Cyclooxygenase and thromboxane/prostaglandin receptor contribute to aortic endothelium-dependent dysfunction in aging female spontaneously hypertensive rats

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Graham DA, Rush JW. Cyclooxygenase and thromboxane/prostaglandin receptor contribute to aortic endothelium-dependent dysfunction in aging female spontaneously hypertensive rats. J Appl Physiol 107: 1059–1067, 2009. First published August 20, 2009; doi:10.1152/japplphysiol.90785.2008.— Cyclooxygenase (COX)-derived vasoconstrictor prostanoids are EDCF that appear to be central to this aortic vasomotor dysfunction: inhibition of COX (9, 11, 13–15, 19, 22, 30) or of thromboxane/prostaglandin (TP) receptors (10, 11, 17, 25, 30) corrects the impaired endothelium-dependent vasomotor response in young SHR (vs. WKY), in young mSHR (vs. fSHR), and in aging mSHR and mWKY (vs. young). COX-generated PGH2 and PGI2 (prostacyclin) have been proposed as two primary candidate EDCFs that contribute to endothelial impairments of mSHR, and to a greater extent with aging (1, 4, 6, 8, 10, 20, 22). Specifically with aging in mSHR, the predominant functional result of PGI2 appears to shift from PGI2 receptor (IP receptor)-mediated vasorelaxation to TP receptor-mediated vascular contraction (4, 8, 22). Interestingly, another common EDCF, thromboxane A2 (TXA2), does not appear to contribute significantly to vasomotor impairments in aorta of SHR (1, 4, 8, 11, 15, 19, 25). Furthermore, expression and activity of several components of the COX-EDCF pathway are altered in aorta of mSHR (vs. mWKY) and in aging males of both strains (vs. young), including elevated COX (6, 8, 20, 33), elevated PGI2 synthase (8, 10, 20, 22), and reduced IP receptor (20).

It is unknown whether aging-related alterations in vasorelaxation occur in fSHR and whether a role exists for COX-derived EDCF in this potential phenomenon. Furthermore, there are no reports comparing the effects of advancing age (i.e., >19 wk old) on vasomotor function between mSHR and fSHR. Characterization of the pathways controlling endothelial vasomotor function in aging fSHR could elucidate sex-specific aging effects in hypertensive vascular dysfunction that may be important from a basic science perspective and potentially from a clinical perspective regarding vascular mechanisms determining the efficacy of specific prevention and treatment modalities before the onset of frank disease.

The present study, therefore, examined endothelium-dependent vasomotor responses mediated by nitric oxide (NO) and COX-derived prostanooids, and the release of PGI2 and TXA2 (assessed as the respective stable metabolites 6-keto-PGF1α and of TXB2), in aorta segments isolated from male and female WKY and SHR at 16 and 30 wk of age. The rationale for using animals at these two ages was that, by 16 wk, SHR had reached a stable elevation in blood pressure (BP) compared with WKY for several weeks. By 30 wk of age, animals would have been exposed to this elevated BP for an additional 14 wk without yet having the confounding influences of secondary conditions related to diseases common in older hypertensive animals (e.g., heart failure) or of cessation of reproductive cycling, which
occurs between 10 and 12 mo of age in fSHR (5, 23). These were important considerations to facilitate distinguishing the effects of aging and hypertension in the two sexes within the age range studied. Moreover, we had observed a consistent pattern in preliminary data from unrelated experiments in which, unlike young mSHR, 16-wk fSHR exhibited unimpaired endothelial vasomotor responses that were similar to those of WKY, while 30-wk fSHR had reductions in function. This prompted our interest in systematically studying age-dependent effects in this age range. It was hypothesized that: 1) endothelium-dependent vasorelaxation would be blunted in 30-wk vs. 16-wk fSHR; 2) a similar rate of deterioration in endothelial function would occur in mSHR and fSHR between 16 and 30 wk old (i.e., 16-wk values will be blunted less in fSHR than in mSHR, and the final extent of decline will be less in 30-wk fSHR vs. mSHR); and 3) the reduced vasorelaxation of 30-wk fSHR would be COX and TP receptor mediated and would be accompanied by increased aortic expression of COX and aortic release of PGI₂.

