Impaired hemodynamics and endothelial vasomotor function via endoperoxide-mediated vasoconstriction in the carotid artery of spontaneously hypertensive rats

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Denniss SG, Rush JWE. Impaired hemodynamics and endothelial vasomotor function via endoperoxide-mediated vasoconstriction in the carotid artery of spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 296: H1038–H1047, 2009. First published January 23, 2008; doi:10.1152/ajpheart.00933.2008.—The fact that endothelial removal increases diameter and compliance in the common carotid artery (CCA) of spontaneously hypertensive rats (SHR) and that improving CCA endothelium-dependent vasorelaxation has been shown to normalize a reduced systolic blood flow through the SHR CCA compared with normotensive Wistar-Kyoto rats (WKY) suggests that endothelial vasomotor dysfunction may be linked to altered large artery hemodynamics in hypertension. The experiments herein were designed to further investigate WKY and SHR CCA hemodynamics and endothelium-dependent vasomotor functions. It was hypothesized that CCA blood flow and conductance would be reduced throughout the cardiac cycle in SHR and that endothelium-dependent contractile activity would impair SHR CCA vasorelaxation. We report that mean, maximal systolic, and diastolic blood flow was reduced in SHR vs. WKY CCA, as was vascular conductance. Pressure was augmented in SHR CCA and accompanied by late systolic flow augmentation so that total flow during systole was indeed no different between strains, possibly explained by earlier lower body wave reflection. While ACh stimulation in isolated precontracted WKY CCA caused a robust nitric oxide (NO)-mediated vasorelaxation, endothelium-dependent, cyclooxygenase (COX)-mediated contractile activity stimulated by high ACh concentration impaired NO- and non-NO/non-COX-mediated vasorelaxation in precontracted SHR CCA. In quiescent CCA, this endothelium-dependent contractile response was COX-1 and thromboxane-prostanoid receptor mediated and modulated by the availability of NO. These data collectively suggest that endothelium-dependent, COX-mediated endoperoxide signaling in the CCA of SHR may elicit vasoconstriction, which could shift the mechanical properties of this conduit artery and contribute to reduced CCA blood flow in vivo.

acetylcholine; endothelium-dependent contraction; carotid pressure and flow augmentation; cyclooxygenase; thromboxane-prostanoid receptor

THE COMMON CAROTID ARTERIES (CCAs) are of great physiological and clinical importance because their internal carotid artery branches contribute ~80% to the total perfusion of the brain in humans and in rodents (16), and CCA intima-media thickness (58) and hemodynamics (47) are strong predictors of arterial atherosclerosis and cardiovascular risk. In humans, CCA blood flow remains relatively similar in early stage essential hypertensive patients compared with age-matched normotensive subjects but is significantly reduced in patients with sustained essential hypertension (7, 20, 32, 37). CCA hemodynamics have not been fully investigated in animal models of hypertension, but preliminary evidence indicates that CCA blood flow is reduced in spontaneously hypertensive rats (SHR), a model of human essential hypertension. Cunha et al. (12) found that CCA blood flow velocity was reduced to a greater extent with age in SHR compared with normotensive Wistar rats. More recently, Iaccarino et al. (31) found that CCA systolic blood flow was reduced in a small sample of young adult SHR compared with Wistar-Kyoto rats (WKY) and that Akt gene transfer to the CCA endothelium normalized the blood flow reduction and corrected a blunted response to vasodilator stimuli in SHR. These findings suggest that CCA endothelial vasomotor dysfunction in hypertensive humans and animals (18) may be associated with altered CCA hemodynamics.

Interestingly, in the absence of an endothelial lining, CCA diameter and compliance are increased in young adult WKY and SHR (38, 42), suggesting a net vasoconstrictive role for the endothelium; an effect that was found to be greater in SHR (9). Indeed, it has been documented in the aorta of adult SHR that ACH, and other vasoactive compounds that elevate endothelial intracellular calcium concentration, can stimulate the release of not only endothelium-derived relaxing factor(s) such as nitric oxide (NO) and endothelium-derived hyperpolarizing factor(s) (EDHF) but also of endothelium-derived contracting factor(s) (EDCF). These EDCF(s) include cyclooxygenase (COX)-derived endoperoxides, which elicit a “net” vasomotor dysfunction by competing with endothelium-derived relaxing factor(s) (18, 59). However, no studies have systematically investigated this pathway as a potential mediator of vasomotor dysfunction in the CCA of SHR. It is therefore unknown whether impaired endothelium-dependent, endoperoxide-mediated, CCA vasomotor function and reduced CCA blood flow indeed coexist in SHR.

