

Remodeled structure and reduced contractile responsiveness of ocular ciliary artery in spontaneously hypertensive rats

Ya-Ru Dong^{1,2}, Claire E Gustafson³, Jun Wang¹, Ji-Zhe Cui¹, Takeshi Yoshitomi²

¹Department of Ophthalmology, the Second Hospital of Jilin University, Changchun 130041, Jilin Province, China

²Department of Ophthalmology, Akita University School of Medicine, Akita 010-8543, Japan

³Division of Immunology and Rheumatology, Department of Medicine, Stanford University, CA 94305-6104, USA

Correspondence to: Ya-Ru Dong. Department of Ophthalmology, the Second Hospital of Jilin University, 218 Ziqiang Street, Changchun 130041, Jilin Province, China. dongyaru@sina.com; Takeshi Yoshitomi. Department of Ophthalmology, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan. yoshitom@med.akita-u.ac.jp

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Abstract

• **AIM:** To investigate the alterations in both structure and contractile responsiveness of ocular ciliary artery (OCA) in spontaneously hypertensive rat (SHR).

• **METHODS:** In this experiment, 20-week-old male SHR and Wistar Kyoto rat (WKY) were studied. The heart rate (HR), the blood pressure (BP; the systolic BP and the diastolic BP) of rats with an electronic sphygmomanometer were measured. Vascular morphometry and isometric tension measurement were used to investigate the alterations in structure and contractility of OCA.

• **RESULTS:** A general narrowing of OCAs was observed in SHR compared to the control WKY. In SHR, the media of OCAs were thicker, the luminal diameters were smaller, and the media-to-lumen ratios were higher when compared with WKY ($P<0.05$). The contractions of OCAs evoked by norepinephrine were smaller in SHR compared to control ($P<0.05$). Then, OCAs were pretreated with iberiotoxin, L-NAME, or indomethacin 30min before norepinephrine-induced contraction. Iberiotoxin (0.1 $\mu\text{mol/L}$) has not changed the norepinephrine-induced contractions in OCAs from both groups. However, L-NAME (100 $\mu\text{mol/L}$) increased the vasoconstrictions, the increased extents were similar in SHR and WKY ($P>0.05$). Indomethacin (10 $\mu\text{mol/L}$) decreased the contractions induced by norepinephrine in OCAs from WKY ($P<0.05$), but did not change those contractions in vessels from SHR ($P>0.05$).

• **CONCLUSION:** Our results demonstrate that the structure and function of OCAs are altered in hypertension. OCAs from SHR are remodeled with decreased lumen diameter and increased media-to-lumen ratio. Moreover, the contractile responsiveness of OCAs from SHR is diminished due to the disruption of vasoconstrictive effect of prostaglandins.

• **KEYWORDS:** remodeled structure; contractile responsiveness; ocular ciliary artery; hypertension; spontaneously hypertensive rat

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INTRODUCTION

Vascular structural and functional impairments can lead to numerous common human diseases. Hypertension, which affects almost one-third of adults in the US, is characterized by the structural and functional macro- and micro-vascular alterations^[1]. These changes result in disturbances of perfusion in brain, heart, kidney, and other organs. Elevated blood pressure (BP) also affects ocular vasculature to cause hypertensive retinopathy, which is a series of extensive or focal retinal micro-vascular impairments. It comprise of retinal microaneurysms, arteriolar thickening, arteriovenous nicking and, in severe cases, Elschnig's spots, flame hemorrhage, cotton wool spots, hard exudates, optic nerve oedema and, serous retinal detachment^[2-3]. These types of lesions indicate the reduction or loss of blood flow in retinal respective area linked to alterations of vessel structure and function^[2].

The wall of small artery can be divided into 3 layers: intima, media and adventitia. There are several indicators that describe the structural properties of small arteries, such as lumen diameter, wall or media thickness, wall- or media-to-lumen ratios. The wall- or media-to-lumen ratio is more physiologically important^[4]. It has been shown that essential hypertension is accompanied by an increase in media-to-lumen

ratio and a decrease in luminal diameter in small arteries^[4]. Thus, the structure of small arteries is remodeled in patients with essential hypertension.