### MATERIALS AND METHODS

**Animals.** Male and female WKY (Harlan, Madison, WI) were raised to 16 or 30 wk of age. Eight experimental groups resulted: 30-wk fSHR would be COX and TP receptor knockout (KO) endothelium-dependent vasorelaxation would be blunted in 16-wk fSHR vs. 16 wk fWKY (n = 15), 30-wk mSHR (n = 16), 30-wk fWKY (n = 16), and 30-wk fSHR (n = 29). Endpoint age ranges were 16.2–17.7 wk (for 16 wk) and 29.7–30.6 wk (for 30 wk). Rats were group housed (4–5/cage) in a temperature- and humidity-controlled room (12:12-h light-dark cycle) and were fed standard chow (Teklad 22/5 Rodent Diet, Harlan) and tap water ad libitum. The University of Waterllo Animal Care Committee approved all animal procedures. All chemicals and drugs were purchased from Sigma (St. Louis, MO) or BioShop (Burlington, ON).

**Blood pressure measurements.** Body mass (BM) was recorded, and anesthesia was induced by pentobarbital sodium (65 mg/kg BM ip). Left common carotid artery was exposed and cannulated with a Mikro-Tip Pressure Transducer catheter (Millar Instruments, Houston, TX), and a stable intra-arterial BP tracing was recorded for several minutes using a PowerLab data-acquisition unit (ADInstruments, Colorado Springs, CO). Mean arterial BP (MAP) was determined from the raw BP tracing using the Cyclic Measurements function in the Chart 5.5 software (ADInstruments). To maintain body temperature during anesthesia and BP measurement, rats were placed on a pump-perfused, water-jacketed heating pad (Gaymar, Orchard Park, NY) set to 37.5°C.

**Tissue harvesting.** Anesthetized animals were euthanized by cardiac excision and exsanguination. Descending thoracic aorta was placed in cooled Krebs-bicarbonate buffer (4°C, pH 7.4: 131.5 mmol/l NaCl, 5.0 mmol/l KCl, 2.5 mmol/l CaCl₂-2H₂O, 1.2 mmol/l NaH₂PO₄·H₂O, 1.2 mmol/l MgCl₂, 11.2 mmol/l d-glucose, 13.5 mmol/l NaHCO₃, 2.5 mmol/l EDTA·2H₂O), and adhering connective tissue was gently removed. Rings (~2 mm) were cut for vasomotor function experiments, and the remainder was gently blotted dry and stored at –80°C for Western blotting. Atria and aortic stump were removed from the heart and discarded. Outer right ventricular wall tissue was gently removed. Rings (8), 16-wk fSHR, 16-wk fWKY, and 30-wk fWKY were exposed to diac excision and exsanguination. Descending thoracic aorta was placed in cooled Krebs-bicarbonate buffer (4°C, pH 7.4) and each exposure was separated by two washes (5 min) and return to baseline tension. Stable contraction to phenylephrine (PE; α₁-adrenoceptor agonist, 10⁻⁶ mol/l) was achieved, and the endothelium-dependent response to cumulative doses of ACh (muscarinic agonist, 10⁻¹⁰–10⁻⁶ mol/l) was performed, followed by three washes. Rings were contracted again (10⁻⁷ mol/l PE), and the endothelium-independent vasorelaxation to cumulative doses of sodium nitroprusside (SNP; NO donor, 10⁻¹⁰–10⁻⁶ mol/l), was assessed.

**Vasomotor function data** were collected from duplicate rings for ND, t-NAME, and SQ-29548, and from singlet rings for Indo and L-NAME. Rings were contracted twice with KCl (60 mmol/l), and each exposure was separated by two washes (5 min) and return to baseline tension. Stable contraction to phenylephrine (PE; α₁-adrenoceptor agonist, 10⁻⁷ mol/l) was achieved, and the endothelium-dependent response to cumulative doses of ACh (muscarinic agonist, 10⁻¹⁰–10⁻⁶ mol/l) was performed, followed by three washes. Rings were contracted again (10⁻⁷ mol/l PE), and the endothelium-independent vasorelaxation to cumulative doses of sodium nitroprusside (SNP; NO donor, 10⁻¹⁰–10⁻⁶ mol/l) was assessed.

