Effect of hypertension and carotid occlusion on brain parenchymal arteriole structure and reactivity

Julie G. Sweet, Siu-Lung Chan, and Marilyn J. Cipolla
Departments of Neurological Sciences, Obstetrics, Gynecology & Reproductive Sciences, and Pharmacology, University of Vermont College of Medicine, Burlington, Vermont

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Sweet JG, Chan SL, Cipolla MJ. Effect of hypertension and carotid occlusion on brain parenchymal arteriole structure and reactivity. J Appl Physiol 119: 817–823, 2015. First published August 20, 2015; doi:10.1152/japplphysiol.00467.2015.—We studied the effect of hypertension and chronic hypoperfusion on brain parenchymal arteriole (PA) structure and function. PAs were studied isolated and pressurized from 18-wk-old Wistar-Kyoto (WKY18; n = 8) and spontaneously hypertensive stroke prone (SHRSP; n = 8) rats. In separate groups, unilateral common carotid artery occlusion (UCCAo) was performed for 4 wk to cause chronic hypoperfusion in 18-wk-old WKY (WKY18-CH; n = 8) and SHRSP (SHRSP18-CH; n = 8). UCCAo caused PAs to have significantly diminished myogenic tone (31 ± 3 vs. 14 ± 6% at 60 mmHg; P < 0.05) and reactivity to pressure from WKY18-CH vs. WKY18 animals. The effect of UCCAo was limited to normotensive animals, as there was little effect of chronic hypoperfusion on vascular reactivity or percent tone in PAs from SHRSP18 vs. SHRSP18-CH animals (53 ± 4 vs. 41 ± 3%; P > 0.05). However, PAs from SHRSP18 and SHRSP5 animals had significantly greater tone compared with WKY18, suggesting an effect of strain and not hypertension per se on PA vasoconstriction. Structurally, PAs from SHRSP18 and SHRSP5 animals had similar sized lumen diameters, but increased wall thickness and distensibility compared with WKY18. Interestingly, chronic hypoperfusion did not affect the structure of PAs from either WKY18-CH or SHRSP18-CH animals. Thus PAs responded to UCCAo with active vasodilation, but not structural remodeling, an effect that was absent in SHRSP. The increased tone of PAs from SHRSP animals, combined with lack of response to chronic hypoperfusion, may contribute to the propensity for ischemic lesions and increased perfusion deficit during hypertension.

hypertension; cerebral arterioles; brain; chronic hypoperfusion; SHRSP

arterial disease and chronic hypoperfusion of the brain are common in adults with large and small vessel disease (SVD) that is associated with increased risk of stroke and cognitive impairment (1, 9, 33, 38). Several animal models have been developed to study chronic hypoperfusion of the brain that involves occlusion or stenosis of one or more carotid and/or vertebral arteries in normotensive animals (2, 4, 14, 32). Depending on the model used, various pathologies have been identified that may be relevant to human disease, including white matter lesions, blood-brain barrier permeability, neuroinflammation, and accelerated cognitive impairment (2, 4, 14, 32). These models have also shown that the cerebral vessels undergo an adaptive response to chronic hypoperfusion involving enlargement of collateral vessels (arteriogenesis) that can restore cerebral blood flow (CBF) to normal over a period of weeks (2, 14, 32). Arteriogenesis of primary and secondary collaterals in response to chronic hypoperfusion is thought to be protective of ischemic injury by enhancing CBF reserve and has been considered as a therapeutic intervention (21, 37). Indeed, enlargement of collaterals due to arteriogenesis after chronic hypoperfusion was shown to decrease infarct size in response to middle cerebral artery (MCA) occlusion (21, 37).

The process of arteriogenesis in response to chronic hypoperfusion occurs in collaterals through increased shear stress that triggers a cascade of events involving endothelial cell activation, recruitment of monocytes and polymorphonuclear cells, and release of various cytokines, chemokines, and growth factors, leading to vessel enlargement (3, 29). Arteriogenesis during chronic hypoperfusion is well-characterized in collaterals, where changes in shear stress are pronounced (30). However, how chronic hypoperfusion affects other segments of the cerebrovasculature that are not collaterals and thus do not experience large changes in shear stress, is less understood. In MCAs, bilateral common carotid artery occlusion (BCCAo) for 15 days caused modest structural remodeling that included hypotrophy of the vessel wall and diminished myogenic tone without a change in passive lumen diameter (22), suggesting adaptive responses to chronic hypoperfusion occur in large, noncollateral cerebral arteries as well.