The main objective of the present study was to quantify CCA blood flow and blood pressure across the cardiac cycle in young adult SHR and WKY and to systematically investigate the relative contributions of the NO synthase (NOS) and COX pathways in mediating ACh-stimulated, endothelium-dependent CCA vasomotor function. We hypothesized that CCA blood flow and conductance would be reduced across the cardiac cycle in the CCA of SHR compared with WKY and that COX and thromboxane-prostanoid (TP)-receptor-medi-
ated, endothelium-dependent vasocontraction would impair endothelium-dependent, NO-mediated vasorelaxation in SHR but not WKY CCA.

MATERIALS AND METHODS

All experiments were performed using young adult rats aged 16–20 wk. WKY and SHR were purchased from Harlan (Indianapolis, IN) at 10 wk of age and were housed four per cage in a temperature-controlled facility (21 ± 1°C) on a reversed 12:12-h light-dark cycle, having free access to standard laboratory chow and tap water. All procedures involving rats were approved by the University of Waterloo Animal Care Committee and were in accordance with the guidelines of the Canadian Council on Animal Care.

An expanded Supplemental Methods appears in the Supplemental Material for this article, which is available online at the Am J Physiol Heart Circ Physiol website.

CCA hemodynamics. The current study took a number of steps to ensure an accurate quantification of blood flow and pressure in the CCA across the cardiac cycle (see Supplemental Methods for details regarding Rationale for procedural approach and Quantification of CCA blood flow and pressure). Blood flow through the left CCA of anesthetized rats was measured using a perivascular flow probe, followed by removal of the flow probe and insertion of a catheter-tip pressure transducer into the left CCA to measure intra-arterial blood pressure, thereby enabling us to subsequently excise from the same rat the structurally undisturbed right CCA for assessment of vasomotor function. Heart rate during flow and pressure waveform collection remained the same (P > 0.500 for differences between cyclic rates from flow waveforms and pressure waveforms; data not shown) signifying a steady cardiovascular state within rats throughout the entire in vivo measurement duration (35–45 min). Accordingly, blood flow and pressure data were treated as though they were recorded simultaneously (44), which allowed for analysis of CCA pressure-flow relationships across the cardiac cycle (see Fig. 1 for details).

Pressure augmentation in the large arteries of the upper body is a consequence of “backward” and/or “forward” traveling reflected...
waves (44). Because in the current study the CCA had to be securely tied around the catheter measuring intra-arterial pressure (see Supplemental Methods), transmission of any reflected waves downstream returning (backward) from sites at or beyond the CCA bifurcation may have been obstructed by the tie and thus not represented in the pressure waveform measured by the catheter-tip transducer; consequently, this pressure waveform presumably included the incident wave and a reflected wave returning (forward) from upstream peripheral sites. The “augmentation index” of this resulting CCA blood pressure waveform was defined as \[ \frac{P_S - P_F}{P_F} \] (Fig. 1, bottom), wherein \( P_S \) is end diastolic pressure, \( P_F \) is the pressure at the inflection (i.e., shoulder) point during systole, and \( P_S \) is peak systolic pressure (44). As recently described by Hirata et al. (29), a blood flow “augmentation index” in the CCA was similarly defined as \[ \frac{F_{i,SF}}{F_S} \] (Fig. 1, bottom), wherein \( F_S \) is end diastolic flow, \( F_S \) is peak systolic flow occurring at about the same point in the cardiac cycle as \( P_S \), and \( F_{i,SF} \) is peak flow occurring late in systole normally at about the same point in the cardiac cycle as \( P_S \).

**Tissue preparations.** Anesthetized animals were killed by removal of the heart. The left ventricle and brain (hemispheres + midbrain + cerebellum) were dissected and weighed. The right CCA was carefully excised, immediately placed in a 4°C Kreb’s bicarbonate buffer solution, and cleaned of fat, surrounding connective tissue, and blood. Some CCA segments were flash-frozen, stored at −80°C, and later homogenized (see Supplemental Methods). From other CCA segments, arterial rings 2.0 mm in axial length were cut in preparation for testing CCA vasomotor function. In a number of cases, arterial rings were mechanically denuded of endothelium by insertion of a 250-μm diameter stainless steel wire through the vessel lumen followed by gentle rolling on Whatman paper wet with 4°C buffer. The efficacy of denudation was later confirmed by the absence of a relaxation response to ACh in precontracted rings.

**CCA vasomotor function.** CCA vasomotor function experiments were adapted from those previously published by our laboratory using WKY and SHR rat aorta (Refs. 27; 49; see Supplemental Methods for additional details regarding CCA vasomotor function experiments). Briefly, each arterial ring was mounted between two 127-mm diameter wires, one fixed to a glass rod and the other connected to a force transducer, and suspended in a 37°C water-jacketed tissue bath containing 10 ml of buffer solution constantly gassed with 95% O2−5% CO2. The resting tension on rings was slowly increased over 60 min to a final resting tension of 2.75–3.00 g, which was found in our preliminary contractile experiments to correspond with the optimal length for drug-stimulated CCA isometric tension production (see Supplemental Fig. S1).