**Table 1. Physical characteristics and MAP**

<table>
<thead>
<tr>
<th></th>
<th>mWKY</th>
<th>mSHR</th>
<th>fWKY</th>
<th>fSHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM, g</td>
<td>310±4</td>
<td>340±3</td>
<td>187±3</td>
<td>187±2b</td>
</tr>
<tr>
<td>30 wk</td>
<td>349±5a</td>
<td>364±4b</td>
<td>214±1a</td>
<td>198±4ab</td>
</tr>
<tr>
<td>LV/BM, mg/g</td>
<td>2.25±0.02</td>
<td>2.56±0.02a</td>
<td>2.50±0.04a</td>
<td>2.73±0.05b</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>22.00±0.03</td>
<td>2.67±0.04a</td>
<td>2.55±0.07a</td>
<td>2.96±0.04ab</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–29 (BM and LV/BM) and n = 6–8 (MAP); mWKY and fWKY, male and female Wistar-Kyoto rats, respectively; mSHR and fSHR, male and female spontaneously hypertensive rats, respectively; BM, body mass; LV, left ventricular mass; LV/BM, LV-to-BM ratio; MAP, mean arterial pressure. P < 0.05 vs. 16-wk mWKY, b16-wk mSHR, c16-wk fWKY, d16-wk fSHR, e30-wk mWKY, f30-wk mSHR, and g30-wk fWKY.

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studies in our laboratory and others (6, 10, 11) indicated that these concentrations of DMSO or ethanol alone had no detectable effects on vasomotor responses to a variety of vasoactive agents as used in this study, compared with the absence of DMSO or ethanol. Absolute vasomotor response (vasoconstriction in g) to a given dose of agonist was determined in triplicate by bicinchoninic acid assay, as described previously (26). Samples were diluted to a total protein concentration of 1.0 mg/ml using 25% of total volume sample buffer (1.46 mol/l sucrose, 7.5 mol/l) potassium chloride (KCl); PE, contractile response to 10^(-7) mol/l phenylephrine before ACh relaxation dose-response; ND, no drug control condition. P < 0.05 vs. *16-wk mWKY, †16-wk mSHR, ‡30-wk mWKY, and §30-wk mSHR.

### Table 2. Final resting tension and contractile responses to PE and KCl stimuli in the ND condition

<table>
<thead>
<tr>
<th>Condition</th>
<th>mWKY</th>
<th>mSHR</th>
<th>fWKY</th>
<th>fSHR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Final resting tension, g</strong></td>
<td>6.96±0.01</td>
<td>6.95±0.01</td>
<td>5.95±0.01</td>
<td>6.45±0.01</td>
</tr>
<tr>
<td>16 wk</td>
<td>7.93±0.01</td>
<td>7.91±0.01</td>
<td>5.47±0.01</td>
<td>6.44±0.01</td>
</tr>
<tr>
<td>30 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>KCl contraction (ND condition), g</strong></td>
<td>1.45±0.05</td>
<td>1.58±0.12</td>
<td>1.45±0.07</td>
<td>1.61±0.09</td>
</tr>
<tr>
<td>16 wk</td>
<td>1.74±0.06*</td>
<td>1.92±0.07†</td>
<td>1.61±0.06</td>
<td>1.76±0.06</td>
</tr>
<tr>
<td>30 wk</td>
<td>1.77±0.06</td>
<td>1.68±0.06</td>
<td>1.33±0.10*</td>
<td>1.54±0.13</td>
</tr>
<tr>
<td><strong>PE contraction (ND condition), g</strong></td>
<td>2.04±0.05*</td>
<td>2.01±0.06‡</td>
<td>1.58±0.10</td>
<td>1.74±0.07§</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–16 in duplicate rings. Final resting tension, tension recording before 30-min drug incubation; KCl, contractile response to 60 mmol/l KCl; PE, contractile response to 10^(-7) mol/l phenylephrine before ACh relaxation dose-response; ND, no drug control condition. P < 0.05 vs. *16-wk mWKY, †16-wk mSHR, ‡30-wk mWKY, and §30-wk mSHR.
endothelial NOS: 1:500, BD Biosciences, Franklin Lakes, NJ), rinsed (3 × 5 min) in TBS-T, transferred to secondary antibody [goat anti-rabbit: 1:5,000 (COX-1) and 1:2,000 (COX-2), Santa Cruz Biotechnology, Santa Cruz, CA; goat anti-mouse: 1:2,000, Santa Cruz Biotechnology], and rinsed (3 × 5 min) in TBS-T. Enhanced chemiluminescence (GE Healthcare/Amersham) and gel documentation (Syngene, Cambridge, UK) were used to detect protein signal. Intergel chemiluminescent Western blot signals were standardized to a common thoracic aortic homogenate run in all gels, and values were then expressed as fold changes relative to 16-wk mWKY. α-Actin was originally used as an internal control to ensure equal protein loading and transfer, but we observed a systematic treatment group effect on α-actin expression. Thus, as an alternative, we performed Ponceau red staining of the membrane and used integration of the densitometry signal from a consistent five-band pattern as a means to rule out lane-to-lane differences in total protein loading and transfer.