Elevated sympathetic neural tone is considered as a contributor to the development and maintenance of hypertension^[5-7]. However, the sympathetic nerve overactivation in hypertension is not a global response, it does not occur in certain hypertensive patients and animal models^[8-10]. Sympathetic nervous release norepinephrine, which in turn stimulate postsynaptic α_1 -adrenergic receptor (α_1 -AR) expressed in vascular smooth muscle cells, and evoke vasoconstriction^[11]. At present, the characteristics of vascular contractile responsiveness in hypertension is poorly understood. The aim of this study was to determine whether the structure and contractile responsiveness of ocular ciliary arteries (OCAs) were altered in spontaneously hypertensive rat (SHR) compared with non-hypertensive Wistar Kyoto rat (WKY). Vascular morphometry and isometric tension measurement were used in this study.

MATERIALS AND METHODS

Ethical Approval This study was approved by the Animal Experiment Committee of Akita University. All rats were treated according to the principles of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Cardiovascular Variables In this experiment, 20-week-old male SHR and WKY were studied. All rats were housed in specific pathogen-free (SPF) at +20°C and under a controlled 12-hour light-dark cycle and fed standard feed and water on demand. We measured the heart rate (HR), the systolic BP (SBP) and the diastolic BP (DBP) of rats with an electronic sphygmomanometer (BP-98A; Tokyo, Japan).

Isolation of the Ocular Ciliary Arteries After measuring the cardiovascular variables, rats were euthanized with ether (Abbott, IL, USA). Then, their eyes were immediately removed to ensure that the optic nerve attached to the eye is as long as possible (Figure 1A). The eyes were placed in a Krebs solution bubbled with 95% O₂ and 5% CO₂. Our previous article has described in detail the composition of Krebs solution^[12]. Under a dissecting microscope, the distal section of OCA (length: 3-4 mm) and surrounding connective tissue were separated and cut off from the optic nerve (Figure 1B). Only one vascular segment was taken from an eyeball.

Vascular Morphometry In order to study the characteristics of the vascular morphology, the OCA segments were treated with several steps, such as 10% formalin fixation (30min), paraffin-embedding, cross-sections (5 μ m) cutting, and hematoxylin-eosin (H&E) staining. Then we used an Olympus microscope ($\times 10$ magnification) to collected pictures. The lumen diameter and media thickness of OCAs were measured, and the ratios of media-to-lumen diameter were calculated. Four sectors (from four animals) of each group were analyzed.

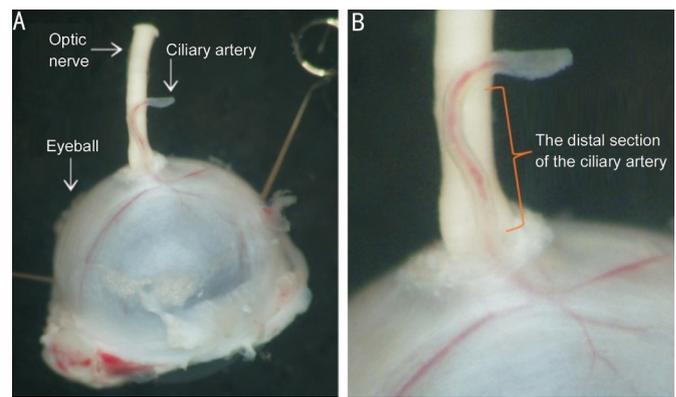


Figure 1 Ocular ciliary artery A: Rat eyeball; B: The vascular segment was cut from the distal section of the ciliary artery.

Isometric Tension Recording Isolated vascular segments (2 mm in length) were mounted in the chamber of Myograph System[®] (JP Trading, Aarhus, Denmark). Our previous reports have described in detail the procedure for mounting the OCAs^[13-14]. After the equilibration period, contractions of the OCA were evoked by a high-K solution and lasted for 20min. Then, 1 μ mol/L carbachol was added to induce relaxation of OCA. Carbachol was a cholinergic agonist acting on vascular endothelial cell receptors^[15]. If high-K-induced contraction was less than 2 mN or carbachol-induced relaxation was less than 0.5 mN, such vascular segments were excluded from this study. After verifying the arterial responsiveness to high-K and carbachol, the medium in the chamber was replaced by Krebs solution.