Rings were preincubated for 30 min in buffer containing either: no drug (ND); Nω-nitro-l-arginine methyl ester (l-NNAME; 10⁻⁴ M in bath) to inhibit NOS (4, 54, 66, 67); indomethacin (Indo; 10⁻⁵ M) to nonselectively inhibit both the constitutive (COX-1) and inducible (COX-2) isoforms of COX (1, 39, 48, 55); l-NNAME + Indo to inhibit both NOS and COX-1/-2; l-NNAME + valeryl salicylate (VAS; 3 × 10⁻³ M) to inhibit COX-1/-2; l-NNAME + SQ29548 (10⁻⁶ M) to inhibit NOS and COX-1 preferentially (25, 26, 64–66, 68); l-NNAME + NS398 (10⁻⁶ M) to inhibit NOS and COX-2 preferentially (1, 22, 26, 64); or l-NNAME + SQ29548 + NS398 (10⁻⁶ M) to inhibit NOS and to antagonize the TP receptor (1, 21, 48). The type and concentration of inhibitors/antagonists used were based on the indicated previous studies in which efficacy has been demonstrated under similar experimental conditions.

Drug-preincubated rings were exposed to one of two protocols. In one protocol, cumulative dose-response relationships to Ach followed by the NO donor sodium nitroprusside (SNP) were measured in rings precontracted with 10⁻⁴ M phenylephrine (PE), shown in our preliminary experiments to elicit a sustained vasoconstriction with an amplitude 80–90% of maximal PE vasoconstriction (see Supplemental Fig. S1). In a second protocol, cumulative dose-response relationships to Ach were measured in quiescent (nonprecontracted) rings. At the beginning of each test of vasomotor function, rings were stimulated to contract twice with exposures to 60 mM KCl.

**Western blotting.** The relative expression of endothelial nitric oxide synthase (eNOS), COX-1, and COX-2 enzymes in the CCA of WKY and SHR was determined by Western blotting. Procedures for gel electrophoresis of prepared CCA homogenate samples, protein transfer to PVDF membranes, and immunoblotting and detection of these enzymes were performed as described in the Supplemental Methods.

**Drugs, reagents, and antibodies.** Kreb’s bicarbonate buffer solution consisted of the following (in mM): 131.5 NaCl, 13.5 NaHCO3, 11.2 glucose, 5.0 KCl, 2.5 CaCl2, 1.2 NaH2PO4, 1.2 MgCl2, and 0.025 EDTA (prepared fresh daily, pH 7.40). All drugs and reagents were purchased from either Sigma-Aldrich (St. Louis, MO via Oakville, ON, Canada) or BioShop Canada (Burlington, ON, Canada) unless indicated. l-NNAME was reconstituted in distilled water, Indo in DMSO, SQ29548 (Cayman Chemical, Ann Arbor, MI) in ethanol, and VAS (Cayman Chemical) and NS398 (Cayman Chemical) in N2-purged ethanol. Primary antibodies specific for COX-1 or COX-2 were purchased from Cayman Chemical and for eNOS were purchased from BD (Franklin Lakes, NJ). Appropriate secondary antibodies were purchased from Santa Cruz (Santa Cruz, CA).

**Statistics.** Data are expressed as means ± SE; n refers to the number of animals/rings per group or drug condition. Statistical analyses were performed using SAS v9.1 or SAS Enterprise Guide v3.0 software (SAS Institute, Cary, NC). Differences were considered statistically significant if \( P < 0.05 \). Two-tailed, independent-sample Student’s t-tests were used for single-point comparisons, and one- or two-way ANOVAs with Bonferroni posttests, when necessary, were used for multiple within and/or between group comparisons.

**RESULTS**

*Reduced blood flow and conductance in the CCA of SHR.* Physical attributes of the WKY and SHR used in these experiments are listed in Table 1. Table 1 also presents WKY and SHR CCA hemodynamics averaged across 5 min of stable waveforms measured in vivo (see Fig. 1, top, for an example of measured CCA pressure and flow waveforms). Mean CCA blood flow was significantly reduced [absolute (abs.), approximately −25% relative to body weight (rel.), approximately −31%] in SHR compared with WKY, as were maximum (abs., approximately −33%; rel., approximately −38%) and minimum (abs., approximately −63%; rel., approximately −65%) flow. Conversely, CCA mean, systolic, diastolic, and pulse pressures were all −1.5- to 2-fold elevated in SHR compared with WKY, and heart rate was on average −105 cycles/min higher in SHR. As a result, there was a marked −65% reduction in mean vascular conductance calculated at the level of the CCA.