Blood supply to the brain parenchyma is via penetrating and parenchymal arterioles (PAs) that branch off pial vessels at right angles and directly connect the pial surface vessels to the capillary bed (5). PAs are high-resistance vessels due to their high-resistance vessels due to their high-resistance arteries in normotensive animals (2, 4, 14, 32). Depending on the model used, various pathologies have been identified that may be relevant to human disease, including white matter lesions, blood-brain barrier permeability, neuroinflammation, and accelerated cognitive impairment (2, 4, 14, 32). These models have also shown that the cerebral vessels undergo an adaptive response to chronic hypoperfusion involving enlargement of collateral vessels (arteriogenesis) that can restore cerebral blood flow (CBF) to normal over a period of weeks (2, 14, 32). Arteriogenesis of primary and secondary collaterals in response to chronic hypoperfusion is thought to be protective of ischemic injury by enhancing CBF reserve and has been considered as a therapeutic intervention (21, 37). Indeed, enlargement of collaterals due to arteriogenesis after chronic hypoperfusion was shown to decrease infarct size in response to middle cerebral artery (MCA) occlusion (21, 37).

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Blood supply to the brain parenchyma is via penetrating and parenchymal arterioles (PAs) that branch off pial vessels at right angles and directly connect the pial surface vessels to the capillary bed (5). PAs are high-resistance vessels due to their large and relatively unbranched architecture with few anastomoses (5, 15). PAs have recently been shown to be the major site of autoregulatory vasodilation during unilateral common carotid artery occlusion (UCCAo) that maintains CBF during decreased cerebral perfusion pressure (36). Importantly, structural and functional changes in PAs are key features of ischemic cerebral SVD that undergo remodeling of the arteriolar wall and reduced lumen diameter, loss of autoregulation, and locally increased blood-brain barrier permeability that is thought to underlie white matter lesions and cognitive impairment (13, 27). SVD is also associated with chronic cerebral hypoperfusion that is pronounced in patients with carotid stenosis, aging, and hypertension (10, 38, 39). In fact, hypertension is considered a main risk factor for SVD, yet only a few studies have investigated the effect of chronic hypoperfusion on arteriogenesis in models of hypertension, and even fewer have studied PAs under these conditions. Jalal et al. (20) used spontaneously hypertensive stroke prone (SHRSP) rats combined with chronic hypoperfusion, and reported that BCCAo is lethal in these animals. In addition UCCAo in SHRSP causes white matter damage in both hemispheres, suggesting intolerance to chronic hypoperfusion during hypertension (20).
Further, studies have shown arteriogenesis of both primary (circle of Willis vessels) and secondary (leptomeningeal anastomoses) collaterals in response to chronic hypoperfusion is impaired during hypertension (26, 34). However, the effect of chronic hypoperfusion on PAs in the setting of hypertension is less understood, but potentially important to understand, given the central role these vessels have in SVD.

In the present study, we investigated the effect of chronic hypoperfusion on PAs under normotensive and hypertensive conditions and measured changes in structural remodeling, vasoreactivity, and endothelial function. We hypothesized that chronic hypoperfusion causes adaptive changes in PAs to increase lumen diameter (outward remodeling and decreased tone) that is prevented during hypertension.

**MATERIALS AND METHODS**

*Animals.* Experiments were performed using female Wistar-Kyoto (WKY) and SHRSP rats (Charles River) at age 5 or 18 wk. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Vermont and complied with the National Institutes of Health guidelines for care and use of laboratory animals. Rats were housed in the Animal Care Facility at the University of Vermont, an American Association for Accreditation of Laboratory Animal Care-accredited facility. Rats were maintained on a 12:12-h light-dark cycle and allowed food and water ad libitum. Animals (n = 8/group) were grouped as naive 5-wk-old SHRSP (SHRSP5), naive 18-wk-old SHRSP (SHRSP18), naive 18-wk-old WKY (WKY18), or 18-wk-old WKY (WKY18-CH) or SHRSP (SHRSP18-CH) with 4-wk chronic UCCAo.

