (95% O_2 + 5% CO_2)). Arteries were then cleaned of any adherent connective tissues and cut into 1- to 1.2-mm segments (1 arterial ring/rat was used). Arterial rings were mounted in a four-chambered myograph (620M, DMT, Denmark) with 20-μm wires and set at a resting tension of 0.1 g. All rings were equilibrated for 45 min with intermittent washes every 15 min. After equilibration, each arterial ring was contracted first by high K^+ solution ((containing in mmol/L) 122.6 KCl, 1.09 $CaCl_2{\cdot}7H_2O$, 1.21 $MgSO_4{\cdot}7H2O$, 1.117 KH_2PO_4, 24.9 $KHCO_3$ and 11.1 glucose) to evaluated rings functionality and then washed. Without contracting response to high K^+ solution, arterial ring was not used for further experiments. In the following steps, prostaglandin F2α (PGF 2α) was used to induce arterial ring contraction, and then 6 doses of acetylcholine (ACh) (10^{-9} ~ 10^{-4} mol/L) or 6 doses of Ang-(1-7) (10^{-9} ~ 10^{-4} mol/L) were administrated in a dose-dependent manner to induce vasodilatation. The degree of relaxation is shown as a percentage of PGF 2α-induced contraction. For the pretreatment with chemicals, the chemicals were added 20 min before the contraction induced by PGF 2α.

2.6. Arteries sample preparation

MA, CA and PA were isolated from rats and flash-frozen in liquid nitrogen and stored at −70 °C, prepared to detect Mas receptor protein expression or immunohistochemistry. For the purpose of detecting the effects of Ang-(1-7) or A-779 on NO, cGMP and PKG levels, CA, MA or PA were isolated from rats and incubated in Krebs-Henseleit solution added with different chemicals for 20 min. These arteries were then quickly removed from the solution and flash-frozen in liquid nitrogen and stored at −70 °C. Lastly, the artery tissues were homogenized in RIPA lysis buffer and centrifuged. The total proteins in the homogenate supernatant were extracted and measured using a protein assay kit

(BCA, Pierce, USA).

2.7. Measurement of Mas receptor protein expression of arteries

The Mas receptor protein expressions in arteries were determined by western blotting as described in previous reports [36,37]. Briefly, proteins in the artery tissues homogenate supernatant were separated by a 10% SDS–PAGE gel and transferred to a nitrocellulose membrane. The membrane was then probed with Mas receptor antibody (1:200, Alomone Labs, Israel), followed by incubation with horseradish peroxidase–conjugated goat anti-rabbit IgG (1:5000; Immunology Consultants Lab, Portland, OR, USA). The bands were visualized by enhanced chemiluminescence using the ECL system (Pierce Chemical, Rockford, IL, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:5000; Bioworld Technology Inc., Louis, MN, USA) protein was used as the loading control. The total amount of the Mas receptor protein levels were normalized to the GAPDH protein level.

2.8. Mas receptor immunohistochemistry

Mas receptor immunohistochemistry of the arteries was performed with an immunohistochemistry kit (Abcam, MA, USA) as our previous report [29]. Briefly, the arteries were processed into coronal sections (5 μm) and were incubated with rabbit polyclonal Mas receptor antibody (1:200, Alomone Labs, Israel) at 4 °C overnight. After washing, sections were incubated with biotinylated goat anti rabbit IgG for 1 h and then stained with DAB according to the manufacturer's instructions. Sections were covered with mounting medium and Mas receptor immunoreactivity was observed under a light microscope (DP70, Olympus, Tokyo, Japan).

2.9. Measurement of NO, cGMP and PKG levels

NO production of arteries was evaluated based on the detection of the concentration of its stable metabolites nitrate and nitrite using a Nitrate/Nitrite Colorimetric Assay Kit (Cayman Chemical Co., Ann Arbor, MI, USA). The cGMP level and PKG protein level of arteries were determined by enzyme immunoassay kits (Cayman Chemical Co., Ann Arbor, MI, USA & Yi Fei Xue Biotechnology, Nanjing, China) following the manufacturer's instruction.

2.10. Chemicals

Ang-(1-7) and D-Alanine-Ang-(1-7) (A-779, an antagonist of Mas receptors) were obtained from Bachem (Bubendorf, Switzerland). N^{ω}-nitro-l-arginine methyl ester (L-NAME, a nitric oxide (NO) synthase inhibitor), 8-Bromoguanosine 3′,5′-cyclic monophosphate (8-Bromo-cGMP, an cGMP analogue), 1H- [1,2,4]oxadiazole [4,3-a]quinoxalin-1-one (ODQ, a guanylate cyclase (GC) inhibitor), DT-2 (a protein kinase G (PKG) inhibitor), Prostaglandin F2α (PGF 2α) and acetylcholine (ACh) were purchased from Sigma Chemical Co (St. Louis, MO, USA). The chemicals were dissolved in normal saline, except ODQ which was dissolved in DMSO.

2.11. Protocols

Protocol 1: The effects of intravenous injection of saline, Ang-(1-7) (10^{-4} mol/L), A-779 (10^{-4} mol/L), A-779 + Ang-(1-7) on mean arterial pressure, renal sympathetic nerve activity and heart rate were determined in 4 groups of WKY and 4 groups of SHR (n = 6 for each group).

Protocol 2: The high K^+ solution induced arterial ring constriction, ACh induced dose-dependent vasodilatation, the effects of pretreatment with saline, A-779 (10^{-4} mol/L), L-NAME (10^{-3} mol/L), DT-2 (10^{-4} mol/L), 0.1% DMSO, ODQ (10^{-4} mol/L) and 8-Bromo-cGMP (10^{-4} mol/L) on Ang-(1-7) induced dose-dependent vasodilatation were determined in 7 groups of WKY and 7 groups of SHR (n = 6 for each group).

Protocol 3: Mas receptor protein expression detected by western blotting and Mas receptor immunohistochemistry of MA, CA and PA were determined in 1 groups of WKY and 1 groups of SHR (n = 6 for each group).

Protocol 4: The effects of saline, Ang-(1-7) (10^{-4} mol/L), A-779 (10^{-4} mol/L) and A-779 + Ang-(1-7) on NO, cGMP and PKG levels of CA, MA or PA were detected in 4 groups of WKY and 4 groups of SHR (n = 6 for each group). CA, MA or PA were isolated from rats and incubated in Krebs-Henseleit solution added with Ang-(1-7) or A-779 for 20 min. A-779 pretreatment was administered 20 min before Ang-(1-7) treatment.

2.12. Statistical analysis

Data are expressed as the mean ± S.E. One-way or two-way ANOVA was used, followed by the Bonferroni test, for post-hoc analysis when multiple comparisons were made. $P < 0.05$ was considered statistically significant.

3. Results

3.1. General data in WKY and SHR

The systolic blood pressure measured in conscious state and the mean arterial pressure measured under anesthesia were significantly increased in SHR, while body weight or heart rate between WKY and SHR had no significant difference (Table 1). Compared with WKY, the high K^+ solution induced vasoconstriction in MA, CA and PA was enhanced significantly in SHR (Table 2). ACh-induced dose-dependent relaxations in MA, CA and PA in SHR were attenuated significantly compared with WKY (Fig. 1).