In SHR, stenoses of the cervical arteries are not likely to account for reduced CCA blood flow since these rats are devoid of overt atherosclerosis (35). Given that the major organ supplied by the CCA, the brain, was found by others (43) and in the current study to be −12–14% reduced in normalized weight in young adult SHR vs. WKY (Table 1) and that hypometabolism already exists in selected brain regions in these SHR (62), it was speculative that reduced SHR brain weight could have to some degree accounted for the 18% reduced compared with WKY (n = 6 per strain; data not shown).

H1040 CAROTID ARTERY HEMODYNAMICS AND VASOMOTOR FUNCTION

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CARRTID ARTERY HEMODYNAMICS AND VASOMOTOR FUNCTION

Table 1. Physical attributes and CCA hemodynamics of young adult WKY and SHR

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
<th>P</th>
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<tbody>
<tr>
<td>Age, wk</td>
<td>17.2 ± 0.2</td>
<td>17.2 ± 0.2</td>
<td>0.948</td>
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<tr>
<td>Weight</td>
<td></td>
<td></td>
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<tr>
<td>Whole body, g</td>
<td>305 ± 4</td>
<td>334 ± 4</td>
<td>&lt;0.001</td>
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<tr>
<td>Left ventricle, mg (or /g body)</td>
<td>685 ± 9 (2.24 ± 0.02)</td>
<td>850 ± 14 (2.54 ± 0.03)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Brain, mg (or /g body)</td>
<td>1832 ± 3 (5.78 ± 0.08)</td>
<td>1730 ± 2 (5.00 ± 0.08)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate, cycles/min</td>
<td>303 ± 9</td>
<td>408 ± 7</td>
<td>&lt;0.001</td>
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<tr>
<td>CCA blood flow, ml/min (or /100 g body)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Maximum</td>
<td>19.20 ± 1.00 (6.47 ± 0.34)</td>
<td>12.84 ± 0.98 (3.99 ± 0.34)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minimum</td>
<td>1.57 ± 0.35 (0.53 ± 0.12)</td>
<td>0.58 ± 0.26 (0.18 ± 0.08)</td>
<td>&lt;0.030</td>
</tr>
<tr>
<td>Mean</td>
<td>5.10 ± 0.38 (1.72 ± 0.14)</td>
<td>3.84 ± 0.32 (1.19 ± 0.11)</td>
<td>&lt;0.020</td>
</tr>
<tr>
<td>CCA blood pressure, mmHg</td>
<td></td>
<td></td>
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<tr>
<td>Systolic</td>
<td>101 ± 4</td>
<td>202 ± 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>69 ± 4</td>
<td>147 ± 5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulse</td>
<td>31 ± 1</td>
<td>55 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean</td>
<td>84 ± 4</td>
<td>172 ± 6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean vascular conductance, mmHg (µl/min) (or /100 g body)</td>
<td>62.3 ± 5.4 (21.1 ± 1.9)</td>
<td>22.4 ± 1.7 (6.9 ± 0.6)</td>
<td>&lt;0.001</td>
</tr>
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</table>

Data represent means ± SE of stable hemodynamic waveforms measured in the left common carotid artery (CCA) of anesthetized Wistar Kyoto rats (WKY; n = 16) and spontaneously hypertensive rats (SHR; n = 12). Due to differences in whole body weight between strains, data were reported in both absolute terms and relative to 100 g of whole body weight (/100 g body) where appropriate.

CCA pressure augmented in SHR and accompanied by late systolic flow augmentation. The relationship between CCA pressure and flow across time-normalized cardiac cycles (i.e., as a fraction of a cardiac cycle; 0.000 and 1.000 representing the beginning and end of the cardiac cycle, respectively; see Fig. 1 for details) averaged within the 16 WKY and 12 SHR is presented in Fig. 1, bottom left and middle. Pressure waveforms analyzed from both strains had a systolic inflection in pressure (P1; SHR, 174 ± 6 mmHg and WKY, 99 ± 4 mmHg; normalized time: SHR, 0.071 ± 0.005 and WKY, 0.113 ± 0.008) before peak pulse pressure (P5; SHR, 204 ± 9 mmHg and WKY, 102 ± 4 mmHg; normalized time: SHR, 0.275 ± 0.008 and WKY, 0.168 ± 0.014), which began earlier and was more prolonged in SHR (all P < 0.001), resulting in a markedly higher pressure augmentation index in SHR compared with WKY (56.5 ± 3.4 vs. 11.6 ± 1.7%; P < 0.001).