*Animal model of chronic UCCAo.* Chronic UCCAo was surgically induced at 14 wk of age and maintained for 4 wk until experimentation at 18 wk old in WKY and SHRSP animals. Animals were anesthetized by inhaled isoflurane (1.5-2%) in oxygen and air. A midline neck incision was placed, and the right common carotid artery and external carotid artery were exposed. The common carotid artery was permanently ligated with two 4-0 sterile silk sutures proximal to the bifurcation of the internal and external carotid arteries. Following surgery, animals received buprenorphine (0.05 mg/kg) subcutaneously near the incision site for pain.

*Blood pressure measurements.* Blood pressures were measured noninvasively at 5 or 18 wk old by determining the tail blood volume with a volume pressure recording sensor and an occlusion tail cuff (Coda 8 system, Kent Scientific, Torrington, CT), as described previously (6). Briefly, animals were placed in individual holders, and both an occlusion cuff and a volume pressure recording sensor cuff were placed near the base of the tail. Volume pressure recording allowed the simultaneous measurement of six blood pressure parameters: systolic blood pressure, diastolic blood pressure, mean blood pressure, heart rate pulse, tail blood volume, and tail blood flow.

*Preparation of isolated PAs and pressurized arteriograph.* Animals were quickly decapitated under isoflurane anesthesia, and the brain was removed and placed in cold, oxygenated physiological saline solution (PSS). PAs branching perpendicularly from the M1 and M2 regions of the right MCA and penetrating into the brain parenchyma were carefully dissected and mounted onto glass cannulas in an arteriograph chamber (Living Systems Instrumentation, Burlington, VT), as previously described (7). In animals that underwent UCCAo, PAs were taken from the side of occlusion.

*Isolated arteriole myogenic tone, reactivity, and structural measurements.* PAs were dissected and mounted in an arteriograph chamber, pressurized to 20 mmHg, and equilibrated for 1 h to allow spontaneous development of myogenic tone. After the equilibration period, myogenic reactivity was assessed with stepwise increases in intravascular pressure up to 100 mmHg and recording lumen diameter once stable. Intravascular pressure was returned to 40 mmHg for the remainder of the experiment. NS309, a small- and intermediate-conductance calcium-activated K+ channel (SK1K) agonist, was cumulatively added to the bath (10⁻⁸-10⁻⁵ M), and diameter measured at each dose. NS309 was washed out of the bath, and PAs were allowed to regain myogenic tone. A single dose of N^o-nitro-L-arginine (L-NNA, 0.1 mM), a nitric oxide synthase (NOS) inhibitor, was added to the bath and diameter measured once stable. Sodium nitroprusside (SNP), a nitric oxide (NO) donor, was cumulatively added to the bath (10⁻⁸-10⁻⁵ M) in the presence of L-NNA, and diameter measured at each concentration. At the conclusion of the experiment, PAs were superfused with papaverine (0.1 mM) and diltiazem (10 μM) in calcium-free PSS to obtain fully passive diameter and structural measurements.

*Drugs and solutions.* All isolated vessel experiments were performed using a bicarbonate-based Ringer’s PSS, the ionic composition of which was as follows (in mmol/l): NaCl 119.0, NaHCO₃ 24.0, KCl 4.7, KH₂PO₄ 1.18, MgSO₄ × 7H₂O 1.17, CaCl₂ 1.6, EDTA 0.026, and glucose 5.5. PSS was made each week and stored without glucose at 4°C. Glucose was added to the PSS before each experiment. PSS was aerated with 5% CO₂, 10% O₂, and 85% N₂ to maintain pH. Diltiazem was purchased from MP Biomedicals (Santa Ana, CA) and made up weekly to a 1 mM stock. Papaverine and L-NNA were purchased from Sigma (St. Louis, MO) and made weekly to 10 mM stocks. NS309 and SNP were purchased from Sigma, and aliquots of 10 mM stocks were frozen at −20°C until use.