Prostanoid release. Prostanoid release was measured in buffer following cumulative ACh exposure, as previously cited in mSHR (11). Isolated aortic rings from a subset (n = 4 per group) of 16- and 30-wk mSHR and fSHR were suspended on a myography system, as described and exposed to cumulative ACh doses (10⁻⁶–10⁻¹⁰ mol/l) in the presence of L-NAME (10⁻⁴ mol/l) and without precontraction. Krebs-bicarbonate buffer (5 ml) was collected and snap frozen in liquid nitrogen after a steady-state tension level was achieved following the last ACh dose. Rings were collected, blotted dry, and snap frozen in liquid nitrogen. Buffer and rings were stored at −80°C until analysis. Levels of TxB₂ (stable metabolite of TxA₂) and 6-keto-PGF₁α (stable metabolite of PGI₂) were determined by EIA kits, according to the manufacturer’s instructions (Cayman Chemical). Buffer samples for measurement of TxB₂ were concentrated 6.37-fold using Clean-Up C18 sorbent-type extraction columns (UCT, Bristol, PA) before the assay was performed. Rings were freeze-dried under vacuum pressure for 3 h at −30°C, followed by 23 h at room temperature. Ring dry weights were then measured on an ultrasensitive balance and used for normalization of prostanoid release to dry tissue mass on a ring-by-ring basis.

Statistics. Values reported are means ± SE. Data were analyzed using ANOVA, and least squares means post hoc was conducted where an interactive term was significant. \( P < 0.05 \) was considered significant. SAS 9.1.3 software (SAS Institute, Cary, NC) was used to perform all statistical analyses.

RESULTS

Physical characteristics and MAP. BM was elevated in males vs. females, and in 30 wk vs. 16 wk, with the exception of fSHR (Table 1). LV/BM was elevated in SHR vs. WKY, females vs. males, and 30-wk vs. 16-wk SHR. MAP was higher in SHR vs. WKY. 16-wk fWKY had higher MAP vs. 16-wk mWKY, whereas the opposite was true within 30-wk WKY. A lower MAP was observed in 30-wk females vs. 16-wk counterparts. Supplementary BP, heart rate, and organ mass measurements are presented in the online data supplement (Supplemental Table I). (The online version of this article contains supplemental data.)

Contractile vasomotor responses. Exposure of ND rings to 60 mmol/l KCl resulted in similar contractions across all 16-wk groups (Table 2). KCl-induced contractions were elevated in 30-wk vs. 16-wk males of both strains, and to a greater extent in SHR. Contractile responses to 10⁻⁷ mol/l PE were higher in males vs. female counterparts, except within 16-wk SHR, where the contractions were similar. PE contractions were also greater in 30-wk vs. 16-wk males. KCl and PE contractile responses in the presence of Indo and SQ-29548 are presented in the online data supplement (Supplemental Table II).
**Endothelium-dependent vasomotor responses.** High-ACh concentrations caused modest re-contraction (i.e., reversal of ACh-mediated relaxation) in both 16-wk and 30-wk mWKY that was slightly accentuated in 30 wk (Fig. 1A). Both 16-wk and 30-wk mSHR exhibited considerable re-contraction to high-ACh doses, and this response was exaggerated in 30 wk (Fig. 1B). Interestingly, mSHR exhibited both greater relaxation to some low-ACh doses (10^{-8.5}–10^{-7.5} mol/l) and greater re-contraction to high-ACh doses (peak re-contraction occurred at 10^{-5.0} mol/l ACh in all groups) vs. age-matched mWKY.

