Functional changes in the uterine artery precede the hypertensive phenotype in a transgenic model of hypertensive pregnancy

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The transgenic female rat containing the human angiotensinogen (hAGN) transgenic rat mated with the male human renin (hREN) transgenic rat is a model of preeclampsia (TgA) that mimics many features of preeclampsia, including high blood pressure and proteinuria in the last third of gestation, characteristics that are absent in the reverse mating (4). At late gestation, TgA rats display increased circulating and uteroplacental levels of angiotensin II (Ang II), whereas in the reverse mating only uteroplacental Ang II is increased (16). Thus species-specific dependency of renin and angiotensinogen for rodent and human proteins and their localization in the placenta and the circulation form the basis of this model. Increased circulating levels of Ang II may derive from the fact that in TgA rats Ang II is upregulated in both the fetal and maternal components of the placenta, whereas the reverse mating showed no increase in Ang II in the maternal but an increase in Ang II in the fetal component of the placenta (6).

During the first half of pregnancy the TgA rat showed no alterations in systolic blood pressure; however, increased blood pressure, proteinuria, and placenta alterations of edema and necrosis are observed during the second half of pregnancy. At the local vascular level, endothelial dysfunction of the uterine artery (UA) has been described in TgA rats on day 21 of gestation (32), and we recently showed an increased Ang II contraction in the UA at early gestation in this model (30).

Vasoconstrictor eicosanoids such as thromboxane A2 (TXA2) are important modulators of vascular function in pregnancy (34), with an increased role of thromboxane analogs in the regulation of blood pressure and renal function in late pregnancy (19). Similarly, in the preeclamptic placenta, TXA2 production is increased more than three times compared with normal placentas (33), an effect proposed to be mediated by increased reactive oxygen species generation (34), suggesting that greater TXA2 levels may mediate systemic vascular alterations in preeclampsia. Recent reports also support the contribution of CYP epoxygenase to the effects on blood pressure, albuminuria, and vascular function observed in the TgA rat at late gestation (17). Thus human and animal studies support the role of eicosanoids in the uteroplacental and systemic pathological changes in preeclampsia.

In this study, we analyzed the role of cyclooxygenase (COX)-derived mediators in contractile and vasodilatory responses of the isolated UA in the TgA rat at early gestation. We hypothesized that vascular alterations, including an increased role for prostanooids, will be detected at a time when the

Preeclampsia is a common disorder of pregnancy that manifests with hypertension and proteinuria in the last term of pregnancy. It is proposed that hypertensive disorders of pregnancy originate from defective trophoblast invasion and result in vascular endothelial dysfunction triggered by placental ischemia (7). An increased response to vasoconstrictor agonists is characteristic of pregnancies at risk of developing preeclampsia (13).

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preeclamptic phenotype has still not developed in this rat model of preeclampsia.

MATERIALS AND METHODS

Animal preparation. Seven-day-pregnant preeclamptic hAGN × hREN (TgA; n = 10) and 7-day-pregnant Sprague-Dawley (SD; n = 8) rats were used. SD rats were obtained from Charles River Laboratories (Wilmington, MA). Transgenic hAGN females and hREN males were obtained from the colony maintained by the Hypertension Research and Vascular Center, Wake Forest University School of Medicine. The animals were housed at a constant room temperature, humidity, and light cycle (12:12-h light-dark), with a global 18% protein-extruded rodent chow (Harlan Laboratories, Indianapolis, IN) and water available ad libitum. All experiments were performed in accordance with the guidelines of and approved by the Wake Forest School of Medicine Institutional Animal Care and Use Committee.

Tissue collections. At 7 days of gestational age (gestation length is 21 days in SD and TgA rats), animals were euthanized by decapitation, trunk blood was collected, and plasma samples were frozen for further analysis. For reactivity studies, both uterine horns were carefully removed, and the main UA was isolated and cleaned of fat and connective tissue while being kept in cold Krebs-Henseleit buffer (KHB) containing (in mmol/l) 118 NaCl, 4.47 KCl, 25 NaHCO3, 1.2 KH2PO4, 1.2 MgSO4, 2.5 CaCl2·2H2O, and 5.5 glucose. For immunohistochemistry studies, some UA segments were incubated in buffered formalin for 24 h and then stored in 70% ethanol. Arteries were sectioned in rings of 5 μm (AML Laboratories, Baltimore, MD).