*Data calculations and statistical analysis.* Results are presented as means ± SE. Myogenic tone was calculated as a percent decrease in diameter from the fully relaxed diameter in calcium-free PSS with diltiazem and papaverine by the following equation: [(1 − (φ_{passive} /φ_{baseline})) × 100%, where φ_{tone} is the inner diameter of the vessel with tone, and φ_{passive} is the inner diameter of the vessel in calcium-free PSS with diltiazem and papaverine. Percent constriction to L-NNA was calculated as a percent change in diameter from baseline by the following equation: [(1 − (φ_{drug}/φ_{baseline})) × 100%, where φ_{drug} is the diameter of vessel in L-NNA, and φ_{baseline} is the diameter before giving drug. Percent reactivity to SNP and NS309 was calculated from the following equation: [(φ_{drug}−φ_{baseline})/φ_{baseline}] × 100%, where φ_{drug} is the diameter at a specific concentration of drug. Percent distensibility was calculated for fully relaxed vessels in calcium-free PSS with diltiazem and papaverine at each pressure by the following equation: (φ_{pressure}−φ_{baseline})/(φ_{baseline}−φ_{baseline}) × 100%, where φ_{pressure} is the passive diameter at a specific pressure, and φ_{5mmHg} is defined as the passive diameter at the lowest pressure (5 mmHg). Outer diameter (OD) was calculated using the equation ID + 2WT, where ID is the measured inner diameter, and WT is the measured wall thickness. Cross-sectional area (CSA) was calculated as π(OD/2)^2 − π(ID/2)^2.

Differences in blood pressure, active diameters, percent tone, percent constriction, percent reactivity, percent distensibility, and passive structural measurements were determined by one-way ANOVA with a post hoc Student-Newman-Keuls test for multiple comparisons. Two-way ANOVA was used to determine the effect of hypertension or UCCAo treatment and their interaction on passive structural characteristics. Differences were considered significant at P < 0.05.

**RESULTS**

*Effect of hypertension on PA myogenic reactivity and tone.* To determine the influence of hypertension on PAs, we used SHRSP animals that were 18 wk old compared with age-matched normotensive WKY and prehypertensive 5 wk old SHRSP in an attempt to distinguish strain differences. Physiological parameters of WKY18, SHRSP18, and SHRSP5 animals are shown in Table 1. Systolic, diastolic, and mean blood pressures were significantly higher in SHRSP18 compared...
with WKY18. Blood pressures of SHRSP5 were significantly lower than those of SHRSP18 and similar to those of WKY18, confirming the prehypertensive state of these animals. The body weight of SHRSP5 was lower than that of all other groups, as expected.

PAs from SHRSP18 had significantly smaller active inner diameters at all pressures studied compared with WKY18 (Fig. 1A). Active inner diameters of PAs from normotensive SHRSP5 were not different from those of SHRSP18 and significantly smaller vs. WKY18. The smaller active diameter of PAs from both SHRSP18 and SHRSP5 groups was at least partially due to increased pressure-induced tone in these vessels (Fig. 1B). Thus increased myogenic tone and smaller inner diameters of PAs were associated with the SHRSP strain and not hypertension per se. In addition, inner diameters of PAs from WKY18, SHRSP18, and SHRSP5 did not change appreciably with increasing pressure, demonstrating those vessels had myogenic reactivity.

**Effect of chronic hypoperfusion on PA myogenic reactivity and tone.** Chronic hypoperfusion by UCCAo for 4 wk decreased myogenic reactivity in PAs from WKY18-CH, such that diameters increased with increasing intravascular pressure (Fig. 1A). In fact, the slope of the diameter vs. pressure curves was minimal in WKY18, SHRSP18, SHRSP18-CH, and SHRSP5 (−0.02 ± 0.02, 0.01 ± 0.02, 0.03 ± 0.02, and 0.04 ± 0.04, but was significantly increased and positive in WKY18-CH (0.18 ± 0.05; P < 0.01 vs. all). In addition, they had significantly larger active diameters at all pressures studied compared with all other groups. PAs from WKY18-CH also had significantly less myogenic tone at 60 mmHg vs. all groups (Fig. 1B). In contrast, SHRSP18-CH had considerably less effect on PAs. Although PAs from SHRSP18-CH animals had inner diameters that were larger than those of SHRSP18, there was no statistical difference between these groups. Similarly, the percentage of tone was less in PAs from SHRSP18-CH compared with SHRSP18, but this was not statistically different. UCC Ao was not performed on 5-wk-old SHRSP.