ACh-mediated relaxations to low doses were slightly greater in 16-wk vs. 30-wk fWKY, and these two groups had similar responses to high doses of ACh with no apparent re-contraction (Fig. 1C). ACh responses of 16-wk and 30-wk fWKY (Fig. 1C) were greater than those of age-matched mWKY counterparts (Fig. 1A) at all ACh concentrations that elicited a detectable response. Sixteen-week fSHR exhibited robust relaxation (Fig. 1D) that was greater than that of 16-wk fWKY to low-ACh doses (10^{-8.5}–10^{-7} mol/l) and similar to this group at high-ACh doses (Fig. 1C). As well, the ACh relaxation response of 16-wk fSHR (Fig. 1D) at all ACh doses was greater than that of 16-wk fSHR (Fig. 1B). In contrast, 30-wk fSHR exhibited a severely impaired ACh response compared with 16-wk fSHR (Fig. 1D); a blunted relaxation to high ACh compared with the 30-wk fWKY group (Fig. 1C); and only slightly greater relaxation to high-ACh doses compared with 30-wk mSHR (Fig. 1B).

Inhibition of COX with Indo eliminated the re-contraction to high concentrations of ACh observed in 16-wk mSHR, 30-wk mSHR, and 30-wk fSHR (Fig. 2, A and B), but enhanced the relaxation to low ACh only in the 30-wk fSHR (Fig. 2, C and D). In the presence of Indo, the robust relaxation exhibited by 30-wk fSHR (Fig. 2D) was similar to that seen in 16-wk fSHR (Fig. 2C) and greater than that of 30-wk mSHR (Fig. 2B). NOS inhibition with L-NAME completely abolished ACh-induced relaxation in all groups, regardless of the presence of Indo (data omitted from figures for clarity).

TP receptor inhibition with SQ-29548 also abolished the re-contractions induced by high doses of ACh in 16-wk mSHR, 30-wk mSHR, and 30-wk fSHR (Fig. 3, A, B, and D). SQ-29548-treated rings from 16-wk mSHR (Fig. 3A) and 30-wk fSHR (Fig. 3D) both had slightly greater maximal relaxation to ACh compared with that from 30-wk mSHR (Fig. 3B). SQ-29548 did not affect relaxation at low ACh, except for a small enhancement in 30-wk mSHR.

**Endothelium-independent vasorelaxation.** Similar maximal relaxation to SNP was observed in curve-fit dose responses of all groups (average 109 ± 1%, Fig. 4). Likewise, EC_{50} values averaged 1.14 ± 0.12 nmol/l across all groups, and there were no age- or hypertension-related group differences. The only significant group difference in EC_{50} values was between 16-wk fSHR (0.54 ± 0.21 nmol/l) and 16-wk mSHR (1.50 ± 0.38 nmol/l, P < 0.0512). Dose-by-dose comparisons revealed some small group differences at certain low doses of SNP.

**Protein expression.** Relative aortic expression of COX-1 protein was lower (33%) in 16-wk fSHR vs. 16-wk mSHR and was elevated in 30-wk fSHR to levels higher (75%) than in 16-wk fSHR and similar to those in 30-wk mSHR (Fig. 5A). In fWKY, COX-1 protein expression was also higher (24%) in 30-wk vs. 16-wk groups. Relative aortic COX-2 protein expression was higher in 16-wk mSHR (54%) and 30-wk mSHR (85%) vs. WKY counterparts (Fig. 5B). In 30-wk mSHR
animals, COX-2 protein levels were higher (+41%) in mSHR vs. fSHR, and in fWKY vs. mWKY (+44%). Relative aortic endothelial NOS protein levels were similar across all groups (n = 4–6, data omitted from figure for clarity).

Prostanoid release. ACh-stimulated release of PGI\(_2\) (assessed as 6-keto-PGF\(_1\alpha\)) was similar in 16-wk mSHR and fSHR (Fig. 6). 6-keto-PGF\(_1\alpha\) was elevated in 30-wk mSHR vs. 16-wk mSHR and 30-wk fSHR. Within fSHR, the ~30% higher group mean for the 30-wk vs. 16-wk group did not reach significance (P < 0.05 vs. 16-wk mWKY, 16-wk mSHR, and 16-wk fWKY).