3.2. Effects of Ang-(1-7) on baseline renal sympathetic nerve activity, mean arterial pressure and heart rate

Intravenous injection of Ang-(1-7) decreased mean arterial pressure (Fig. 2A) but had no significant effect on renal sympathetic efferent nerve activity (Fig. 2B) and heart rate (Fig. 2C) in both WKY and SHR. This hypotensive effect of Ang-(1-7) lasted for at least 1 h, and was blocked by Mas receptor antagonist A-779 pretreatment. Intravenous injection of A-779 alone, increased mean arterial pressure, and had no significant effect on renal sympathetic nerve activity and heart rate in both WKY and SHR (Fig. 2).

3.3. Ang-(1-7) induced relaxation in MA, CA and PA

Ang-(1-7) dose-dependently caused MA, CA and PA relaxation in both WKY and SHR (Fig. 3).

Table 1
Body weight, SBP, MAP and HR in one representative group of WKY and SHR.

	WKY	SHR
Body Weight, g	318.7 ± 4.2	313.5 ± 3.0
SBP, mm Hg	112.6 ± 5.1	192.2 ± 4.6 *
MAP, mm Hg	90.8 ± 3.8	137.3 ± 4.9 *
HR, beats/min	352.3 ± 10.1	349.6 ± 14.4

Systolic blood pressure (SBP) of tail artery was measured in conscious state by use of a noninvasive computerized tail-cuff system, mean arterial pressure (MAP) and heart rate (HR) were measured with a pressure transducer in the right carotid artery under anesthesia. Values are expressed as mean ± SE. *P < 0.05 compared with the WKY. n = 6 for each group.

Table 2
The high K^+ induced contraction (mg/mm) in MA, CA and PA in one group of WKY and SHR.

	WKY	SHR
MA	432.7 ± 63.2	1042.1 ± 80.8*
CA	82.3 ± 12.5	191.8 ± 32.7*
PA	111.6 ± 15.5	163.0 ± 9.5*

Values are mean ± SE. *P < 0.05 compared with WKY. n = 6 for each group.

3.4. Effect of A-779, L-NAME, DT-2, ODQ and 8-Bromo-cGMP on Ang-(1-7) induced vascular relaxation

Compared with saline or 0.1% DMSO, pretreatment with Mas receptor antagonist A-779 (Fig. 3A), endothelial NO synthase (eNOS) inhibitor L-NAME (Fig. 3B), PKG inhibitor DT-2 (Fig. 4A) or GC inhibitor ODQ (Fig. 4B) significantly inhibited the Ang-(1-7) induced MA, CA and PA relaxation in both WKY and SHR. Conversely, 8-Bromo-cGMP, a cGMP analogue, enhanced the Ang-(1-7) induced vascular relaxation in MA in WKY or SHR (Fig. 4C). Saline, A-779, 0.1% DMSO, ODQ, and 8-Bromo-cGMP had no significant influence on basal vascular tension in WKY or SHR, while L-NAME increased basal vascular tension in MA, CA and PA in SHR, and DT-2 increased basal vascular tension in CA in SHR, respectively (Table 3).

3.5. Mas receptor expression of arteries

Compared with WKY, the Mas receptor protein expressions in MA, CA, and PA of SHR detected by either western blotting (Fig. 5A) or immunohistochemistry (Fig. 5B) were lower than that of WKY.

3.6. NO, cGMP and PKG levels in MA, CA, and PA

The NO (Fig. 6A), cGMP (Fig. 6B) and PKG protein levels (Fig. 6C) in MA, CA, and PA in SHR were much lower than WKY. Ang-(1-7) increased or normalized NO, cGMP and PKG levels in MA, CA, and PA in SHR. The effect of Ang-(1-7) was abolished by pretreatment with A-779 on arteries. However treatment with A-779 alone on arteries had no significant effect on NO, cGMP and PKG levels in both WKY and SHR (Fig. 6).

4. Discussion

Numerous studies have shown that endothelial dysfunction is a hallmark of hypertension. Enhanced vasoconstriction and attenuated vasodilatation due to the endothelial dysfunction of MA, results in increased total peripheral resistance and sustained high blood pressure in hypertension [38]. Endothelial dysfunction induced impaired coronary arterial relaxation reduces the blood supply to the heart and subsequently causes myocardial ischemia and angina in hypertension [11,39,40]. Attenuated pulmonary arterial relaxation increases pulmonary vascular resistance and increases the risk of pulmonary hypertension [19,20]. The present study demonstrates new findings: 1) High K^+ solution induced vasoconstriction were enhanced and acetylcholine-induced dose-dependent relaxations in MA, CA and PA in SHR were attenuated significantly in SHR compared with WKY; 2) Ang-(1-7) caused dose-dependent relaxation in MA, CA, and PA, and this relaxation was blocked by pretreatment with Mas receptor antagonist (A-779), NO synthase inhibitor (L-NAME), GC inhibitor (ODQ), and PKG inhibitor (DT-2); 3) Ang-(1-7) decreased, while A-779 increased arterial blood pressure and abolished the effect of Ang-(1-7); 4) The Mas receptor expression, NO, cGMP and PKG levels of the arteries of SHR decreased compared with WKY; 5) Ang-(1-7) increased NO, cGMP and PKG levels of arteries of SHR, which was blocked by A-779. These results indicate that exogenous Ang-(1-7) improved the endothelial dysfunction in SHR. Activation of Mas receptor by Ang-(1-7) relaxes MA, CA and PA of SHR through the NO-cGMP-PKG pathway, which contributes to the decrease of arterial blood pressure induced by intravenous injection of Ang-(1-7) in SHR.

In the present study, we found that the SBP and the mean arterial pressure were significantly increased in SHR compared with WKY. High K^+ solution induced vasoconstriction was enhanced significantly in SHR. Acetylcholine stimulates the endothelial cells to release NO and then induces vasodilatation. Therefore, acetylcholine-induced vasodilatation was most often used to evaluate arterial endothelial function. We found that acetylcholine-induced endothelium-dependent relaxation in MA, CA, and PA of SHR was impaired significantly compared with that in WKY. These results indicate endothelial dysfunction in small arteries, thus initiating enhanced vasoconstriction and attenuated vasodilatation in SHR. Finding a way to improve endothelial function is important for inhibition of the development and progression of hypertension and organ damage.