**Effect of hypertension on PA reactivity to NO and SK/IK channel activation.** Figure 2A shows the percent constriction to L-NNA in PAs from all groups. All vessels constricted to NOS inhibition with L-NNA, demonstrating PAs have basal NO that inhibits tone in these vessels. The percent constriction was not different between WKY18 and SHRSP18; however, it was significantly decreased in PAs from SHRSP5. Figure 2B shows the reactivity to the NO donor SNP in PAs from all groups. Dilation to SNP was significantly increased in SHRSP18 compared with WKY18 and SHRSP5, demonstrating an effect of hypertension on sensitivity to SNP, but not strain. Figure 3 shows percent reactivity to the SK/IK channel opener NS309. All vessels dilated significantly to NS309, and there was no different reactivity between SHRSP5 and SHRSP18; however, PAs from WKY18 animals were modestly more sensitive to SK/IK channel activation that was significant at 0.3 μM.

**Effect of chronic hypoperfusion on PA reactivity to NO and SK/IK activation.** Chronic hypoperfusion decreased constriction to L-NNA in PAs from WKY18 (WKY-CH) but not SHRSP (SHRSP-CH) (Fig. 2A). The constriction to L-NNA in PAs from SHRSP18-CH was unaffected by chronic hypoper-

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Table 1. Physiological characteristics of 18-wk-old WKY and SHRSP and 5-wk-old SHRSP animals

<table>
<thead>
<tr>
<th></th>
<th>WKY18</th>
<th>SHRSP18</th>
<th>SHRSP5</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>209 ± 4</td>
<td>198 ± 3</td>
<td>91 ± 2†</td>
</tr>
<tr>
<td>Arterial blood pressure, mmHg</td>
<td>119 ± 3</td>
<td>161 ± 7‡</td>
<td>127 ± 5</td>
</tr>
<tr>
<td>Systolic</td>
<td>82 ± 3</td>
<td>122 ± 7‡</td>
<td>86 ± 7</td>
</tr>
<tr>
<td>Diastolic</td>
<td>94 ± 2</td>
<td>135 ± 7‡</td>
<td>100 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. WKY18, 18-wk-old Wistar-Kyoto rats; SHRSP18 and SHRSP5, 18- and 5-wk-old spontaneously hypertensive stroke prone rats, respectively. *P < 0.01 vs. WKY18; †P < 0.01 vs. SHRSP18; and ‡P < 0.01 vs. SHRSP5 by one-way ANOVA.
fusion. Figure 2B shows the reactivity to SNP in PAs from all groups except WKY18-CH. In those vessels, the decrease in tone and substantial vasodilation did not allow for a dilatory curve to be performed. Chronic hypoperfusion of SHRSP had no effect on SNP dilation of PAs that was similar between SHRSP18 and SHRSP18-CH. Figure 3 shows the dilation of PAs to NS309. Again, dilation to NS309 was not done in WKY18-CH because these vessels were already dilated. There was no difference in reactivity to NS309 between SHRSP18 and SHRSP18-CH.

Effect of hypertension on PA structural remodeling. Structural characteristics of PAs from all groups are shown in Table 2. Structural measurements were compared at 5 and 60 mmHg to evaluate the effect of hypertension on PAs in an unpressurized state (5 mmHg), where differences in distensibility would not influence the diameters, and at a physiological pressure (60 mmHg). There were no statistical differences in inner or outer diameters at either 5 or 60 mmHg by one-way ANOVA between WKY18, SHRSP18, and SHRSP5. However, SHRSP5 had increased wall thickness compared with WKY18 at 5 mmHg, whereas SHRSP18 wall thickness was increased at 5 and 60 mmHg vs. WKY18. CSA was increased only in SHRSP18 at 60 mmHg, but not at 5 mmHg or in SHRSP5 vs. WKY18. Figure 4 shows the passive distensibility of PAs from all groups. Passive distensibility was significantly increased in both SHRSP18 and SHRSP5 compared with WKY18, suggesting a strain difference and not hypertension per se that affected vessel stiffness.