The contractile responsiveness of the isolated OCAs to norepinephrine was determined. Generally, the segment on the resting tone was maintained for 30min. Then 0.1-100 μ mol/L norepinephrine was added in a cumulative manner every ten minutes. To investigate whether prostaglandins, nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF) were implicated in norepinephrine-induced contraction, 10 μ mol/L indomethacin, a cyclooxygenase inhibitor^[16]; 100 μ mol/L N^G-nitro-L-arginine methylester (L-NAME), a NO synthase inhibitor^[17]; or 0.1 μ mol/L iberiotoxin, a large-conductance calcium-activated K⁺ (BK_{Ca}) channel blocker^[18] was applied. Our previous study has demonstrated that the BK_{Ca} channels are utilized in EDHF signaling pathway in OCA^[19]. Indomethacin, L-NAME or iberiotoxin was added 30min prior to norepinephrine-induced contractions.

Drugs The following drugs were used: norepinephrine, L-NAME, indomethacin, iberiotoxin, and carbachol hydrochloride (Sigma, St. Louis, MO, USA). The concentrations of these drugs were referred to the molarity in the myograph chambers.

Statistical Analysis Means \pm standard deviations were used to express the measured values and *n* represented the number of studied vessel segments unless specifically indicated. Unpaired two-tailed *t*-test was performed to analyze the

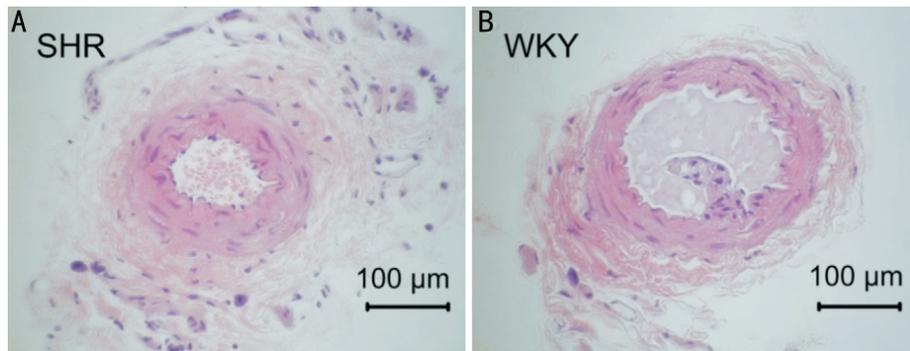


Figure 2 Cross sections of ocular ciliary artery A: SHR; B: WKY. The vessels were stained with hematoxylin-eosin (H&E).

statistical differences between the values. One-way analysis of variance was used to determine the differences between these concentration-response curves. Statistical significant was set at probability values less than 0.05.

RESULTS

Cardiovascular Variables We measured the cardiovascular variables of SHR and WKY to confirm SHR have developed sustaining hypertension. At 20wk of age, although the HR didn't differ between SHR and WKY, both SBP and DBP were higher in SHR compared with WKY ($P < 0.05$, Table 1).

However, these BP values appear overestimated (in WKY, SBP/DBP: $146.2 \pm 8.2 / 104.8 \pm 12.7$ mm Hg). It may be that we have measured the tail artery BP and restraining the animals that tends to make them stressed. Thus, before measuring the BP, we have let the rats move freely on the table for several minutes to calm them down. The first measure was more likely higher, and subsequent measurements would be stable. So, we measured BP four times per rat.

Structure of Ocular Ciliary Artery In SHR, common pathological changes of OCAs caused by hypertension were observed. These changes include lumen diameter and media-to-lumen ratios and they are summarized in Table 2. An overall narrowing of vessels was observed in SHR compared to WKY. The media of vessels became thicker, the lumen became smaller, and the media-to-lumen ratio became larger in SHR compared with WKY ($P < 0.05$; Figure 2).

Norepinephrine-Induced Contraction The norepinephrine-induced concentration-response curve of OCAs were weaker in SHR ($n=7$) compared with WKY ($n=6$, $P < 0.05$; Figure 3). The contractions at $100 \mu\text{mol/L}$ norepinephrine were 3.83 ± 0.78 mN for SHR and 6.30 ± 1.10 mN for WKY.