The discovery of Ang-(1-7) in the last two decades adds a new important component to the RAS family. Ang-(1-7) has been found to play important roles in the regulation of cardiovascular activity [23,41]. Dense Mas receptors are found in vascular endothelial cells [42], VSMCs [43], and many sites of brain [44]. Within vascular tissue, the Mas receptors are more concentrated in the endothelium [42]. Some studies have shown that Ang-(1-7) infusion decreases blood pressure [45] and induces the mesenteric arterial relaxation in normotensive rats [46,47]. Ang-(1-7) improves endothelial function and delays the development of cardiac remodeling and heart failure in rats with myocardial infarction [48]. We found that Ang-(1-7) in different parts of body has different effects on modulating the blood pressure and sympathetic activity. Our recent studies have found that microinjection of Ang-(1-7) into either PVN or RVLM, two important sites in central nervous system for regulating sympathetic outflow and blood pressure, increases renal sympathetic nerve activity and mean arterial pressure in renovascular hypertensive rats [28,29]. In the present study, we

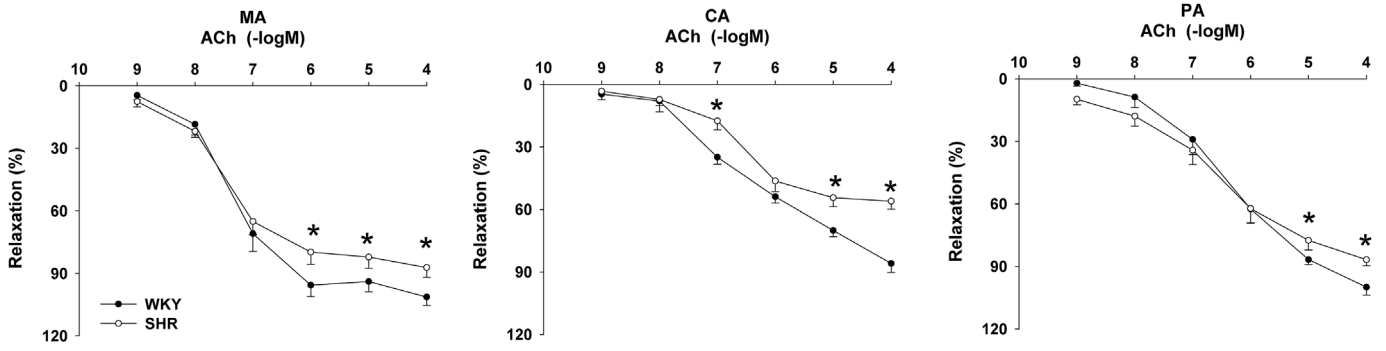

Fig. 1. Dose-response curves of ACh-induced relaxation in MA, CA and PA in WKY and SHR. Values are mean ± SE. *P < 0.05 compared with WKY. n = 6 for each group.

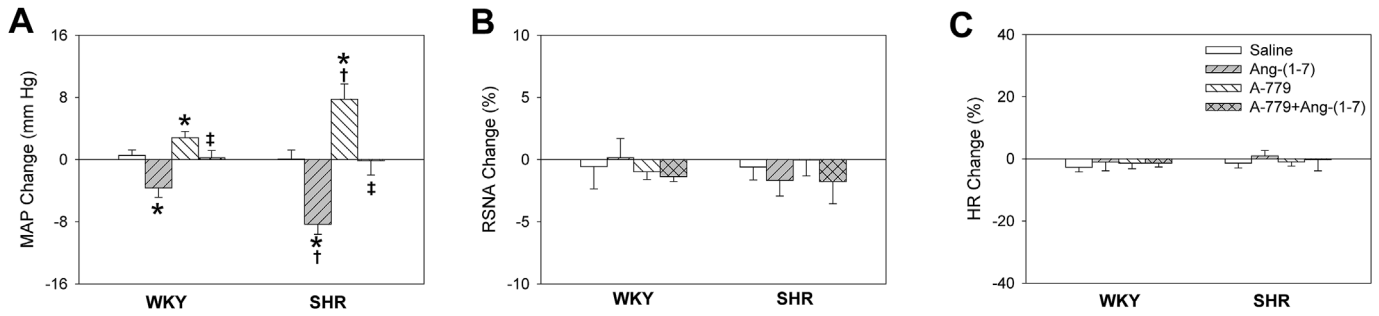

Fig. 2. Effects of intravenous injection of saline, Ang-(1-7) (10^{-4} mol/L), A-779 (10^{-4} mol/L), A-779 + Ang-(1-7) on the mean arterial pressure (MAP) (A), baseline renal sympathetic nerve activity (RSNA) (B) and heart rate (HR) (C) in WKY and SHR. Values are mean ± SE. *P < 0.05 compared with Saline. †P < 0.05 compared with WKY. ‡P < 0.05 compared with Ang-(1-7) alone. n = 6 for each group.

reported that intravenous injection of Ang-(1-7) decreased mean arterial pressure, while Mas receptor antagonist, A-779, increased mean arterial pressure, and that neither had a significant effect on renal sympathetic nerve activity, in either SHR or WKY. Ang-(1-7) caused dose-dependent vascular relaxation in MA, CA and PA in SHR and WKY. The effects of Ang-(1-7) on blood pressure and vascular tension were blocked by pretreated arteries with A-779. These findings indicate a beneficial effect of Ang-(1-7) on the Mas receptor functions within arteries in states of hypertension, by decreasing vascular tension and blood pressure. Ang-(1-7) decrease mean arterial pressure by means of its capacity to induce MA relaxation, thereby decrease the total peripheral resistance in hypertension. Ang-(1-7) induces coronary arterial relaxation, possibly increases the blood supply to the heart and improves myocardial ischemia in hypertension. Ang-(1-7) also induces pulmonary arterial relaxation, which decreases pulmonary vascular resistance and lower the risk of pulmonary arterial hypertension in cases of hypertension. In addition, we found that the Mas receptor expressions in MA, CA, and PA in SHR were much lower than that in WKY. We speculate that Ang-(1-7)/Mas receptor activity was impaired by some unknown mechanisms which inhibited the synthesis of Mas receptor in arteries, which would be studied in the future. Impaired Ang-(1-7)/Mas receptor activity may be an important cause of endothelial dysfunction and subsequent enhanced vasoconstriction and attenuated vasodilatation in hypertension. Therefore, to improve Ang-(1-7)/Mas receptor activity should be beneficial for inhibiting the development and progression of hypertension.

It has been reported that NO mediates some effects of Ang-(1-7) via Mas receptors in some tissues. Ang-(1-7) induces relaxation of canine middle cerebral arteries through NO from endothelial cells [49]. The Ang-(1-7)/Mas axis stimulates eNOS activation and NO production in human aortic endothelial cell and in Mas-transfected Chinese hamster ovary cells [50] and in cardiomyocytes [51]. We know that, endothelial cells release NO, and NO then induces VSMC relaxation through the intracellular cGMP-PKG signaling pathway. However, whether the NO-cGMP-PKG signal pathway mediates the effects of Ang-(1-7) on arteries in SHR remain uncertain. The present study found that pretreating arteries with the eNOS inhibitor L-NAME, significantly blocked the Ang-(1-7)-induced vascular relaxation in MA, CA, and PA in WKY and SHR. Pretreatment with GC inhibitor ODQ or PKG inhibitor DT-2 on arteries, also inhibited the Ang-(1-7)-induced MA, CA, and PA relaxation, while 8-Bromo-cGMP, a cGMP analogue, enhanced the Ang-(1-7)-induced vascular relaxation in MA in both WKY and SHR. Both NO level and cGMP level of MA, CA, and PA in SHR were much lower than that in WKY, further suggesting the presence of endothelial dysfunction in