Plasma thromboxane levels. Plasma levels of the stable metabolite thromboxane B2 (TXB2) were measured in plasma samples from SD and TgA animals taken in indomethacin- and EDTA-containing tubes. Plasma was extracted with ethanol and then acidified to pH 4.0 with acetate buffer. Sep-Pak columns were then prepared by washing with methanol. The sample was applied and washed with ultrapure water, followed by hexane. Samples were eluted with ethyl acetate and 1% methanol. TXB2 was measured by ELISA specific for TXB2 (Cayman Chemicals, Ann Arbor, MI).

Vascular reactivity. Arterial segments, maximum of 2 mm in length, were mounted between an isometric force transducer (DSC 6; Kistler Morce, Seattle, WA) and a displacement device on a myograph (Multi Myograph, Model 620M; Danish Myo Technologies, Aarhus, Denmark) using two stainless steel wires (diameter 40 μm), as described previously (28, 29). The myograph organ bath (5 ml) was filled with KHB maintained at 37°C and aerated with 95% O2-5% CO2. In a subgroup of arteries from SD and TgA rats, the endothelium was destroyed by passing a human hair through the lumen as described (29).

Resting wall tension-internal circumference relationship. The vessels were washed and incubated for 30 min before the normalization procedure was performed. Arterial segments were normalized to 0.9/L100, with L100 being the internal circumference the vessels would have if they were exposed to a transmural pressure of 100 mmHg (24). Each arterial segment was stretched in a stepwise manner (50 μm steps), and the internal circumference (L) and corresponding wall tension (T) at each stretch level were calculated and plotted to produce a resting wall tension-internal circumference curve for that particular artery using the DMT Normalization Module (ADInstruments). For each arterial segment (72 arteries from 9 TgA and 56 arteries from 7 SD rats), values of tension and internal circumference were fitted to an

Fig. 1. Stretch response in uterine artery (UA) from Sprague-Dawley (SD) and transgenic (TgA) rats. Resting wall tension-internal circumference relationship in UA from SD and TgA rats expressed as normalized tension (T/T100) vs. normalized internal circumference (L/L100). Inset: values for the length constant (B) obtained from the equation T = T100 × exp[(L-L100)/L100]/B) in UA from SD (n = 7) and TgA (n = 9) rats. *P < 0.05 vs. SD.

Fig. 2. Immunolocalization of actin and staining for collagen in UA from control and TgA rats at early gestation. UA segments from control (SD; n = 5) (A and B) or TgA rats (n = 5) (C and D) were incubated with an anti-α-smooth muscle actin antibody (B and D). Control incubations for SD (A) and TgA UA (C) did not contain primary antibody. Signals were developed by incubation with 3,3′-diaminobenzidine (brown) and counterstained with hematoxylin (nuclei; blue). UA segments from control (SD: E) (n = 5) or TgA rats (F) (n = 5) were stained for collagen with Picro Sirius Red staining. Representative pictures and average values obtained by the reciprocal intensity method in each group are shown. Scale bar, 200 μm. *P < 0.05 vs. SD. AU, arbitrary units.
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Cayman Chemical), or monoclonal anti-actin α-smooth muscle antibody (cat. no. AS5228, 1.25 μg/ml Sigma). After washing three times in PBS, incubations with the corresponding secondary antibodies (goat anti-mouse IgG for eNOS and actin, goat anti-rabbit IgG for COX-2, all at 1:400 dilutions) were performed in blocking solution for 1 h at room temperature. Signals were developed using the ABC Vectastain kit (Vector Laboratories, Burlingame, CA), and sections were counterstained with hematoxylin. Staining for collagen was performed, following the Picro Sirius Red staining procedure (Sigma, St. Louis, MO). Images were acquired at ×400 magnifications using a Leica DM 4000B upright microscope (Leica Microsystems, Bannockburn, IL). Illumination settings were held constant for image capture sessions (Retiga 1300R charge-coupled device digital camera; QImaging, Surrey, BC, Canada, and SimplePCI version 6; SimplePCI, Cranberry Township, PA). Regions of interest (ROI) were defined using the open source Fiji software (http://fiji.sc/Fiji; ImageJ, National Institutes of Health) covering smooth muscle and endothelial layers (for actin, collagen and COX-2) or just the endothelial layer (for eNOS) in each arterial segment. Intensity of the staining in five ROIs per segment was quantified as described previously by us (30), following the reciprocal intensity method (26).