Effect of chronic hypoperfusion on PA structural remodeling. Two-way ANOVA was used to determine the influence of hypertension and chronic hypoperfusion on PA remodeling in 18-wk-old animals only. Passive inner diameters of PAs at 5 mmHg were significantly smaller from SHRSP18-CH compared with WKY18-CH (Table 2). Similarly, wall thickness at 5 and 60 mmHg and CSA at 60 mmHg were significantly greater in PAs from SHRSP18-CH vs. WKY18-CH. However, despite smaller lumens and thicker walls of SHRSP18-CH compared with WKY18-CH, chronic hypoperfusion did not affect structural characteristics of PAs from either SHRSP or WKY, as there were no significant differences in any parameter between WKY18 vs. SHRSP18-CH or SHRSP18 vs. SHRSP18-CH. Passive distensibility was increased in WKY18-CH compared with WKY18, although this was not significantly different (Fig. 4). Again, although distensibility of PAs was increased in SHRSP18 and SHRSP18-CH compared with WKY, there was no effect of chronic hypoperfusion on distensibility in these vessels.

DISCUSSION

The major findings of this study were that 4 wk of UCC Ao caused substantial vasodilation in PAs from WKY18 (WKY18-CH) animals, such that there was significantly decreased myogenic tone and reactivity to pressure. The effect of chronic hypoperfusion was largely prevented in SHRSP18 (SHRSP18-CH) animals, suggesting hypertension impaired this adaptive response to hypoperfusion. Interestingly, the effect of chronic hypoperfusion in WKY animals was limited to vasoactive responses, as there was no effect of UCC Ao on passive structural characteristics (passive diameters, wall thickness, or distensibility) of PAs from either WKY18-CH or SHRSP18-CH animals compared with their naive counterparts. In addition, we found that PAs from SHRSP18 animals had smaller active lumen diameters that was due to increased...
myogenic tone and not structural changes in the vessel wall (i.e., inward remodeling). Lastly, prehypertensive SHRSP5 animals had PAs with similarly increased tone as hypertensive SHRSP18 animals, suggesting that it was the SHRSP strain and not hypertension per se that caused this effect.

PAs from normotensive WKY rats appeared to undergo an adaptive vasodilatory response to UCC Ao. In fact, PAs from WKY18-CH animals had little myogenic tone and responded in a passive manner to increased intravascular pressure, suggesting loss of myogenic reactivity. Although we attempted to investigate underlying mechanisms by which vascular responses might be different, including dilation to NO and SK/IK channel activation, PAs from WKY18-CH animals were so potently dilated they could not perform dilation curves. We speculate that the smooth muscle from these vessels is in a hyperpolarized state, potentially due to changes in ion channel expression or activity, such as voltage-dependent or large-conductance calcium-activated K\(^+\) channels that can promote hyperpolarization of smooth muscle. While this vasodilatory response may be an important adaptation during UCC Ao, the diminished reactivity to pressure would likely cause loss of local CBF auto-regulation, one of the proposed mechanisms leading to white matter injury during cerebral ischemic SVD (13, 27, 36).

The adaptive vasodilatory response of PAs to UCC Ao that was noted in WKY18-CH animals was not seen in SHRSP18-CH that had similar tone and reactivity as vessels from SHRSP18 animals. While it is not clear why PAs from SHRSP did not respond to UCC Ao in the same manner as PAs from WKY, it is possible that there was an overall lack of adaptation in other (upstream) vessels as well, such as the collateral systems, and that CBF was not restored to normal in SHRSP18-CH animals. Although we did not measure CBF in either group of animals during the 28 days of UCC Ao, studies have shown that hypertension prevents arteriogenesis of both circle of Willis and leptomeningeal anastomoses vessels in response to UCC Ao, suggesting this may be the case (26, 34). In addition, Jalal et al. (20) reported that BCCA Ao in SHRSP animals was lethal, further supporting the concept that arteriogenesis of collaterals during chronic hypoperfusion is an important protective response of the cerebral circulation to maintain CBF that is impaired during hypertension. Although we do not know if the level of CBF during UCC Ao was reduced in SHRSP compared with WKY, CBF has been shown to be decreased in male SHRSP at 20 wk of age (41), which would likely decrease further with UCC Ao.

It is not entirely surprising that PAs from WKY18-CH or SHRSP18-CH did not undergo structural enlargement. The primary driver for arteriogenesis is increased shear stress that occurs in collaterals when blood flow is redirected during an occlusion (3, 29, 30). PAs are not collaterals, but end vessels that directly connect the pial circulation to the capillaries. Thus the stimulus for arteriogenesis may be absent in PAs, although this was not directly measured in this study. It is worth noting that, in studies using BCCA Ao, structural remodeling of noncollateral vessels has been found (21, 22, 32). Kim et al. (21) found that 4 wk of BCCA Ao caused enlargement of the posterior cerebral and communicating arteries, as well as increased size of parenchymal vessels on immunohistochemistry. Márquez-Martín et al. (22) found that 15 days of BCCA Ao caused decreased wall thickness and CSA, but not structural enlargement of MCA. However, BCCA Ao does not engage collaterals to the same extent as UCC Ao, and thus vascular remodeling in noncollateral vessels may be more related to ischemia during BCCA Ao than to changes in shear stress.