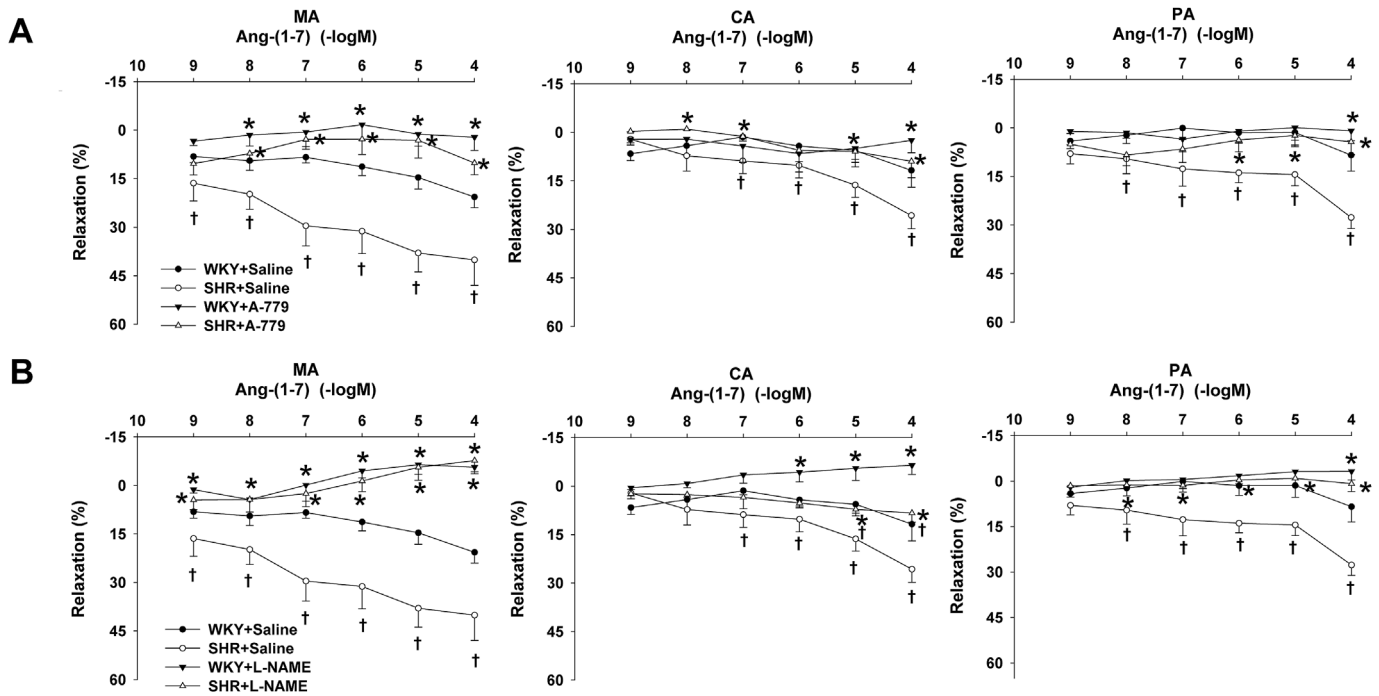

Fig. 3. Effects of saline, A-779 (10^{-4} mol/L) and L-NAME (10^{-3} mol/L) on Ang-(1-7)-induced dose-dependent relaxation in MA, CA and PA in WKY and SHR. Values are mean ± SE. *P < 0.05 compared with Saline. †P < 0.05 compared with WKY. n = 6 for each group.

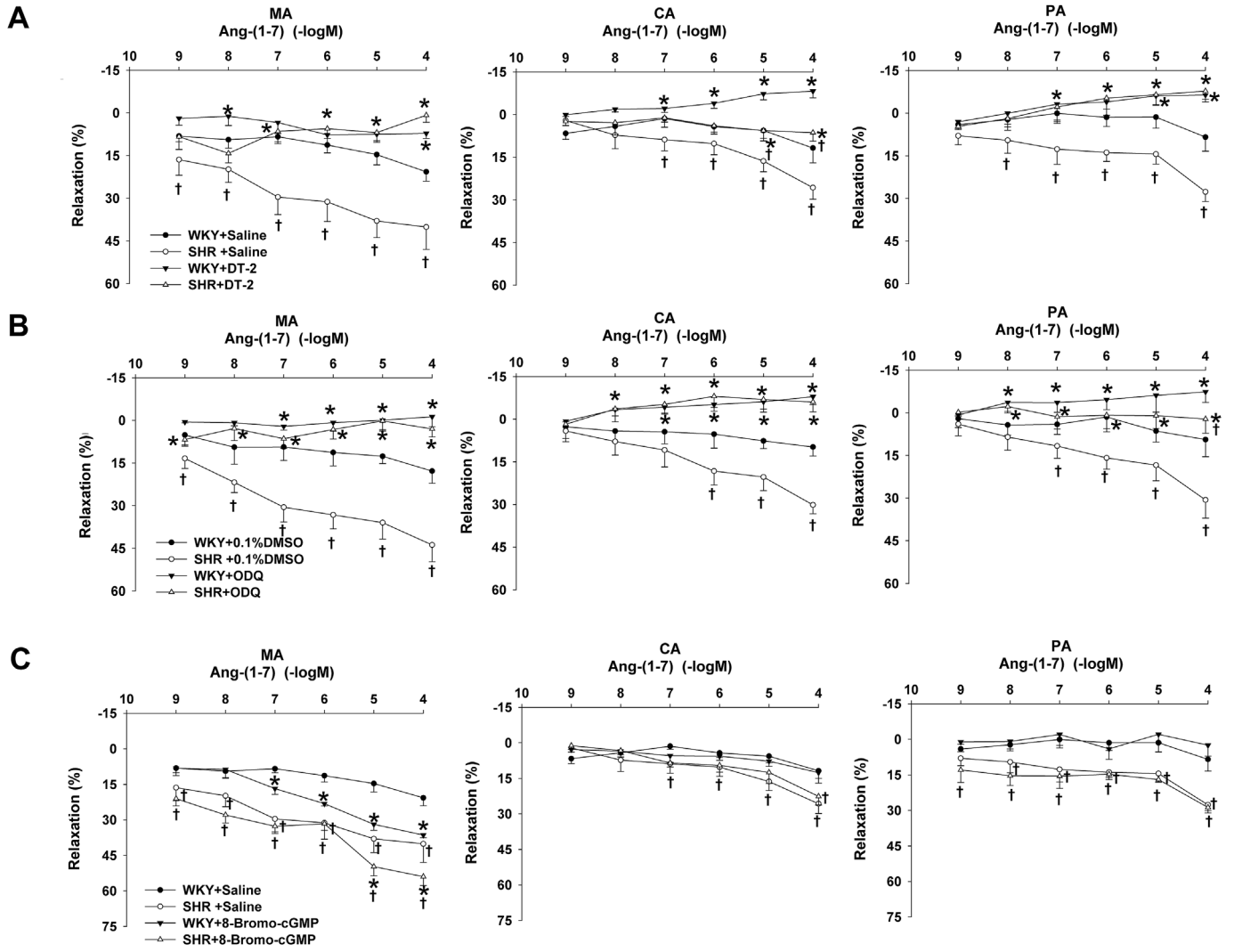

Fig. 4. Effects of saline, DT-2 (10^{-4} mol/L), 0.1% DMSO, ODQ (10^{-4} mol/L) and 8-Bromo-cGMP (10^{-4} mol/L) on Ang-(1-7)-induced dose-dependent relaxation in MA, CA and PA in WKY and SHR. Values are mean ± SE. *P < 0.05 compared with Saline or 0.1% DMSO. †P < 0.05 compared with WKY. n = 6 for each group.