F5, which was ~30% lower in SHR vs. WKY (13.21 ± 1.12 vs. 19.19 ± 1.10 ml/min; P = 0.002), occurred in early systole in both strains just after P1 (normalized time: SHR, 0.115 ± 0.008 and WKY, 0.131 ± 0.004). After F5, although pressure, especially in the case of SHR, continued to rise, flow began to decrease but much more quickly in WKY than in SHR so that total flow during systole (the 0.000 to 0.390 normalized time interval in both strains) was not statistically different between strains when considering the greater number of cardiac cycles per minute in SHR (SHR, 2.83 ± 0.24 vs. WKY, 3.31 ± 0.26 ml in 1 min; P = 0.197). Indeed, in all SHR animals there was a secondary peak in F5SP (7.25 ± 0.62 ml/min) just after PS (normalized time: 0.276 ± 0.015), corresponding to an average flow augmentation index of 50.3 ± 3.7%. In WKY, flow waveforms from only 9 of 16 animals possessed a secondary peak in F5LSP (5.55 ± 0.44 ml/min; P = 0.037 vs. SHR), occurring much after PS (normalized time: 0.321 ± 0.011), corresponding to a markedly lower average flow augmentation index of 21.1 ± 2.1% (P < 0.001 vs. SHR). During diastole (0.400 to 1.000 normalized time interval), total flow was ~40% lower in SHR vs. WKY (1.19 ± 0.15 vs. 2.10 ± 0.22 ml in 1 min; P = 0.003). As a result of these pressure-flow interactions, SHR maintained a markedly lower CCA vascular conductance (Fig. 1, bottom right) throughout the entire cardiac cycle compared with WKY (averaged approximately −55% in systole and approximately −70% in diastole; P < 0.001).

Endothelium-dependent, COX-mediated contractile activity impairs NOS- and non-NOS/non-COX-mediated relaxation responses in the PE-precontracted CCA of SHR. After measures of left CCA hemodynamics, the right CCA was excised for assessment of the relative contributions of NOS and COX pathways in mediating vasomotor functions in PE-precontracted arterial rings preincubated with either ND, l-NAME, Indo, or l-NAME + Indo (Fig. 2). Control experiments confirmed that there were no significant differences in vasomotor functions between CCA harvested from animals after hemodynamic measures and CCA freshly harvested from age-matched WKY and SHR (see Supplemental Methods). Contractile responses to 10−6 M PE were 40–50% lower in SHR than WKY across all drug conditions (P < 0.001), and this persisted when considered relative to their drug condition-specific KC1-stimulated contractions, which were not different between strains. Incubation with l-NAME and l-NAME + Indo compared with ND and Indo, respectively, caused a moderate but significant increase in developed tension to KC1 in both WKY and SHR (10–15%; P = 0.002) and a much more significant increase in developed tension to PE (80–95%; P < 0.001). There was no difference between strains in the PE-l-NAME-to-PE-ND developed tension ratio, a putative indicator of an NO effect on basal tone (15, 28, 46, 57). A similar pattern of response existed between drug conditions within and between strains in the full PE dose-response relationships evaluated in our preliminary contractile experiments (see Supplemental Fig. S1).

ACh-stimulated responses in PE-precontracted WKY and SHR CCA. Control experiments (see supplemental methods) confirmed that all ACh-stimulated vasomotor activity in PE-precontracted CCA (Fig. 2, bottom, left and right) was endothelium dependent. ACh elicited a concentration-dependent relaxation in WKY CCA and Indo caused an ~10% increase in both the maximum amplitude and area under the curve (AUC) compared with ND (see Fig. 2, inset). Incubation with l-NAME or l-NAME + Indo caused an ~90% decrease in both

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maximum amplitude and AUC compared with ND and Indo, respectively. Compared with L-NAME, L-NAME + Indo significantly increased the maximum amplitude.

The response to ACh in ND and L-NAME SHR CCA was biphasic: relaxing from a precontracted baseline in a sigmoid-like shape to a maximum amplitude (stimulatory phase: −9.0 to approximately −6.5 logM) followed by recontraction in a sigmoid-like shape (inhibitory phase: approximately −6.0 to −4.0 logM). Thus these responses were found to be best fit to a bell-shaped dose-response curve that sums the two sigmoidal dose-response relationships wherein EC501 and EC502 define the ACh concentration ([ACh]) resulting in 50% of the theoretical plateau in amplitude for the stimulatory (relaxation) and inhibitory (recontraction) phases, respectively. In ND SHR CCA, ACh first stimulated relaxation reaching maximum amplitude at −6.1 ± 0.1 logM after which the rings recontracted through to −4.0 logM. L-NAME caused a significant blunting of the relaxation phase of the ACh response, inducing an ~75% decrease in maximum amplitude and a lower EC501. The recontraction phase was also present in the L-NAME SHR CCA; the extent of recontraction was 2.5-fold greater than in ND. Notably, Indo completely abolished the recontraction phase of both ND and L-NAME SHR CCA, thus markedly increasing both maximum amplitude and AUC compared with similar conditions without Indo.