**Drugs**. *L*-NAME, PE, acetylcholine, and SNP were purchased from Sigma (St. Louis, MO), and stock solutions were prepared in distilled water. Indomethacin was dissolved in 50 mM NaCl in KHB. All other chemical reagents were purchased from Sigma.

**Data analysis.** All data analysis was performed using the GraphPad Prism version 5 statistical analysis package (GraphPad Software, La Jolla, CA). Data of TBX2 plasma levels were compared using Student’s *t*-test. Maximal contractile responses to KCl were expressed in absolute values, whereas maximal responses to PE were expressed as a percent of the maximal response induced by KCl (%KMAX). Vasodilatory responses to ACh and SNP were expressed as percent of preconstricted tone. Concentration-response curves were analyzed by fitting individual experimental data to a logistical curve to determine the maximal response and sensitivity. The curve was of the form $Y = \frac{Y_{\text{max}}}{1 + 10^{(X-X_{50})/X_{\text{slope}}}}$ where $X$ is the logarithm of the concentration and $Y$ is the response; the sensitivity values reported are derived from these fits. The contractile response to KCl was expressed in mN/mm as units of arterial wall tension (AWT) ($\text{AWT} = \text{force/2 × length of vessel}$). For each arterial segment, the maximal response to KCl was calculated, and the response to contractile agonists was expressed as percent of the maximal contraction to phenylephrine in isolated UA from SD (●; n = 7) and TgA (○; n = 9) animals. Parallel experiments in intact arteries (A), denuded arteries (B), and intact arteries preincubated with 10⁻³ M indomethacin (C) or 10⁻⁴ M N⁶-nitro-L-arginine methyl ester hydrochloride (*L*-NAME) (D) are shown. *P < 0.05 vs. SD.

**Response to potassium chloride (KCl).** After equilibration, to test the viability of the arterial preparations and determine the response to non-receptor-mediated contraction, UA segments were exposed to 75 mM KCl in KHB (Na⁺ replaced with K⁺ in KHB to get 75 mM K⁺) for 5 min, and after washing the stimulation was repeated twice.

**Response to acetylcholine.** Arteries were washed and stimulated with a submaximal dose of phenylephrine (PE) between 10⁻⁶ and 3 × 10⁻⁵ M to attain an equivalent level of contraction. A dose–response curve to acetylcholine (10⁻¹¹–10⁻⁵ M) was performed. In parallel experiments, different arterial segments were denuded or preincubated for 15 min with the COX inhibitor indomethacin (10⁻⁵ M) or the nitric oxide synthase inhibitor N⁶-nitro-L-arginine methyl ester hydrochloride (*L*-NAME; 10⁻⁴ M); denuded arteries showed <10% relaxation to acetylcholine.

**Response to PE.** After washing and resting for 20 min, UA segments were exposed to a cumulative concentration-response curve of PE by exposing arteries to 14 (10⁻⁸–10⁻⁴ M) increasing concentrations in fourth-log steps, with each subsequent dose being introduced only after a steady response had been reached. In parallel experiments; different arterial segments were denuded or preincubated for 15 min with the COX inhibitor indomethacin (10⁻⁵ M) or the nitric oxide synthase inhibitor *L*-NAME (10⁻⁴ M).

**Response to sodium nitroprusside.** After washing in KHB for ≥30 min, arterial segments were stimulated with PE 10⁻⁸M, and after a stable contraction was reached, increasing concentrations of sodium nitroprusside (SNP; 10⁻⁹–10⁻³ M) were added at 3-min intervals.