SHR. Ang-(1-7) increased or normalized the NO and cGMP levels in MA, CA, and PA in SHR, which was abolished by A-779. These results suggest that the NO-cGMP-PKG signal pathway in endothelial cell and VSMC is an important signaling mechanism mediating the effects of Ang-(1-7) on vascular tension. Activation of Mas receptors on the endothelial cell membrane by Ang-(1-7), first stimulated NO generation, which then relaxed the VSMC through the intracellular cGMP-PKG signal pathway.

In conclusion, enhanced vasoconstriction and attenuated vasodilatation due to the endothelial dysfunction occurred and the activity of the Ang-(1-7)/Mas receptor was impaired in SHR. Exogenous Ang-(1-7) improved the endothelial dysfunction in SHR. Activation of Mas receptors by Ang-(1-7) relaxes MA, CA, and PA through the NO-cGMP-PKG pathway, contributing to a drop in blood pressure in SHR. A summary flow diagram showing the role and mechanisms of Ang-(1-7) in vasodilatation was shown in Fig. 7. Impaired Ang-(1-7)/Mas receptor activity inside of artery might be an important reason for the persistence of high blood pressure in SHR. Improvement of arterial Ang-(1-7)/Mas receptors activity might be a therapeutic strategy for treating cardiovascular diseases hypertension.

Table 3
Influences of saline, A-779 (10^{-4} mol/L), L-NAME (10^{-3} mol/L), DT-2 (10^{-4} mol/L), 8-Bromo-cGMP (10^{-4} mol/L), 0.1% DMSO and ODQ (10^{-4} mol/L) on the basal vascular tension (mg/mm) in WKY and SHR.

		Saline	A-779	L-NAME	DT-2	8-Bromo-cGMP	0.1% DMSO	ODQ
WKY	MA	1.39 ± 3.33	−2.40 ± 1.44	−0.50 ± 3.35	2.70 ± 2.57	1.15 ± 1.60	−0.23 ± 3.79	−1.08 ± 1.73
	CA	5.35 ± 4.65	3.40 ± 2.81	4.38 ± 3.29	4.35 ± 1.80	−3.66 ± 1.94	2.11 ± 5.03	2.82 ± 1.26
	PA	5.95 ± 4.25	5.77 ± 3.02	2.77 ± 2.87	−0.47 ± 2.23	−3.33 ± 2.63	4.34 ± 3.85	8.83 ± 1.14
SHR	MA	4.99 ± 2.41	3.91 ± 3.23	24.82 ± 2.17*†	14.88 ± 4.79	4.23 ± 2.46	1.76 ± 4.13	6.62 ± 3.34
	CA	2.80 ± 4.62	8.18 ± 3.24	46.53 ± 4.98*†	49.33 ± 5.52*†	0.40 ± 5.83	−1.80 ± 5.11	10.59 ± 6.40
	PA	2.11 ± 5.16	4.96 ± 2.19	49.46 ± 6.61*†	11.43 ± 5.40	−1.26 ± 6.27	3.49 ± 4.77	9.19 ± 4.28

Data showed the changes (mg/mm) of vascular tension from the values before chemical intervention. Values are expressed as mean ± SE. *P < 0.05 vs. the Saline or 0.1% DMSO, †P < 0.05 vs. WKY. n = 6 for each group.

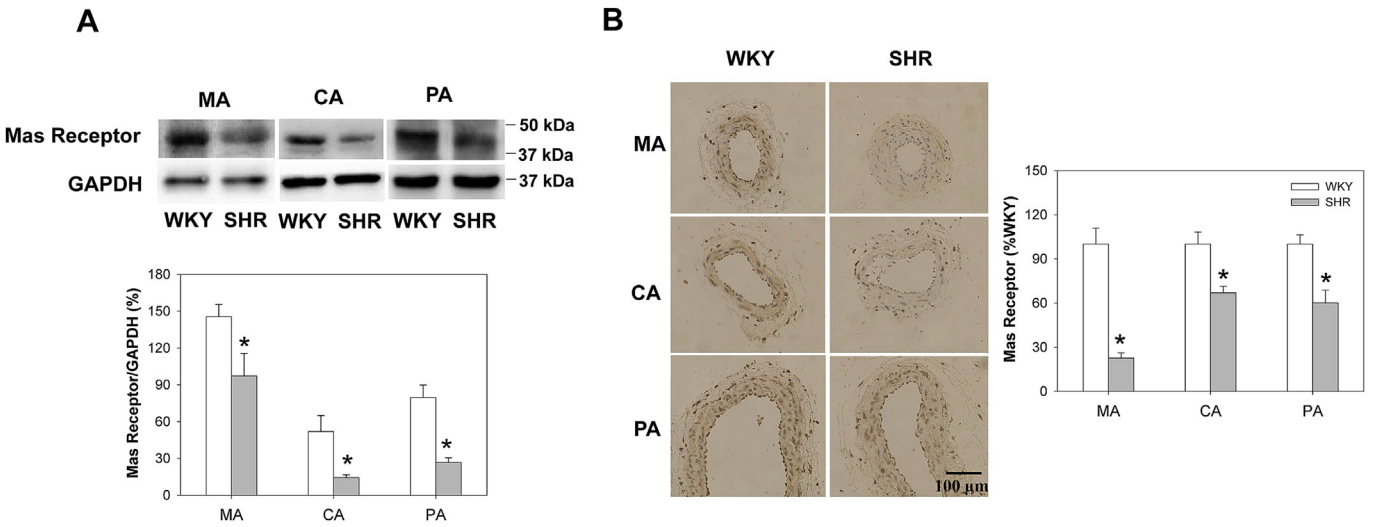

Fig. 5. Mas receptor protein expression detected by western blotting (A) and immunohistochemistry (B) of MA, CA and PA in WKY and SHR. Values are mean ± SE. *P < 0.05 compared with WKY. n = 6 for each group.

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

All authors contributed to the work in this paper. Y.H. conceived and designed the experiments. F.Z., X.S.R., Y.X., Y.R.L and S.S