WKY vs. SHR. Peak relaxation (maximum amplitude) to ACh was distinctly lower in ND SHR vs. WKY CCA (P < 0.001); moreover, a dose-dependent recontraction phase occurred at a higher [ACh] (approximately −6.0 to −4.0 logM) but only in SHR. In contrast, at a lower [ACh] (−9.0 to approximately −6.5 logM), relaxation was indeed augmented in ND SHR vs. WKY CCA (EC501; P < 0.001), resulting in no significant difference in overall AUC between strains (P = 0.130). This augmented relaxation response in SHR at a lower [ACh] persisted in the presence of L-NAME, Indo, or L-NAME + Indo (P < 0.001). Similar peak relaxation and AUC occurred in Indo SHR compared with Indo WKY CCA, and SHR CCA incubated with L-NAME or L-NAME + Indo.
had a higher peak relaxation and increased AUC compared with responses than both L-NAME and L-NAME + Indo WKY CCA (P < 0.001).

SNP, used to assess vascular smooth muscle (VSM) sensitivity to exogenous NO, elicited comparable sigmoidal-shaped concentration-dependent relaxation responses in all WKY and SHR CCA from all drug conditions (see Supplemental Fig. S2).

ACh-stimulated contraction in quiescent SHR CCA is endothelium dependent, COX and TP-receptor mediated, and it is modulated by the availability of NO. To further investigate the recontraction response to ACh more than −6.5 logM in PE-precontracted SHR CCA (Fig. 2), experiments were performed with freshly harvested, intact, or endothelium-denuded quiescent (i.e., not precontracted) WKY and SHR CCA preincubated with either ND, L-NAME, or Indo (l-NAME + valeryl salicylate (L-NAME + Indomethacin) (L-NAME) compared with ND in SHR CCA, consistent with the effect of l-NAME on ACh-stimulated recontraction in PE-precontracted SHR CCA (Fig. 2) and further confirming that SHR conduit artery contraction in response to ACh is markedly amplified by inhibiting the production of NO (4, 54, 67). This ACh-stimulated contraction was completely abolished by endothelial denudation, Indo (l-NAME + Indo), or the COX-1 preferential inhibitor VAS (l-NAME + VAS), while the COX-2 preferential inhibitor NS398 (l-NAME + NS398) only partially attenuated the response. The TP-receptor antagonist SQ29548 (l-NAME + SQ29548) abolished ~90% of the contractile response.

Increased COX-1 and eNOS protein expression in the CCA of SHR. Immunoblot analyses revealed significant increases in the protein expression of eNOS (30%) and COX-1 (57%) in CCA of SHR relative to those of WKY (Fig. 4), whereas no statistically significant difference was detected for COX-2.

**DISCUSSION**

The present study provides evidence that blood flow through the CCA of young adult SHR is reduced at peak systole and throughout diastole compared with WKY, whereas MAP and PP are much higher throughout the entire cardiac cycle, resulting in a persistently decreased vascular conductance in SHR. Furthermore, this study demonstrates that coexistent with the altered CCA hemodynamics, ACh-stimulated vasorelaxation in CCA excised from SHR is blunted as a result of a competitive, COX- and TP-receptor-mediated vasocontractile response that is exacerbated by inhibiting the production of NO. Together, these findings support the working hypothesis that CCA vasmotor dysfunction, via endothelium-dependent, COX- and TP-receptor-mediated contractile activity, could be associated with altered CCA hemodynamics in SHR.

CCA hemodynamics across the cardiac cycle in WKY and SHR. Our findings that mean, maximum systolic, and minimum diastolic CCA flow and conductance were significantly reduced in young adult SHR compared with age-matched WKY are congruent with previous reports of reduced CCA blood flow in sustained essential hypertensive patients (7, 20, 32, 37) and in young adult SHR (12, 31). To our knowledge, the present study is the first in the WKY-SHR model of hypertension to have analyzed CCA pressure and flow across the cardiac cycle to and to have investigated their interrelationships, an approach that produced a number of novel findings. A markedly reduced total flow during diastole accounted for the greatest part of the ~25% reduction in mean CCA blood flow in SHR. Although peak systolic flow was also significantly reduced, total systolic flow per minute of cardiac cycles was no different between strains because of a higher heart rate in SHR vs. WKY and a prolonged post-systolic peak decay in SHR.
CCA flow that may have been the result of a much higher pressure augmentation during this same time (i.e., postpeak-to-late systole). CCA pressure augmentation measured in our study presumably represents a reflected waveform returning early from upstream peripheral sites, e.g., due to aortic degeneration and increased pulse wave velocity (11, 29). This forward reflected waveform in systole would have accelerated (or prolonged the decay of) forward CCA flow, thus augmenting total systolic flow. Late systolic flow augmentation, attributed to pressure wave reflection returning early from the lower body, has recently been reported in the aging human CCA and was implicated in cerebral microvascular damage (29). In the case of the young adult SHR in the present study wherein total CCA blood flow was reduced predominantly because of a reduction in flow during diastole, it could be speculated that the late systolic flow augmentation may indeed assist in preventing hypoperfusion of downstream brain tissues.