### Table 2. Effect of hypertension and chronic hypoperfusion on passive structural characteristics of parenchymal arterioles

<table>
<thead>
<tr>
<th></th>
<th>WKY18</th>
<th>WKY18-CH</th>
<th>SHRSP18</th>
<th>SHRSP18-CH</th>
<th>SHRSP5</th>
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<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
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<tr>
<td>ID(_5)</td>
<td>48.2 ± 4.6</td>
<td>52.7 ± 2.8</td>
<td>40.9 ± 3.3</td>
<td>41.0 ± 3.2†</td>
<td>38.4 ± 3.0</td>
</tr>
<tr>
<td>ID(_{60})</td>
<td>55.2 ± 3.9</td>
<td>68.2 ± 2.6</td>
<td>61.5 ± 5.3</td>
<td>63.0 ± 4.7</td>
<td>54.0 ± 3.1</td>
</tr>
<tr>
<td>OD(_5)</td>
<td>62.2 ± 4.8</td>
<td>68.0 ± 2.6</td>
<td>59.4 ± 3.6</td>
<td>59.8 ± 4.1</td>
<td>55.9 ± 3.4</td>
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<tr>
<td>OD(_{60})</td>
<td>67.8 ± 9.4</td>
<td>80.8 ± 2.6</td>
<td>77.5 ± 5.5</td>
<td>78.5 ± 5.2</td>
<td>67.3 ± 3.8</td>
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<tr>
<td>WT(_5)</td>
<td>7.00 ± 0.26</td>
<td>7.67 ± 0.42</td>
<td>9.25 ± 0.45§</td>
<td>9.39 ± 0.53‡</td>
<td>8.75 ± 0.31§</td>
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<td>WT(_{60})</td>
<td>6.33 ± 0.21</td>
<td>6.33 ± 0.33</td>
<td>8.00 ± 0.50§</td>
<td>7.75 ± 0.53‡</td>
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<td>CSA(_5)</td>
<td>1220 ± 123</td>
<td>1452 ± 89</td>
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<td>CSA(_{60})</td>
<td>1220 ± 187</td>
<td>1473 ± 99</td>
<td>1758 ± 176*</td>
<td>1756 ± 211†</td>
<td>1287 ± 145</td>
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Values are means ± SE; n, no. of animals. WKY18-CH and SHRSP18-CH, WKY18 and SHRSP18 rats with chronic hypertension, respectively; ID\(_5\) and ID\(_{60}\), inner diameter at 5 or 60 mmHg, respectively; OD\(_5\) and OD\(_{60}\), outer diameter at 5 or 60 mmHg, respectively; WT\(_5\) and WT\(_{60}\), wall thickness at 5 or 60 mmHg, respectively; CSA\(_5\) and CSA\(_{60}\), cross-sectional area at 5 or 60 mmHg, respectively. *P < 0.05 vs. WKY18 and †P < 0.01 vs. WKY18 by one-way ANOVA. ‡P < 0.05 vs. WKY18-CH and §P < 0.01 vs. WKY18-CH by two-way ANOVA.
PAs from SHRSP18 had significantly increased tone and smaller lumen diameters actively compared with WKY18. Numerous other studies have shown that pial arteries and arterioles from spontaneously hypertensive and SHRSP rats have increased vasoconstriction compared with normotensive strains that is thought to be partially responsible for increased cerebrovascular resistance and shifting the CBF autoregulatory curve to higher pressures in those animals (17, 18). The increased tone in PAs from SHRSP18 animals does not appear to be related to diminished NO responsiveness, as constriction to NOS inhibition with l-NNA was not different from WKY18, but dilation to SNP was significantly increased. Thus PA smooth muscle from SHRSP18 appears more sensitive to NO, not less sensitive, as would be expected if the increased tone was due to a diminished effect of NO on inhibiting tone. PAs from SHRSP18 animals did have modestly diminished dilation to the SK/IK channel opener NS309. SK/IK channels are expressed only in cerebrovascular endothelium and are shown to be critical to the endothelium-derived hyperpolarization pathway (16). Although endothelium-derived hyperpolarization does not appear to oppose basal tone in large cerebral arteries, we have shown that SK/IK channels are basally active in PAs and inhibit tone (7). Thus impairment of SK/IK channel activity or the ability to promote hyperpolarization in PAs from SHRSP18 animals could contribute to the increased tone in those vessels.