**Immunohistochemistry.** Immunostaining for α-actin, endothelial nitric oxide synthase (eNOS), and COX-2 in UA was performed as described (30, 35). Fixed arterial rings were deparaffinized and rehydrated, and unspecific binding sites were blocked with 10% normal goat serum, 0.1% Triton X-100, and 0.1% BSA in phosphate-buffered saline (PBS) for 30 min at room temperature. Sections were incubated with primary antibodies overnight as follows: monoclonal mouse anti-eNOS (cat. no. 610297; 1:100 dilution; BD Transduction Laboratories), polyclonal anti-COX-2 (cat no. 160126; 1:200 dilution; with a submaximal dose of phenylephrine (PE) between 10⁻⁵ B, and intact arteries preincubated with 10⁻³ M indomethacin (C) or 10⁻⁴ M N⁶-nitro-L-arginine methyl ester hydrochloride (*L*-NAME) (D) are shown. *P < 0.05 vs. SD in maximal response; **P < 0.05 vs. SD in sensitivity.
corresponding maximal response to KCl. Vasodilatory responses were expressed as percent of preconstricted tone. Sensitivity was expressed as pD2 (pD2 = −log [EC50]), with EC50 being the concentration of agonist producing 50% of the maximal response. Data are expressed as means ± SE. One-way analysis of variance with Bonferroni’s multiple comparisons was used to determine significant differences. P < 0.05 was accepted as an indication of statistical significance.

RESULTS

Response to stretch in UA from SD and TgA rats at early gestation. Figure 1 shows the resting wall tension-internal circumference relationship obtained for UA isolated from SD and TgA rats. At low levels of stretch, arteries from TgA showed a lower tension at equivalent internal circumference; the calculated values for the length constant β were lower in TgA rats compared with arteries from SD (P < 0.05; Fig. 1, inset). At the end of the curve (at high levels of stretch), similar values were observed, and consequently the calculated optimal diameters showed no differences between SD and TgA UA (317 ± 4 vs. 331 ± 14 μm, P > 0.05).

Resting and active tension in UA from SD and TgA at early gestation. Intact arteries from SD and TgA animals showed no difference in resting tension (0.98 ± 0.05 vs. 0.90 ± 0.07 mN/mm, P > 0.05). Active tension (maximal response to 75 mM KCl) was greater in intact arteries from TgA animals (4.56 ± 0.41 vs. 3.76 ± 0.28 mN/mm, P < 0.05), and this difference was abolished by endothelial denudation (3.1 ± 0.25 vs. 2.8 ± 0.7 mN/mm, P > 0.05).

Actin and collagen levels in UA from SD and TgA at early gestation. Immunolocalization of smooth muscle α-actin showed similar signal intensity in SD and TgA UA (P > 0.05; Fig. 2, B and D). Staining with Picro Sirus Red showed lower levels of collagen in TgA UA (Fig. 2F) compared with SD UA (P < 0.05; Fig. 2E).

Vasodilatory response to acetylcholine in UA from SD and TgA at early gestation. Arterial segments preconstricted with PE were incubated with increasing concentrations of acetylcholine (10⁻¹⁰–10⁻⁴ M) in half-log steps. Dose-dependent relaxations were observed in both groups. At 3 × 10⁻⁵ M acetylcholine, intact arteries from both groups reached maximal relaxations of 64 ± 8 and 75 ± 6% in SD and TgA UA, respectively (P > 0.05). At doses of acetylcholine greater than 3 × 10⁻⁶ M, intact arteries from TgA rats displayed a contraction (P < 0.05; Fig. 3A). Endothelial denudation completely abolished responses in both groups (Fig. 3B), whereas blockade of prostaglandin generation with indomethacin at 10⁻⁵ M eliminated the contraction in TgA UA, making responses in both groups similar (ACHMAX 78 ± 6 vs. 83 ± 11%, pD2 6.92 ± 0.16 vs. 7.08 ± 0.27, SD vs. TgA UA, respectively, P > 0.05, Fig. 3C). Blockade of NO production with L-NAME at 10⁻⁵ M abolished relaxation in arteries from SD control animals, with some residual relaxation still present in arteries from TgA animals (Fig. 3D).

Contractile response to PE in UA from SD and TgA at early gestation. Increasing concentrations of PE (10⁻⁸–10⁻⁴ M) applied in fourth-log steps dose-dependently increased contraction in both groups (Fig. 4). In SD UA, endothelium denudation or preincubation with indomethacin had no effect on contraction, whereas preincubation with L-NAME increased maximal contraction and sensitivity (P < 0.05; Fig. 4D and Table 1). In TgA UA, endothelium denudation (Fig. 4B) or preincubation with L-NAME (Fig. 4D) increased sensitivity to PE (P < 0.05; Table 1), whereas pre incubation with indomethacin (Fig. 4C) decreased it. Compared with SD, maximal contraction to PE was decreased in TgA denuded arteries and in arteries preincubated with indomethacin or L-NAME (Fig. 4, B–D, and Table 1).