OCAs were pretreated with iberiotoxin, L-NAME, or indomethacin 30min before norepinephrine-induced contraction. Iberiotoxin ($0.1 \mu\text{mol/L}$) has not changed the norepinephrine-induced contraction in OCAs from SHR ($n=7$) or WKY ($n=6$, $P > 0.05$; Figure 4A, 4B). L-NAME ($100 \mu\text{mol/L}$) increased the norepinephrine-induced contraction in vessels from both SHR ($n=7$) and WKY ($n=6$, $P < 0.05$; Figure 5A, 5B). However, the increased extents were similar in SHR and WKY

Table 1 Comparison of cardiovascular variables between SHR and WKY

Parameters	<i>n</i>	HR (BPM)	SBP (mm Hg)	DBP (mm Hg)
SHR	33	382.3 ± 30.0	218.4 ± 14.9	153.8 ± 16.0
WKY	30	376.5 ± 22.0	146.2 ± 8.2	104.8 ± 12.7
<i>P</i>		>0.05	<0.05	<0.05

SHR: Spontaneously hypertensive rats; WKY: Wistar Kyoto rats; HR: Heart rate; BPM: Beats per min. SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

Table 2 Morphological analyses of OCAs

Parameters	<i>n</i>	Lumen diameter	Media width	Media/lumen
SHR	4	$91.6 \pm 5.7 \mu\text{m}$	$78.4 \pm 6.9 \mu\text{m}$	85.6%
WKY	4	$176.3 \pm 9.8 \mu\text{m}$	$46.4 \pm 8.4 \mu\text{m}$	26.3%
<i>P</i>		<0.05	<0.05	<0.05

SHR: Spontaneously hypertensive rats; WKY: Wistar Kyoto rats.

($P > 0.05$; Figure 5C). Indomethacin ($10 \mu\text{mol/L}$) decreased the contraction curves of OCAs from WKY ($n=6$, $P < 0.05$; Figure 6A), but did not change those contractions of OCAs from SHR ($n=7$, $P > 0.05$; Figure 6B). The contraction curves of WKY incubated with indomethacin were equal to that of SHR without indomethacin ($P > 0.05$; Figure 6C).

DISCUSSION

In this study, we compared the structure and contractile responsiveness of OCAs between SHR and WKY. Vessels from SHR demonstrated the significant remodeling with an increase of media-to-lumen ratio and a reduction of lumen diameter. On the other hands, vascular contractility was uniformly decreased in SHR, in conjunction with sustained elevated BP.

Remodeling of small arteries is a crucial factor in the pathological process of essential hypertension^[20]. It has been strongly suggested that small arteries in patients with essential hypertension had the structural alterations: an increase of media-to-lumen ratio and a decrease of lumen volume^[21]. The present study has shown that OCAs from SHR are remodeled in the same way as human essential hypertension. Similarly, corrosion cast/scanning electron microscopy showed that the choroidal arteries of SHR were tortuosity, caliber irregularity and generalized narrowing^[22]. Micro-vascular alterations can

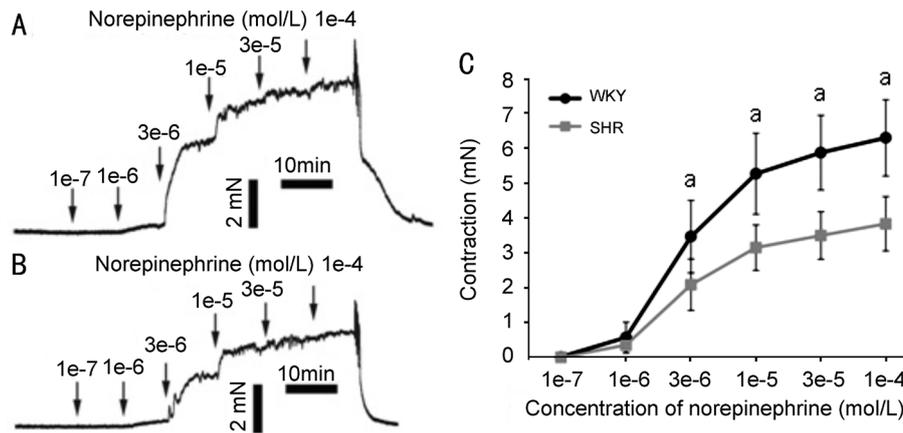


Figure 3 Norepinephrine-induced contractions A representative contraction curve of OCAs from WKY (A) or SHR (B) induced by norepinephrine in a cumulative manner. Horizontal axis is time (min) and vertical axis is isometric tension in millinewtons (mN). C: Greater contraction occurred in OCAs of WKY compared with those of SHR. ^a*P*<0.05.