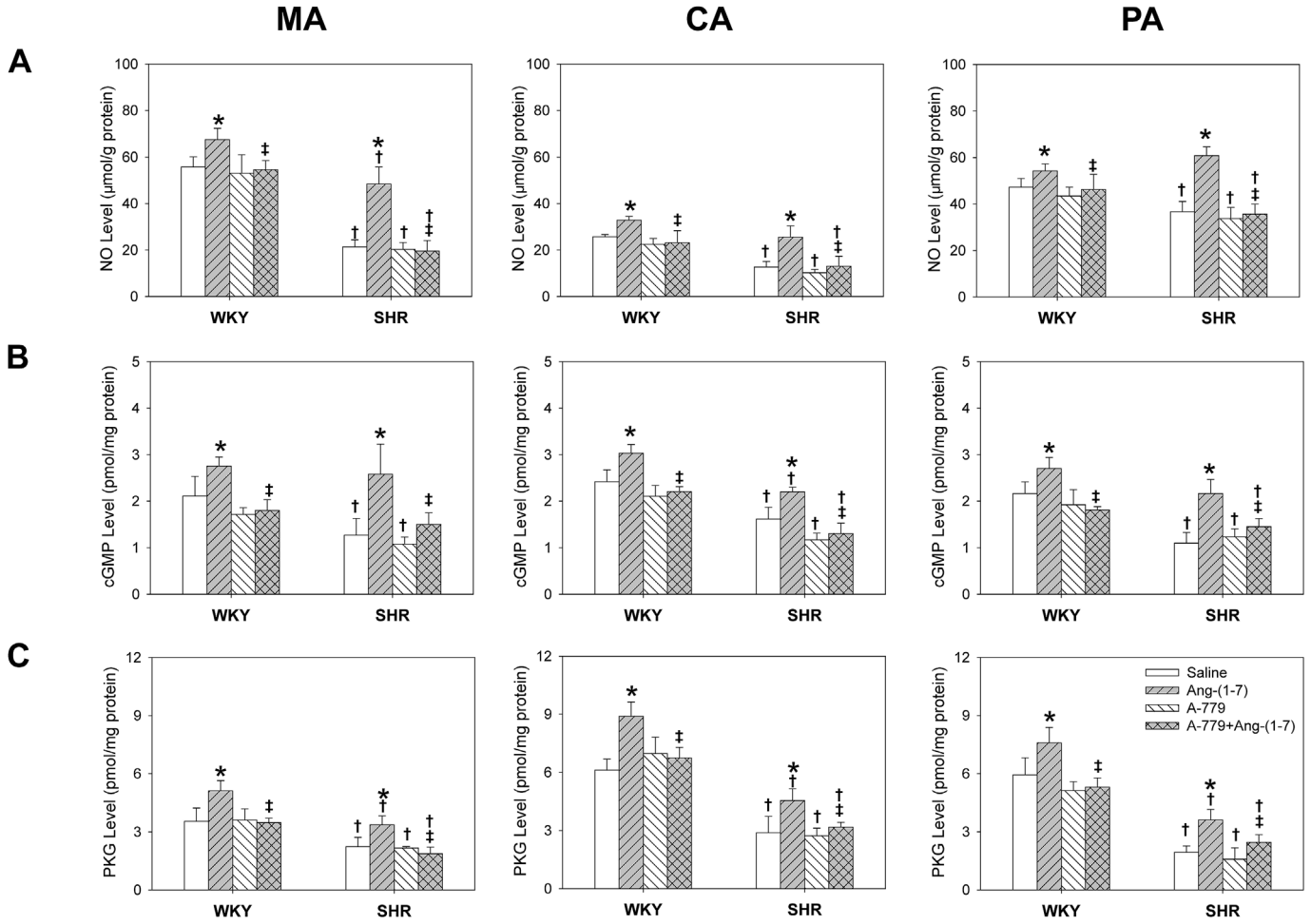

Fig. 6. Effects of the saline, Ang-(1-7) (10^{-4} mol/L), A-779 (10^{-4} mol/L), A-779 + Ang-(1-7) on the NO level (A), cGMP level (B) and PKG protein level (C) of MA, CA, and PA in WKY and SHR. Values are mean ± SE. *P < 0.05 compared with Saline. †P < 0.05 compared with WKY. ‡P < 0.05 compared with Ang-(1-7) alone. n = 6 for each group.

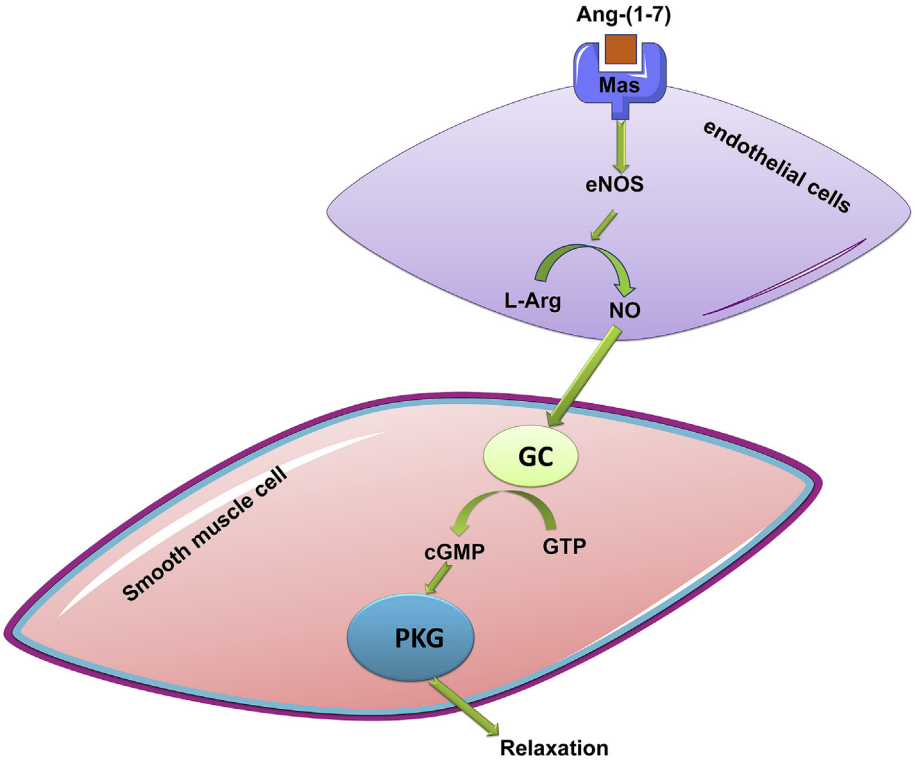

Fig. 7. A summary flow diagram showing the role and mechanisms of Ang-(1-7) in vasodilatation.

performed the experiments. F.Z., P.L. and A.D.C analyzed the data. Y.H. and H.T wrote or contributed to the writing of the manuscript. H.T provided intellectual suggestions and critically reviewed the manuscript.

Author disclosure statement

No competing financial interests exist.

Sources of funding

This work was supported by the National Natural Science Foundation of China [81470538 & 31571168], Jiangsu Qing Lan Project and the Natural Science Basis Research Plan in Shanxi Province of China (2018JC-012).