Response to SNP in UA from SD and TgA animals at early gestation. Response to SNP in PE-preconstricted arteries showed lower maximal relaxation in TgA UA (P < 0.05; Fig. 5A and Table 2). This difference was abolished after endothelial denudation (Fig. 5B) or preincubation with indomethacin (Fig. 5C), whereas in L-NAME-preincubated vessels, the response to SNP in TgA UA showed a lower level of relaxation than in SD UA (P < 0.05; Fig. 5D and Table 2).

Immunostaining of COX-2 and eNOS in SD and TgA UA at early gestation. Immunolocalization of COX-2 and eNOS revealed the presence of both enzymes in UA. COX-2 signals

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Table 1. Contractile responses to phenylephrine in UA from SD and TgA rats at early gestation

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>TgA</th>
<th>Denuded</th>
<th>+Indomethacin</th>
<th>+L-NAME</th>
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<tbody>
<tr>
<td><strong>PEMAX, %KMAX</strong></td>
<td>159 ± 11</td>
<td>134 ± 12</td>
<td>165 ± 11</td>
<td>134 ± 8*</td>
<td>179 ± 16</td>
</tr>
<tr>
<td>pD2 (Log EC50)</td>
<td>6.09 ± 0.09</td>
<td>6.08 ± 0.06</td>
<td>6.15 ± 0.12</td>
<td>6.53 ± 0.10††</td>
<td>5.92 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE. UA, uterine artery; SD, Sprague-Dawley; TgA, transgenic; PEMAX, maximal contraction to phenylephrine; L-NAME, Nω-nitro-L-arginine methyl ester hydrochloride. PEC50 is expressed as %KMAX and sensitivity as pD2. Results are shown for intact denuded arteries and intact arteries preincubated with indomethacin (10⁻⁵ M) or L-NAME (10⁻⁴ M) as indicated. *P < 0.05 vs. SD; †P < 0.05 vs. intact.

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Fig. 5. Response to sodium nitroprusside in UA from SD and TgA rats. Relaxation to sodium nitroprusside in isolated UA from SD (•; control, n = 5) and transgenic TgA (○; n = 5) animals preconstricted with 10⁻⁵ M phenylephrine. Parallel experiments in intact arteries (A), denuded arteries (B), and intact arteries preincubated with 10⁻⁵ M indomethacin (C) or 10⁻⁴ M L-NAME (D) are shown. *P < 0.05 vs. SD in maximal response.
showed no differences between SD and TgA animals \((P<0.05;\) Fig. 7). This lower response in TgA UA suggests a cause for stiffening and buckling pressure (3, 9). Our observations of a particular, treatment with collagenase has been shown to affect the mechanical properties of the vascular wall, decreasing stiffness and buckling pressure (3, 9). These results are in agreement with a prostanoid-dependent endothelial dysfunction suggested for TgA UA on day 18 of gestation (32). In TgA at late gestation, Verlohren et al. (32) showed that the increased contraction to acetylcholine was prevented by blocking thromboxane receptors, which was in agreement with an important role that thromboxane receptors have in mediating some of the pathophysiological effects on Ang II-dependent hypertension (10).

Isolated TgA UA showed a lower response to stretch compared with SD UA. This response is critically dependent on the content of elastin and collagen of the arterial wall (9); in particular, treatment with collagenase has been shown to affect the mechanical properties of the vascular wall, decreasing stiffness and buckling pressure (3, 9). Our observations of a lower expression of collagen in TgA UA suggest a cause for this lower stretch response in TgA UA. This lower response may be a reflection of a greater distensibility of the arterial wall and related to the unexpectedly lower resistance index reported previously in the UA in this model at 18 and 21 days of gestation (14, 15).