CCA vasomotor function in WKY and SHR. In young adult SHR conduit arteries, \( \alpha \)-adrenergic-stimulated contractile responses have previously been found to be either similar to WKY (1, 2, 31, 39, 40, 55, 60) or lower (23, 24, 27, 39) as in the current study. Since there was no difference between our WKY and SHR CCA in KCl-stimulated contractile responses or in the PE-NAME-to-PE-ND developed tension ratio, neither a general impairment of contractile function (23) nor a NO-mediated suppression of contractile activity (2, 14, 15), respectively, likely accounts for the differences in PE responsiveness. Adaptations in CCA \( \alpha \)-adrenergic receptors and their intracellular signaling mechanisms themselves may provide an explanation (23, 61). Importantly, there is no apparent reason to suspect that these differing PE responses corrupted our evaluation of WKY and SHR CCA ACh-stimulated vasomotor functions and the elucidation of the COX-EDCF-TP-receptor mechanism.

The current study demonstrates that whereas ACh initiates a robust endothelium-dependent, NOS-mediated relaxation response in the PE-precontracted CCA of WKY, ACh initiates two distinct competing responses in the CCA of SHR: an endothelium-dependent relaxation response to lower \([ACh]\) mediated by both NOS and non-NOS/non-COX signaling, and an endothelium-dependent contractile response to higher \([ACh]\) mediated by COX. Previous studies have shown impaired ACh-stimulated vasorelaxation but no overt recontraction in CCA from SHR vs. WKY aged 12–24 wk (30, 31, 39), which is presumed to be NO dependent (18), even though NOS inhibitors were not systematically used. Indo has elicited robust vasorelaxation in SHR or stroke-prone SHR CCA in some (10, 52) but not other (39) studies. There does not appear to be a single compelling potential explanation for the discrepancies between the past and the current study of the SHR CCA, but important factors might include the following: the source and/or exact age of the animals investigated (30, 31, 39); the precontractile agonist used (30, 39); and the range of \([ACh]\) evaluated, especially the exclusion of higher \([ACh]\) in past studies.

Fig. 4. Expression of endothelial nitric oxide synthase (eNOS), cyclooxygenase (COX)-1, and COX-2 protein in the CCA of WKY and SHR by Western blot. Representative immunoblots of 1 standard (STD), 6 SHR, and 6 WKY CCA samples are shown. Bar graph data represent means ± SE of protein expression in 12 SHR and 12 WKY CCA assayed in duplicate, quantified by densitometry, and expressed relative to STD.
works (30, 31). The current study was designed to provide a more definitive assessment by including the higher [ACh] that elicit the recontraction phase, systematically evaluating NOS and COX blockade effects and elucidating the involvement of the COX-EDCF-TP-receptor axis.

Our results demonstrate that impaired endothelium-dependent relaxation in SHR CCA can be caused exclusively by competitive COX-mediated vasoconstriction. Data from the NOS inhibition, eNOS expression, and sensitivity to SNP experiments do not support a major role for altered NO bioavailability in causing the SHR CCA ACh-stimulated vasomotor dysfunction. This interpretation is consistent with reports from SHR aorta (18, 59). Conversely, nonspecific COX inhibition completely abolished the recontraction response to higher [ACh] in precontracted SHR CCA, resulting in a similar peak relaxation to that observed for WKY CCA. The endothelium-dependent contracile response to cumulative [ACh] in quiescent SHR CCA was also completely abolished by nonsel ective COX inhibition and by preferential inhibition of COX-1 but not of COX-2. Likewise, this ACh-stimulated contraction in quiescent SHR CCA was almost completely abolished by TP-receptor antagonism. Supporting these vasomotor findings, we further showed that COX-1, but not COX-2, was significantly overexpressed in the CCA tissue of SHR compared with WKY, consistent with previous aortic studies (22, 56). Thus it is likely that a TP-receptor-binding endoperoxide product(s) of arachidonic acid metabolism by COX-1 was responsible for the ACh-stimulated CCA contractile activity. In SHR aorta, COX-1-derived EDCF(s) generated in response to ACh have been identified as PGH2 and PGI2, which activate VSM TP receptors (18, 59). It should be noted that very recent evidence in Sprague-Dawley rat femoral arteries suggests that VAS at the dosage used herein might have TP-receptor antagonist properties in addition to its COX-1 inhibition effects (51). The possibility that this also occurs in the current study does not change the overall interpretation, however, since even if the VAS experiment is ignored, the combination of the Indo and NS398 results suggests that COX-1 is the source of the EDCF(s) and the SQ29548 results suggest that the TP receptor is the EDCF target.