A robust vasorelaxation to ACh occurred in arteries from 16-wk female animals and was similar between 16-wk fSHR and 16-wk fWKY at high-ACh doses. By 30 wk of age, however, fSHR had a greatly blunted ACh response compared with both 16 wk and WKY counterparts, maintaining only a slightly greater response to high-ACh doses compared with 30-wk mSHR, which exhibited deteriorating vasomotor function compared with the younger males in the present study and others (10, 14, 15). These results demonstrate a decline in function in arteries of fSHR between 16 wk and 30 wk of age, confirming our first hypothesis. The data further suggest that the rate of impairment of endothelial vasomotor function was accelerated in fSHR compared with mSHR between the ages of 16 wk and 30 wk, in contrast to our second hypothesis. This finding is a simple function of the facts that 1) at 16 wk old, the response was already blunted in males but not females; and 2) that by 30 wk old, the male and female responses were reduced to similar levels.

The differences between impairments of endothelial vasomotor function of mSHR and fSHR as they age from 16 wk to 30 wk of age observed in the present study are contrasted by very modest functional reductions across this age range in WKY that are similar in males and females. Previous studies have also reported a greater decline in endothelium-dependent vasomotor function in SHRs across the age range studied occurred at a greater rate in females than in males; and 3) the COX-TP receptor axis contributed to the vasomotor impairments of 30-wk fSHR based on functional responses using pharmacological blockers and assessments of COX-1 expression and PGI\(_2\) release.

DISCUSSION

The present study examined the endothelium-dependent aortic vasomotor responses of male and female WKY and SHR at 16 and 30 wk of age and mechanisms contributing to these responses. The major novel findings of this study are that 1) ISHR exhibited reductions in endothelium-dependent vasomotor responses between 16 and 30 wk of age; 2) the decline of endothelium-dependent vasomotor function in SHR across the age range studied occurred at a greater rate in females than in males; and 3) the COX-TP receptor axis contributed to the vasomotor impairments of 30-wk fSHR based on functional responses using pharmacological blockers and assessments of COX-1 expression and PGI\(_2\) release.
The decline of endothelium-dependent vasomotor function as fSHR age from 16 wk to 30 wk old in the present study was abolished by blockade of the COX-TP receptor pathway in vitro at two levels: COX inhibition with Indo, and TP receptor inhibition with SQ-29548. Additionally, elevation of COX-1 expression occurred in aorta of 30 wk vs. 16 wk fSHR, and the aging-related increases in 6-keto-PGF1α (stable metabolite of PGI2) release from aorta and endothelial dysfunction were significantly positively correlated. Collectively, these data confirm the first two parts of our third hypothesis and support a COX- and TP receptor-mediated decline in endothelium-dependent vasomotor function as fSHR age from 16 wk to 30 wk old.

A role for COX-1-derived EDCF, most likely in the form of PGH2 and/or PGI2, has been established in the aortic endothelial vasomotor dysfunction of aging mSHR (1, 4, 6, 8, 10, 20, 22). Inhibition of COX in the present study and others (9, 14, 15, 22) and of the TP receptor in the present study and others (10, 17) enhanced endothelium-dependent relaxation and reduced endothelium-dependent contraction in aging mSHR. The elevated 6-keto-PGF1α release in the present 30-wk vs. 16-wk mSHR corroborates previous observations (20) and adds to the growing support for a role for PGI2 in the reduced endothelial function in aging mSHR (1, 4, 6, 8, 10, 20, 22). The low levels of TXB2 (stable metabolite of TxA2) that were released equally across mSHR and fSHR of both ages, on the other hand, suggest a limited role for this prostanoid in the present sex and aging effects on vasomotor dysfunction observations and align with previous conclusions in mSHR (1, 4, 8, 11, 15, 19, 25). Overall, the present results suggest that a common COX-TP receptor pathway largely contributes to the aging-related impairment of aortic endothelium-dependent vasorelaxation in both fSHR and mSHR across the age range studied. While PGI2 appears to play a prominent role in the aging effect in both mSHR and fSHR, it is possible that the proportional contribution of COX-derived EDCF, such as PGI2 and PGH2, to aging-related endothelial impairments differs between the sexes. This issue was not specifically studied in the present experiments, but the difference in the degree to which PGI2 release is affected by age in mSHR vs. fSHR is consistent with this idea. Additionally, it is unclear at present what stimuli ultimately determine the sex difference in COX- and TP receptor-mediated vasomotor responses in 16 wk-old SHR and how this changes with aging over the 16- to 30-wk period.