References

[1] A. Yannoutsos, B.I. Levy, M.E. Safar, G. Slama, J. Blacher, Pathophysiology of hypertension: interactions between macro and microvascular alterations through endothelial dysfunction, J. Hypertens. 32 (2014) 216–224.
[2] G.E. Boari, D. Rizzoni, C. De Ciuceis, E. Porteri, D. Avanzi, C. Platto, M. Mazza, A. Brignani, C.A. Rosei, D. Ricotta, L. Caimi, E.A. Rosei, Structural alterations in subcutaneous small resistance arteries predict changes in the renal function of hypertensive patients, J. Hypertens. 28 (2010) 1951–1958.
[3] A. Virdis, A. Bacca, R. Colucci, E. Duranti, M. Fornai, G. Materazzi, C. Ippolito, N. Bernardini, C. Blandizzi, G. Bernini, S. Taddei, Endothelial dysfunction in small arteries of essential hypertensive patients: role of cyclooxygenase-2 in oxidative stress generation, Hypertension 62 (2013) 337–344.
[4] Y. Higashi, S. Sasaki, K. Nakagawa, H. Matsuura, T. Oshima, K. Chayama, Endothelial function and oxidative stress in renovascular hypertension, N. Engl. J. Med. 346 (2002) 1954–1962.
[5] A.C. Henriques, F.H. Carvalho, H.N. Feitosa, R.H. Macena, R.M. Mota, J.C. Alencar, Endothelial dysfunction after pregnancy-induced hypertension, Int. J. Gynaecol. Obstet. 124 (2014) 230–234.
[6] P.Y. Chen, P.W. Sanders, Role of nitric oxide synthesis in salt-sensitive hypertension in Dahl/Rapp rats, Hypertension 22 (1993) 812–818.
[7] O. Boonla, U. Kukongviriyapan, P. Pakdeechote, V. Kukongviriyapan, P. Pannangpetch, P. Prachaney, S.E. Greenwald, Curcumin improves endothelial dysfunction and vascular remodeling in 2K-1C hypertensive rats by raising nitric oxide availability and reducing oxidative stress, Nitric Oxide 42 (2014) 44–53.
[8] X. Zhang, Y. Fu, X. Xu, M. Li, L. Du, Y. Han, Y. Ge, PERK pathway are involved in NO-induced apoptosis in endothelial cells cocultured with RPE under high glucose conditions, Nitric Oxide 40 (2014) 10–16.
[9] U. Forstermann, E.I. Closs, J.S. Pollock, M. Nakane, P. Schwarz, I. Gath, H. Kleinert, Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions, Hypertension 23 (1994) 1121–1131.
[10] S. Kerr, M.J. Brosnan, M. McIntyre, J.L. Reid, A.F. Dominiczak, C.A. Hamilton, Superoxide anion production is increased in a model of genetic hypertension: role of the endothelium, Hypertension 33 (1999) 1353–1358.
[11] U. Landmesser, H. Drexler, Endothelial function and hypertension, Curr. Opin. Cardiol. 22 (2007) 316–320.
[12] V. Chinnathambi, M. Balakrishnan, J. Ramadoss, C. Yallampalli, K. Sathishkumar, Testosterone alters maternal vascular adaptations: role of the endothelial NO system, Hypertension 61 (2013) 647–654.
[13] J.E. Brush Jr., D.P. Faxon, S. Salmon, A.K. Jacobs, T.J. Ryan, Abnormal endothelium-dependent coronary vasomotion in hypertensive patients, J. Am. Coll. Cardiol. 19 (1992) 809–815.
[14] A. Deussen, V. Ohanyan, A. Jannasch, L. Yin, W. Chilian, Mechanisms of metabolic coronary flow regulation, J. Mol. Cell. Cardiol. 52 (2012) 794–801.
[15] E. Gutierrez, A.J. Flammer, L.O. Lerman, J. Elizaga, A. Lerman, F. Fernandez-Aviles, Endothelial dysfunction over the course of coronary artery disease, Eur. Heart J. 34 (2013) 3175–3181.
[16] M.T. Olivari, C. Fiorentini, A. Polese, M.D. Guazzi, Pulmonary hemodynamics and right ventricular function in hypertension, Circulation 57 (1978) 1185–1190.
[17] C. Fiorentini, P. Barbier, C. Galli, A. Loaldi, G. Tamborini, E. Tosi, M.D. Guazzi, Pulmonary vascular overreactivity in systemic hypertension. A pathophysiological link between the greater and the lesser circulation, Hypertension 7 (1985) 995–1002.
[18] S. Aharinejad, D.E. Schraufnagel, P. Bock, C.A. MacKay, E.K. Larson, A. Miksovsky, S.C. Marks Jr., Spontaneously hypertensive rats develop pulmonary hypertension and hypertrophy of pulmonary venous sphincters, Am. J. Pathol. 148 (1996) 281–290.
[19] S. Gomart, C. Damoiseaux, P. Jespers, M. Makanga, N. Labranche, S. Pochet, C. Michaux, G. Berkenboom, R. Naeije, K. McEntee, L. Dewachter, Pulmonary vasoreactivity in spontaneously hypertensive rats–effects of endothelin-1 and leptin, Respir. Res. 15 (2014) 12.
[20] H. Christou, H. Hudalla, Z. Michael, E.J. Filatava, J. Li, M. Zhu, J.S. Possomato-Vieira, C. Dias-Junior, S. Kourembanas, R.A. Khalil, Impaired pulmonary arterial vasoconstriction and nitric oxide-mediated relaxation underlie severe pulmonary hypertension in the sugen-hypoxia rat model, J. Pharmacol. Exp. Ther. 364 (2018) 258–274.
[21] S. Keidar, M. Kaplan, A. Gamliel-Lazarovich, ACE2 of the heart: from angiotensin I to angiotensin (1-7), Cardiovasc. Res. 73 (2007) 463–469.
[22] C. Mercure, A. Yogi, G.E. Callera, A.B. Aranha, M. Bader, A.J. Ferreira, R.A. Santos, T. Walther, R.M. Touyz, T.L. Reudelhuber, Angiotensin(1-7) blunts hypertensive cardiac remodeling by a direct effect on the heart, Circ. Res. 103 (2008) 1319–1326.