The robust ACh-stimulated WKY CCA relaxation in the current study was almost exclusively mediated by NO signaling, since this response was blunted ~90% by L-NAME and negligibly affected by Indo. In contrast, the SHR CCA relaxation phase stimulated by lower [ACh], which was augmented compared with WKY over the same dose range, again was negligibly affected by Indo but interestingly was blunted only ~70–80% by L-NAME or L-NAME + Indo. Together these data suggest that this augmented ACh-stimulated response in SHR CCA was mediated by a non-NOS/non-COX-dependent signaling mechanism, traditionally considered to involve an EDHF pathway (17, 19). Others (15, 27, 46, 57) have found augmented relaxation to lower [ACh] in the aorta and/or CCA from SHR or SPSHR aged 8–20 wk speculated to be an age-dependent compensation to hypertension development (15, 18, 46). However, there have been no attempts to systematically investigate the mechanism responsible; notably, none have determined the effect of blocking calcium-activated K+ channels, which are ubiquitously involved in EDHF signaling (19). In SHR vasculature, peak VSM hyperpolarization (13) and ACh-stimulated EDHF-mediated relaxation are generally found to be impaired (17, 19) but not in all cases (8, 50), which may be related to age and/or the vascular wall remodeling state (17). Although our results might suggest that an EDHF may in fact be responsible for the augmented low [ACh]-stimulated SHR CCA relaxation, there are no additional data directly substantiating this supposition.

**Perspectives**

Congruent with the working hypothesis that CCA endothelial vasomotor dysfunction could be associated with altered CCA hemodynamics in hypertension, Laccarino et al. found that improving vasomotor dysfunction with Akt gene transfer to the CCA endothelium corrected a ~30% reduction in peak systolic CCA flow in SHR. The authors (31) suggested this improvement was a result of increased vessel wall compliance, thus reducing CCA resistance to flow. This is consistent with findings by Levy and associates (9, 38, 42) that the endothelium can alter CCA diameter and compliance in SHR, which might be attributed to the disappearance of a contracting factor produced in the SHR endothelium (42). Although it is impossible with our methodological approach to directly link the in vitro vasomotor function responses with the in vivo hemodynamics, in light of the findings of Laccarino et al. (31) and Levy and associates (9, 38, 42), it is intriguing to speculate from our data that endothelium-dependent, endoperoxide-mediated signaling may elicit vasoconstriction in the CCA of SHR and contribute to the ~25% reduced mean blood flow through this artery in vivo. This would likely contribute most to the ~33% reduced peak systolic flow we observed, whereas the ~63% reduced total flow we found during diastole would likely be more dependent on elevated downstream arterial/arteriolar resistance in SHR due to luminal narrowing, rarefaction, and endothelial vasomotor dysfunction (5). This overall interpretation highlights that the vascular system is a continuum (53), with both conduit and resistance vessel endothelial vasomotor dysfunction contributing to altered CCA hemodynamics in hypertension.

**Limitations**

No in vivo experiments to manipulate CCA NO or endoperoxide signaling were performed in the current study to establish whether these vasomotor pathways indeed do affect blood flow through the CCA of SHR. Therefore, this study cannot conclude with causality that the overactive endoperoxide signaling and enhanced ability of the SHR CCA to contract played a physiologically important role in reducing CCA blood flow in vivo. Rather, this perspective is put forth as a working hypothesis to be further investigated. In such an investigation, it will be necessary to ensure that experiments directly manipulating CCA endoperoxide or NO signaling in vivo, as was accomplished by Laccarino et al. (31) via selective gene transfer to the CCA endothelium, avoid concomitant effects on downstream cerebrovascular tone and/or uncontrollable systemic compensations, which may occur with acute or chronic infusions or topical application of drugs, which, consequently, would corrupt interpretation of CCA flow changes.

**Clinical relevance**

Because the CCAs contribute ~80% to the total perfusion of the brain (16) and normal brain function is highly reliant on
CCA blood flow (45), a 25% reduction in this parameter could result in significant hyperperfusion that may contribute to the predisposition of SHR to localized neurological deficits (3, 41). Future investigation of the SHR CCA circulation will provide insight into the roles of reflected pressure waveforms in altering CCA blood flow (6, 34) and in this way may help elucidate mechanisms that govern CCA and cerebral blood flow in human essential hypertension (7, 29, 36). Future investigation of the COX- and TP-receptor-mediated vasocontractile characteristics of the SHR CCA will provide insight into the possible role of operative endoperoxide signaling in shifting the mechanical properties of the CCA (i.e., stiffness/compliance) affecting blood flow, which could have both downstream (cerebral perfusion) and upstream (left ventricular afterload) consequences. Accordingly, this signaling pathway may prove to be an important therapeutic target in the prevention and treatment of hypertensive complications related to ventricular–vascular coupling (63).

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