Although there is good general agreement between the data presented in the present study and previous work in this area, there has been one previous report (34), which disagrees with the finding of impaired ACh responses in 16-wk-old mSHR vs. fSHR reported in the present study and others (13). Additionally, one previous study (17) disagrees with the finding in the present study and others that inhibition of the TP receptor (10, 11) or of COX (10, 11) restored a robust ACh response in mSHR between the ages of 30 wk and 80 wk old. Possible reasons for the sex and aging discrepancies between these previous studies (17, 34) and the present data are not readily apparent from the methods described in the papers in question. It is noteworthy, however, that the endothelium-dependent relaxation responses in these two dissenting studies (17, 34) and the present data are not readily apparent from the methods described in the papers in question.

The decline of endothelium-dependent vasomotor function as fSHR age from 16 wk to 30 wk old in the present study was abolished by blockade of the COX-TP receptor pathway in vitro at two levels: COX inhibition with Indo, and TP receptor inhibition with SQ-29548. Additionally, elevation of COX-1 expression occurred in aorta of 30 wk vs. 16 wk fSHR, and the aging-related increases in 6-keto-PGF1α (stable metabolite of PGI2) release from aorta and endothelial dysfunction were significantly positively correlated. Collectively, these data confirm the first two parts of our third hypothesis and support a COX- and TP receptor-mediated decline in endothelium-dependent vasomotor function as fSHR age from 16 wk to 30 wk old.

A role for COX-1-derived EDCF, most likely in the form of PGH2 and/or PGI2, has been established in the aortic endothelial vasomotor dysfunction of aging mSHR (1, 4, 6, 8, 10, 20, 22). Inhibition of COX in the present study and others (9, 14, 15, 22) and of the TP receptor in the present study and others (10, 17) enhanced endothelium-dependent relaxation and reduced endothelium-dependent contraction in aging mSHR. The elevated 6-keto-PGF1α release in the present 30-wk vs. 16-wk mSHR corroborates previous observations (20) and adds to the growing support for a role for PGI2 in the reduced endothelial function in aging mSHR (1, 4, 6, 8, 10, 20, 22). The low levels of TXB2 (stable metabolite of TxA2) that were released equally across mSHR and fSHR of both ages, on the other hand, suggest a limited role for this prostanoid in the present sex and aging effects on vasomotor dysfunction observations and align with previous conclusions in mSHR (1, 4, 8, 11, 15, 19, 25). Overall, the present results suggest that a common COX-TP receptor pathway largely contributes to the aging-related impairment of aortic endothelium-dependent vasorelaxation in both fSHR and mSHR across the age range studied. While PGI2 appears to play a prominent role in the aging effect in both mSHR and fSHR, it is possible that the proportional contribution of COX-derived EDCF, such as PGI2 and PGH2, to aging-related endothelial impairments differs between the sexes. This issue was not specifically studied in the present experiments, but the difference in the degree to which PGI2 release is affected by age in mSHR vs. fSHR is consistent with this idea. Additionally, it is unclear at present what stimuli ultimately determine the sex difference in COX- and TP receptor-mediated vasomotor responses in 16 wk-old SHR and how this changes with aging over the 16- to 30-wk period.

Although there is good general agreement between the data presented in the present study and previous work in this area, there has been one previous report (34), which disagrees with the finding of impaired ACh responses in 16-wk-old mSHR vs. fSHR reported in the present study and others (13). Additionally, one previous study (17) disagrees with the finding in the present study and others that inhibition of the TP receptor (10, 11) or of COX (10, 11) restored a robust ACh response in mSHR between the ages of 30 wk and 80 wk old. Possible reasons for the sex and aging discrepancies between these previous studies (17, 34) and the present data are not readily apparent from the methods described in the papers in question. It is noteworthy, however, that the endothelium-dependent relaxation responses in these two dissenting studies (17, 34) and the present data are not readily apparent from the methods described in the papers in question.

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did not exhibit the characteristic re-contraction to high-ACh doses that has been commonly observed: in SHR in the present study and others (10, 11, 13–16, 19, 30); with aging in SHR in the present study and others (10, 14, 15); and to a greater extent in mSHR compared with fSHR in the present study and others (13).