[23] J.C. Velez, K.J. Ryan, C.E. Harbeson, A.M. Bland, M.N. Budisavljevic, J.M. Arthur, W.R. Fitzgibbon, J.R. Raymond, M.G. Janech, Angiotensin I is largely converted to angiotensin (1-7) and angiotensin (2-10) by isolated rat glomeruli, Hypertension 53 (2009) 790–797.
[24] R.A. Santos, A.C. Simoes e Silva, C. Maric, D.M. Silva, R.P. Machado, I. de Buhr, S. Heringer-Walther, S.V. Pinheiro, M.T. Lopes, M. Bader, E.P. Mendes, V.S. Lemos, M.J. Campagnole-Santos, H.P. Schultheiss, R. Speth, T. Walther, Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 8258–8263.
[25] R.A. Santos, M.J. Campagnole-Santos, N.C. Baracho, M.A. Fontes, L.C. Silva, L.A. Neves, D.R. Oliveira, S.M. Caligiorne, A.R. Rodrigues, C. Gropen Junior, et al., Characterization of a new angiotensin antagonist selective for angiotensin-(1-7): evidence that the actions of angiotensin-(1-7) are mediated by specific angiotensin receptors, Brain Res. Bull. 35 (1994) 293–298.
[26] R.A. Santos, A.S. Haibara, M.J. Campagnole-Santos, A.C. Simoes e Silva, R.D. Paula, S.V. Pinheiro, M.F. Leite, V.S. Lemos, D.M. Silva, M.T. Guerra, M.C. Khosla, Characterization of a new selective antagonist for angiotensin-(1-7), D-pro7-angiotensin-(1-7), Hypertension 41 (2003) 737–743.
[27] R. Faria-Silva, F.V. Duarte, R.A. Santos, Short-term angiotensin(1-7) receptor MAS stimulation improves endothelial function in normotensive rats, Hypertension 46 (2005) 948–952.
[28] Y. Han, H.J. Sun, P. Li, Q. Gao, Y.B. Zhou, F. Zhang, X.Y. Gao, G.Q. Zhu, Angiotensin-(1-7) in paraventricular nucleus modulates sympathetic activity and cardiac sympathetic afferent reflex in renovascular hypertensive rats, PLoS One 7 (2012) e48966.
[29] P. Li, H.J. Sun, B.P. Cui, Y.B. Zhou, Y. Han, Angiotensin-(1-7) in the rostral ventrolateral medulla modulates enhanced cardiac sympathetic afferent reflex and sympathetic activation in renovascular hypertensive rats, Hypertension 61 (2013) 820–827.
[30] X. Ren, F. Zhang, M. Zhao, Z. Zhao, S. Sun, D.R. Fraidenburg, H. Tang, Y. Han, Angiotensin-(1-7) in paraventricular nucleus contributes to the enhanced cardiac sympathetic afferent reflex and sympathetic activity in chronic heart failure rats, Cell. Physiol. Biochem. 42 (2017) 2523–2539.
[31] Y. le Tran, C. Forster, Angiotensin-(1-7) and the rat aorta: modulation by the endothelium, J. Cardiovasc. Pharmacol. 30 (1997) 676–682.
[32] R.A. Marangoni, A.K. Carmona, R.C. Passaglia, D. Nigro, Z.B. Fortes, M.H. de Carvalho, Role of the kallikrein-kinin system in Ang-(1-7)-induced vasodilation in mesenteric arterioles of Wistar rats studied in vivo-in situ, Peptides 27 (2006) 1770–1775.
[33] J. Stegbauer, V. Oberhauser, O. Vonend, L.C. Rump, Angiotensin-(1-7) modulates vascular resistance and sympathetic neurotransmission in kidneys of spontaneously hypertensive rats, Cardiovasc. Res. 61 (2004) 352–359.
[34] M. Durik, R. van Veghel, A. Kuipers, R. Rink, M. Haas Jimoh Akanbi, G. Moll, A.H. Danser, A.J. Roks, The effect of the thioether-bridged, stabilized Angiotensin-(1-7) analogue cyclic ang-(1-7) on cardiac remodeling and endothelial function in rats with myocardial infarction, Int. J. Hypertens. 2012 (2012) 536426.
[35] Y. Han, Y.E. Cho, R. Ayon, R. Guo, K.D. Youssef, M. Pan, A. Dai, J.X. Yuan, A. Makino, SGLT inhibitors attenuate NO-dependent vascular relaxation in the pulmonary artery but not in the coronary artery, Am. J. Physiol. Lung Cell Mol. Physiol. 309 (2015) L1027–L1036.
[36] P. Li, F. Zhang, H.J. Sun, F. Zhang, Y. Han, Angiotensin-(1-7) enhances the effects of angiotensin II on the cardiac sympathetic afferent reflex and sympathetic activity in rostral ventrolateral medulla in renovascular hypertensive rats, J Am Soc Hypertens 9 (2015) 865–877.
[37] H.J. Sun, P. Li, W.W. Chen, X.Q. Xiong, Y. Han, Angiotensin II and angiotensin-(1-7) in paraventricular nucleus modulate cardiac sympathetic afferent reflex in renovascular hypertensive rats, PLoS One 7 (2012) e52557.
[38] Y.J. Gao, R.M. Lee, Hydrogen peroxide induces a greater contraction in mesenteric arteries of spontaneously hypertensive rats through thromboxane A(2) production, Br. J. Pharmacol. 134 (2001) 1639–1646.
[39] D. Hasdai, R.J. Gibbons, D.R. Holmes Jr., S.T. Higano, A. Lerman, Coronary endothelial dysfunction in humans is associated with myocardial perfusion defects, Circulation 96 (1997) 3390–3395.
[40] J. Shaw, T. Anderson, Coronary endothelial dysfunction in non-obstructive coronary artery disease: risk, pathogenesis, diagnosis and therapy, Vasc. Med. 21 (2016) 146–155.
[41] A.Q. Gomes da Silva, C.H. Xavier, M.J. Campagnole-Santos, S.M. Caligiorne, O.C. Baltatu, M. Bader, R.A. Santos, M.A. Fontes, Cardiovascular responses evoked by activation or blockade of GABA(A) receptors in the hypothalamic PVN are attenuated in transgenic rats with low brain angiotensinogen, Brain Res. 1448 (2012) 101–110.
[42] N. Alenina, P. Xu, B. Rentzsch, E.L. Patkin, M. Bader, Genetically altered animal models for Mas and angiotensin-(1-7), Exp. Physiol. 93 (2008) 528–537.
[43] F. Zhang, X. Ren, M. Zhao, B. Zhou, Y. Han, Angiotensin-(1-7) abrogates angiotensin II-induced proliferation, migration and inflammation in VSMCs through inactivation of ROS-mediated PI3K/Akt and MAPK/ERK signaling pathways, Sci. Rep. 6 (2016) 34621.
[44] R. Metzger, M. Bader, T. Ludwig, C. Berberich, B. Bunnemann, D. Ganten, Expression of the mouse and rat mas proto-oncogene in the brain and peripheral tissues, FEBS Lett. 357 (1995) 27–32.
[45] A.N. Braga, M. da Silva Lemos, J.R. da Silva, W.R. Fontes, R.A. dos Santos, Effects of angiotensins on day-night fluctuations and stress-induced changes in blood pressure, Am. J. Physiol. Regul. Integr. Comp. Physiol. 282 (2002) R1663–R1671.
[46] G. Raffai, M.J. Durand, J.H. Lombard, Acute and chronic angiotensin-(1-7) restores vasodilation and reduces oxidative stress in mesenteric arteries of salt-fed rats, Am. J. Physiol. Heart Circ. Physiol. 301 (2011) H1341–H1352.
[47] W.O. Sampaio, A.A. Nascimento, R.A. Santos, Systemic and regional hemodynamic effects of angiotensin-(1-7) in rats, Am. J. Physiol. Heart Circ. Physiol. 284 (2003) H1985–H1994.
[48] J. Wang, W. He, L. Guo, Y. Zhang, H. Li, S. Han, D. Shen, The ACE2-Ang (1-7)-Mas receptor axis attenuates cardiac remodeling and fibrosis in post-myocardial infarction, Mol. Med. Rep. 16 (2017) 1973–1981.
[49] K. Feterik, L. Smith, Z.S. Katusic, Angiotensin-(1-7) causes endothelium-dependent relaxation in canine middle cerebral artery, Brain Res. 873 (2000) 75–82.
[50] W.O. Sampaio, R.A. Souza dos Santos, R. Faria-Silva, L.T. da Mata Machado, E.L. Schiffrin, R.M. Touyz, Angiotensin-(1-7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways, Hypertension 49 (2007) 185–192.
[51] M.F. Dias-Peixoto, R.A. Santos, E.R. Gomes, M.N. Alves, P.W. Almeida, L. Greco, M. Rosa, B. Fauler, M. Bader, N. Alenina, S. Guatimosim, Molecular mechanisms involved in the angiotensin-(1-7)/Mas signaling pathway in cardiomyocytes, Hypertension 52 (2008) 542–548.