Interestingly, we found that PAs from SHRSP18 animals did not have structurally smaller lumens, but did present with medial hypertrophy (thicker walls) and increased passive distensibility compared with WKY18. The structure of PAs in the brain has been studied extensively in SHRSP animals to understand cerebrovascular lesions and more recently the impact of hypertension on progressing SVD. Structural changes in PAs in SHRSP animals appear to depend on age, location, and diet. Schreiber et al. (31) performed a time course study of vascular pathology using MRI and histology in SHRSP animals from 12 to 42 wk of age and found that small vessel diameters were not different at any of these ages, except in the basal ganglia, where they were larger. A similar result was found in a study of PAs isolated from humans undergoing tumor resection, with and without essential hypertension (28). PAs from hypertensive patients had similar inner diameters, but increased wall-to-lumen ratios (28), a finding that is similar to the present study. However, other studies on SHRSP animals have found PAs with increased wall thickness, smaller lumen diameters, and fibrinoid degeneration of the arteriolar wall (11, 12, 19, 24, 25, 35, 40, 41). These pathologic findings are progressing with age and in animals that have the highest blood pressure or that are on a high-salt diet (8, 23, 35, 42). Thus it is possible that lack of inward remodeling in PAs from SHRSP18 in the present study was due to their relatively young age and modestly increased blood pressures that are lower in females than males. It is worth noting that increased wall thickness and distensibility were seen in PAs from both prehypertensive and hypertensive SHRSP, suggesting an effect of strain and not hypertension per se on vascular structure. In addition, because vascular lesions are progressive with age and degree of hypertension, there may be a genetic effect that makes the vasculature more sensitive to the effects of hypertension in SHRSP animals, as has been suggested previously (40).

PAs from SHRSP18 were compared with prehypertensive SHRSP5 animals to distinguish between the effects of hypertension vs. strain differences. We found that the majority of responses, including increased tone, reactivity to NS309, increased wall thickness, and distensibility were present in PAs from both hypertensive and prehypertensive SHRSP animals. While it is tempting to conclude that it was the strain and not hypertension that caused these effects in PAs, we did not have a control for young age, i.e., 5-wk-old WKY. However, it is important to consider that strain may have a significant influence on vascular reactivity and structure of the cerebrovasculature, independent of hypertension.

There are several limitations to this study that are worth considering. First, we did not measure CBF in any of animals, and, therefore, we do not know the relationship between PA vasoactivity, structure, and CBF during UCCAo or if the potent vasodilatory response in normotensive animals contributes to restoring CBF in those animals. Second, we did not measure changes in the structure and function of collagen, especially the circle of Willis vessels that would be expected to undergo arteriogenesis and influence CBF. It is possible that there is an overall effect of hypertension that impairs arteriogenesis in both noncollateral and collateral vessels that is not specific for PAs. Third, the comparison of vessels from 18-wk-old animals to 5-wk-old animals was not straightforward, considering the young animals were considerably smaller in size and were still undergoing development. To better understand the influence of strain vs. hypertension on PAs, further studies are needed.

**REFERENCES**


**AUTHOR CONTRIBUTIONS**

Author contributions: J.G.S. and S.-L.C. performed experiments; J.G.S., S.-L.C., and M.J.C. analyzed data; J.G.S., S.-L.C., and M.J.C. interpreted results of experiments; J.G.S. and M.J.C. prepared figures; J.G.S., S.-L.C., and M.J.C. drafted manuscript; J.G.S., S.-L.C., and M.J.C. contributed to the final version of the manuscript; M.J.C. conceived and designed the research; J.G.S., S.-L.C., and M.J.C. approved the final version of the manuscript; M.J.C. drafted the manuscript; J.G.S., S.-L.C., and M.J.C. edited and revised the manuscript.