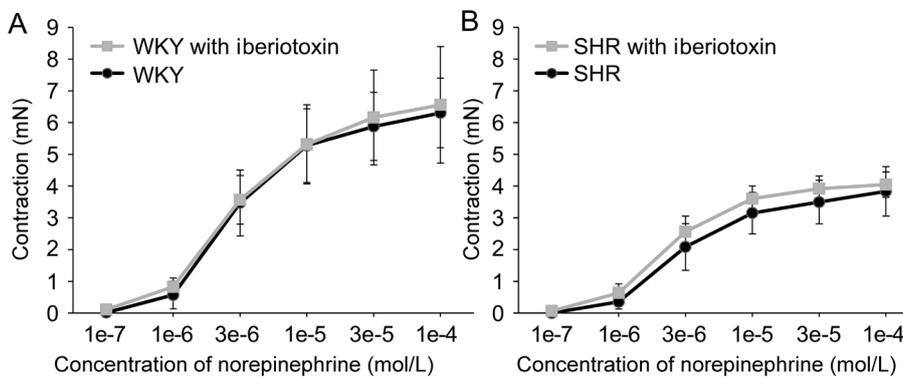


Figure 4 Effect of iberiotoxin on norepinephrine-induced contraction of OCAs Iberiotoxin (0.1 μmol/L) has not changed the contraction curves of OCAs from WKY (A) or SHR (B).

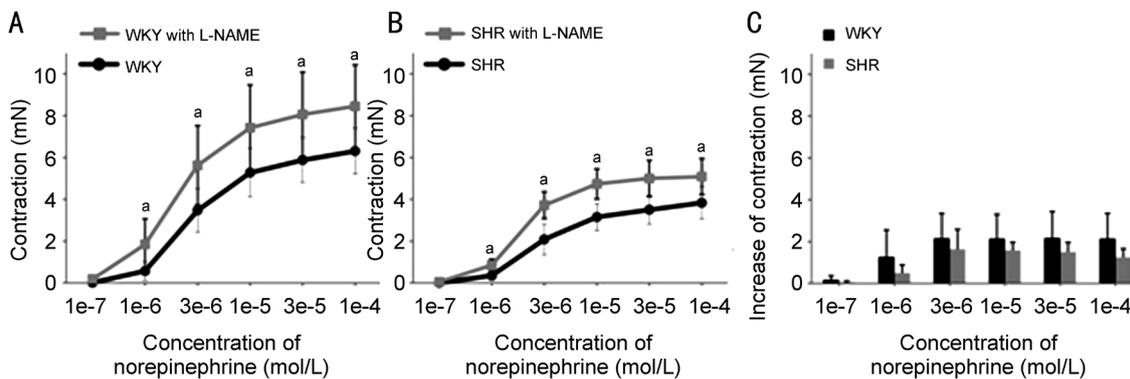


Figure 5 Effect of L-NAME on norepinephrine-induced contraction of OCAs L-NAME (100 μmol/L) increased the contraction curves of OCAs from both WKY (A) and SHR (B). C: The increased extents of vasoconstrictions were similar in WKY and SHR. ^a*P*<0.05.

lead to the tissue perfusion disorder and increased susceptibility to ischemia^[1]. A remodeled OCA reduces blood and oxygen supply to the eye, causes ocular circulatory insufficiency and eventually leads to hypertensive retinopathy. Notably, patients suffering from hypertension develop deteriorated tissue blood perfusion that cause visual dysfunction and damage. For example, hypertension is a frequent risk for nonarteritic anterior ischemic optic neuropathy (NAION) characterized by sudden visual loss in the patients older than 50y^[23]. The pathogenesis of NAION is loss of the ciliary circulation

perfusion at the optic disc, causing ischemia and hypoxia, resulting in axonal swelling and, capillary dilatation and fluid leakage.

On the other hands, our study has shown that concentration-dependent contraction curves to norepinephrine were uniformly diminished in OCA segments from SHR compared with WKY. Regulation of vasomotor activity is achieved through the complex interactions of two types of vascular regulatory factors, vasoconstrictors and vasodilators, and the combined effect of these factors determines the vascular tone^[24].