The greater contraction to KCl we observed in TgA UA suggests that altered responses of the UA of TgA animals are already detected at early gestation in this model and agrees with the reported observation of a greater content of smooth muscle than controls in TgA spiral arteries at 18 days of gestation (14). At early gestation, however, immunostaining of \(\alpha\)-smooth muscle actin showed similar signals between SD and TgA UA. Moreover, the greater active contraction in TgA UA is not maintained after endothelium denudation, suggesting an additional endothelium-derived constrictor factor as responsible for this increased response in TgA UA at early gestation.

TgA UA relaxed to acetylcholine similarly to SD UA; however, higher doses of acetylcholine induced a contraction in TgA UA. Since this contraction is inhibited by preincubation with indomethacin, it appears to be mediated by cyclooxygenase-derived products. These results are in agreement with a prostanoid-dependent endothelial dysfunction suggested for TgA UA on day 18 of gestation (32). In TgA at late gestation, Verlohren et al. (32) showed that the increased contraction to acetylcholine was prevented by blocking thromboxane receptors, which was in agreement with an important role that thromboxane receptors have in mediating some of the pathophysiological effects on Ang II-dependent hypertension (10).

Our results indicate that this endothelial dysfunction may already be in place at early gestation in this model. TgA UA also displayed a small residual relaxation to acetylcholine after nitric oxide (NO) production blockade with \(\text{L-NAME}\), and thus, considering the similar relaxation in the presence of indomethacin, this observation suggests an increased contribution of an additional endothelium-derived factor in TgA UA. Non-NO nonprostaglandin relaxations are associated with an endothelium-derived hyperpolarization factor (EDHF), and this activity has been shown to play a role in the uterine contractility.

<table>
<thead>
<tr>
<th>SNPmax</th>
<th>Intact</th>
<th>TgA</th>
<th>pD2</th>
<th>Intact</th>
<th>TgA</th>
<th>TgA</th>
<th>+L-NAME</th>
<th>TgA</th>
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<tr>
<td>SD</td>
<td>92 ± 2</td>
<td>74 ± 5*</td>
<td>7.73 ± 0.26</td>
<td>7.32 ± 0.39</td>
<td>88 ± 2</td>
<td>83 ± 4</td>
<td>90 ± 3</td>
<td>84 ± 3</td>
</tr>
<tr>
<td>TgA</td>
<td>7.43 ± 0.18</td>
<td>7.95 ± 0.33</td>
<td>7.83 ± 0.29</td>
<td>7.91 ± 0.43</td>
<td>7.99 ± 0.26</td>
<td>7.56 ± 0.03</td>
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Values are means ± SE. SNP, sodium nitroprusside; SNPmax, maximal relaxation to sodium nitroprusside. SNPmax is expressed as loss of preconstricted tone (in %) and sensitivity as pD2. Results are shown for intact denuded arteries and intact arteries preincubated with indomethacin \((10^{-7} \text{M})\) or \(\text{L-NAME}\) \((10^{-5} \text{M})\) as indicated. *\(P<0.05\) vs. SD.

**DISCUSSION**

In this study, we showed that the UA from the hAGN \(\times\) hREN (TgA) rat displays functional alterations prior to the installation of the preeclampsic syndrome. We described an increased role for prostanoids in the regulation of in vitro vasodilatory and vasoconstrictor responses at early gestation in this model of hypertensive pregnancy.

The TgA rat is an established model that shares some of the characteristics of preeclampsia in humans, such as proteinuria, the presence of agonistic autoantibodies to the Ang II AT1 receptor, and hypertension in late pregnancy (4, 8). Previously, we reported that TgA rats displayed normal systolic blood pressure and increased Ang II contraction of UA at 7 days of gestation (30). This is consistent with the increased sensitivity to Ang II contraction as a marker for the development of preeclampsia (12, 31).

Isolated TgA UA showed a lower response to stretch compared with SD UA. This response is critically dependent on the content of elastin and collagen of the arterial wall (9); in particular, treatment with collagenase was detected in the endothelial layer (Fig. 6, E and F). Using the reciprocal intensity method, similar levels were observed in signal intensity for COX-2 or eNOS in both groups.

**Plasma values of TXB2.** Levels of TXB2 in plasma samples showed no differences between SD and TgA animals \((P>0.05;\) Fig. 7).