Limitations. The unexpected finding of reductions in MAP recorded in the 30-wk fWKY and fSHR compared with 16-wk counterparts in this study is inconsistent with previous observations that BP did not decrease between 4 and 8 mo of age in fSHR (5) and, indeed, with other preliminary data from our own laboratory. Endpoint carotid arterial BP measurements, recorded over a relatively small time period under anesthetized conditions, were obtained for the purpose of simply documenting that SHR were hypertensive compared with WKY counterparts, which was confirmed. We did not design our BP assessment approach to take ambulatory BP measurements or to compare the BP changes with vascular function changes among groups. This is an experimental design limitation of this study, as it is possible that the age-related BP reductions in female animals reported herein could be particularly compromised by limitations in the procedures employed (e.g., timing of collection, anesthesia, etc.).

PE-mediated precontractions were not similar across treatment groups. This is explicitly illustrated in Table 2. In keeping with convention, we expressed dose-response data to dilatory agonists as a percentage of the preceding PE-mediated contraction. This is the most suitable manner in which to express the relaxation data from a common precontractile stimulus level (dose of PE) as it normalizes the response for differences that might result from potentially different mass of the rings across treatment groups (12, 34), which we did not directly assess in the present study. Group differences in optimum final resting tension and in tension development to 60 mmol/l KCl followed a similar pattern to that observed in the precontraction response to PE, suggesting a common, rather than a specific, mechanism (e.g., altered aortic smooth muscle content).

A central role has been attributed to sex hormones in governing the sex differences in vasomotor function in young (i.e., 16- to 17-wk-old) SHR (3, 13, 32). Precise characterization of the mechanism(s) through which the COX-TP receptor pathway is differentially controlled in mSHR vs. fSHR and changes with aging in SHR would contribute to the field. These experiments, however, would require very specific manipulation of sex hormones (e.g., gonadectomy with and without sex hormone replacement) and are beyond the scope of the present study, which was designed to functionally characterize sex- and aging-dependent effects and to establish whether the COX-TP receptor axis was involved specifically in the female aging response in hypertension. The vasomotor function changes observed with aging between 16 and 30 wk old in fSHR in this study, however, likely did not depend on changes in estradiol per se, as it has been previously reported that SHR do not stop cycling until 10–12 mo of age (5, 23). This does not discount the possibility that some aspect of changes in sex hormone production or sensitivity may contribute, as might be revealed in studies designed to specifically elucidate this involvement.

Conclusions. This is the first report characterizing endothelium-dependent aortic relaxation occurred in fSHR between the ages of 16 wk old, when relaxations were normal and robust, and 30 wk old, when the blunted responses of females were nearly as impaired as those of age-matched mSHR. Across this age range, fSHR exhibit a greater rate of impairment than mSHR. In the presence of the COX inhibitor, Indo, or the TP receptor inhibitor, SQ-29548, the endothelium-dependent vasomotor response of 30-wk-old fSHR was restored to the level of 16-wk counterparts. Increased aortic protein levels of COX-1 in 30- vs. 16 wk-old fSHR does not prove, but is consistent with, the suggestion that this isoform could be involved in coordinating the aging-related blunting of endothelium-dependent relaxation observed in these animals. While the potential role of TxA2 as an EDCF in the age-related endothelial dysfunction in fSHR appears to be limited, PGI2 likely does contribute to this dysfunction, although differential roles for PGI2 and PGH2 between male and female aging effects could exist. Furthermore, it must be noted that differences in sensitivity of the vascular smooth muscle to prostanoid products from the endothelium could contribute to age-, sex-, and hypertension-related functional differences. This issue has not been addressed in the present study. The results of this study, implicating the COX-EDCF-TP receptor axis as a contributing factor to the vasomotor impairment that develops as fSHR age from 16 to 30 wk old, parallel previously published observations in aging mSHR (6, 8–10, 14, 15, 17, 20, 22), and our own observations of mSHR across the age range reported herein. Therefore, the endothelium-dependent vasomotor dysfunction that develops in fSHR between 16 and 30 wk of age appears, at least to a significant extent, to be COX (likely COX-1) dependent and to be mediated through the TP receptor, thus sharing commonalities with the mechanisms implicated in the vasomotor dysfunction occurring in aging mSHR.

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REFERENCES


22. Rapoport RM, Williams SP. Role of prostaglandins in acetylcholine-induced contraction of aorta from spontaneously hypertensive and Wistar-Kyoto rats. *Hypertension* 28: 64–75, 1996.