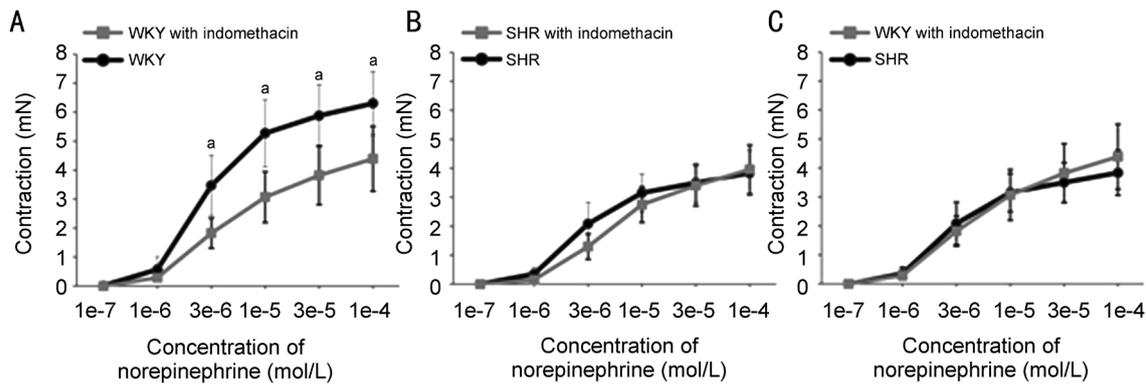


Figure 6 Effect of indomethacin on norepinephrine-induced contraction of OCAs A: Indomethacin (10 μ mol/L) decreased the contraction curves of OCAs from WKY; B: Indomethacin has not changed the contraction of OCAs from SHR; C: The contraction curves of WKY incubated with indomethacin were equal to that of SHR without indomethacin. ^a $P < 0.05$.

Norepinephrine stimulates postsynaptic α_1 -AR expressed in vascular smooth muscle cells, and regulates arterial contraction by acting as a vasoconstrictor^[11]. Vascular endothelial cells can release both vasoconstrictor prostaglandins and vasodilators, such as EDHF, NO, and prostacyclin^[25].

In present study, the involvement of EDHF, NO, or prostaglandins in norepinephrine-induced vasoconstriction was separately analyzed by incubation with iberiotoxin, L-NAME, or indomethacin, respectively. In both experimental groups, iberiotoxin has not changed norepinephrine-induced vasoconstrictions, suggesting that there is not participation of EDHF in the vasoconstrictions. However, the norepinephrine-induced contractions were increased by L-NAME, and the extents of increases were similar in SHR and WKY. This result indicated that NO participates in the regulation of vasoconstriction and is not affected by hypertension. Unlike iberiotoxin and L-NAME, the effects of indomethacin on vasoconstrictions were different between WKY and SHR. Indomethacin decreased the contraction curves of WKY, but did not change those of SHR. It means that the effect of prostaglandins on norepinephrine-induced contraction was destroyed in SHR. Indomethacin could block the production of both vasodilator and vasoconstrictor prostaglandins. It has been reported that the integrated vascular regulatory effect of both kinds of prostaglandins is vasoconstriction in rats^[26]. The present study also proved this finding. A recent study by Blixt *et al*^[27] showed an increase of endothelin-1 mediated vasoconstriction occurring in the ophthalmic artery at 48h after ischemia. In their study, the endothelium of the ophthalmic artery was shown to be intact, and did not have an influence on the contractile response. Our results demonstrated that hypertension disrupted the vasoconstrictive effect of endothelium-derived prostaglandins.

The decreased contractile responsiveness of OCAs in SHR may actually be a protective mechanism for ocular disease caused by hypertension. Unchecked decline in the vascular lumen

can lead to tissue ischemia in hypertension. Disruption of vasoconstrictive effect of prostaglandins results in diminished vascular contraction and, in turn, prevents continual decline in the vascular lumen and further tissue ischemia.

Taken together, our study demonstrated the fact that the structure and function of OCAs were altered in hypertension. OCAs from SHR were remodeled with decreased lumen diameter and increased media-to-lumen ratio. Moreover, the contractile responsiveness of OCAs from SHR was diminished due to the disruption of vasoconstrictive effect of prostaglandins. Although a remodeled OCAs might cause decreased ocular blood supply, the reduced contractile responsiveness of these vessels suggest that it could inhibit further diminishment of the vascular lumen and prevent extensive tissue ischemia.

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