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**Fig. 6. Immunolocalization of cyclooxygenase-2 (COX-2) and endothelial nitric oxide synthase (eNOS) in UA from SD and TgA rats at early gestation.** UA segments from control \((n = 5)\) (SD; B and E) or TgA rats \((n = 5)\) (C and F) were incubated with anti-COX-2 (B and C) or anti-eNOS (E and F) antibodies. Signals were detected at early gestation. UA segments from control (A and TgA UA (D) did not contain primary antibodies. Representative pictures and average values of relative intensity units obtained by the reciprocal intensity method in each group are shown. Scale bar, 200 μm.
carnation, where K⁺ channel inhibitors eliminated residual uterine vasoconstriction in response to acetylcholine (7a, 11). Thus our results suggest a slightly increased EDHF activity in TgA UA.

The uterine circulation displays increased responses to α-adrenergic stimulation compared with the systemic circulation (21), and this adrenergic contraction in the UA depends on α₁-adrenergic receptors (18). A previous report in the TgA model indicated that contraction to PE was increased compared with SD UA at late gestation (32). Our observation of a similar contraction to PE in intact arteries of SD and TgA UA indicates that this difference appears later in gestation, consistent with the increased sensitivity to α-adrenergic stimulation in late pregnancy compared with arteries from nonpregnant animals (27), and may be related to the great degree of remodeling that this vascular bed endures during gestation (22). Contraction to PE was greatly affected by preincubation with indomethacin in TgA, lending further support to an increased role for prostanoids in the local vascular response in TgA UA at early gestation.

Endothelium-independent vasodilation, expressed as the response to the NO donor SNP, was lower in TgA UA, suggesting that the smooth muscle portion of the NO-dependent vasodilatory pathway is altered in TgA vessels. This effect appears to be dependent on endothelium and prostaglandin generation since it was absent in denuded arteries or intact arteries preincubated with indomethacin. The relaxation induced by NO donors is mediated by activation of the enzyme guanylyl cyclase, and reports indicate that this enzyme may be inhibited in the vasculature by endogenous NO (5, 23) or thromboxane receptor activation (1). It is conceivable that increased production of endothelium-derived prostaglandins is modulating endothelium-independent vasodilation in TgA UA.

In TgA rats, a role for thromboxane (32) and 5,6-epoxyeicosatrienoic acid (17) in modulating vascular responses has been described at late gestation, and increased CYP epoxide-nase expression has been proposed as a contributor to vascular dysfunction in this model (17). It is conceivable that circulating cytotoxic factors released by hypoperfusion and hypoxia caused by defective placentation in this model of preeclampsia would increase the expression of eicosanoid-generating pathways. TgA rats also have increased Ang II levels in the uteroplacental unit (6), and given the ability of Ang II to modulate prostaglandin levels in vascular tissues (2), these increased Ang II levels may induce increased expression of eicosanoid-generating enzymes, thus contributing to the vascular alterations we observed.

Our observation of similar plasma levels of the vasoconstrictor eicosanoid thromboxane between groups contrasts with the increased levels observed at late gestation in this model (32). This observation highlights the role of local more than systemic alterations at early gestation in this model of hypertensive pregnancy. How alterations in the different eicosanoid-generating pathways are modulating local responses of the uterine circulation in this model remains to be studied.

Figure 7. Plasma thromboxane levels in SD and TgA rats at early gestation. Plasma levels of thromboxane B2 (TXB₂) were measured in SD (open bars; n = 8) and TgA (closed bars; n = 10) rats.

**Perspectives and Significance**

The functional alterations in vascular function we observed in vitro at early gestation in the preeclamptic model hAGN × hREN suggested that uterine circulation is affected in this model before the establishment of the preeclamptic phenotype. The changes we observed on vasoconstricter and vasodilatory responses indicate an increased role of prostanoids in controlling uterine vascular tone at early gestation, and studies remain to be done to identify the role of these agents in vivo. Identification of the specific lipid mediators controlling vascular reactivity at early gestation will increase our understanding of the vascular alterations contributing to the development of a hypertensive pregnancy.

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**Disclosures**

The authors have no conflicts of interest, financial or otherwise, to declare.

**Author Contributions**


**References**


VASCULAR REACTIVITY AT EARLY GESTATION IN PREECLAMPSIA


- Bibliographic references for the text are not provided, but the text is a comprehensive review of vascular reactivity at early gestation in preeclampsia, covering various aspects of the disease from the molecular to the clinical level.