COX2 inhibition during nephrogenic period induces ANG II hypertension and sex-dependent changes in renal function during aging

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COX2 inhibition during nephrogenic period induces ANG II hypertension and sex-dependent changes in renal function during aging, Am J Physiol Renal Physiol 306: F534–F541, 2014. First published December 13, 2013; doi:10.1152/ajprenal.00535.2013.—This study was performed to test the hypothesis that ANG II contributes to the hypertension and renal functional alterations induced by a decrease of COX2 activity during the nephrogenic period. It was also examined whether renal functional reserve and renal response to volume overload and high sodium intake are reduced in 3–4- and 9–11-mo-old male and female rats treated with vehicle or a COX2 inhibitor during the nephrogenic period (COX2np). Our data show that this COX2 inhibition induces an ANG II-dependent hypertension that is similar in male and female rats. Renal functional reserve is reduced in COX2np-treated rats since their renal response to an increase in plasma amino acids levels is abolished, and their renal ability to eliminate a sodium load is impaired (P < 0.05). This reduction in renal excretory ability is similar in both sexes during aging but does not induce the development of a sodium-sensitive hypertension. However, the prolonged high-sodium intake at 9–11 mo of age leads to a greater proteinuria in male than in female (114 ± 12 μg/min vs. 72 ± 8 μg/min; P < 0.05) COX2np-treated rats. Renal hemodynamic sensitivity to acute increments in ANG II is unaltered in both sexes and at both ages in COX2np-treated rats. In summary, these results indicate that the reduction of COX2 activity during nephrogenic period programs for the development of an ANG II-dependent hypertension, reduces renal functional reserve to a similar extent in both sexes, and increases proteinuria in males but not in females when there is a prolonged increase in sodium intake.

COX2; sex- and aging-dependent changes; fetal programming; hypertension; renal function; renal reserve

THE INCREASE IN BLOOD PRESSURE (BP) and a deteriorated renal function as a consequence of an altered renal development are sex-dependent and probably related to the degree that renal development is affected (2, 6, 23, 25, 26, 29, 31, 36). The involvement of cyclooxygenase-2 (COX2)-derived metabolites in the regulation of renal morphogenesis has been demonstrated in studies showing that COX2 inhibition during the perinatal period reduces nephron endowment by 17% and induces the development of hypertension in both sexes but leads to a progressive deterioration of renal function in male, but not in female rats (26). It is also known that COX2-deficient mice show a thin nephrogenic cortex, immature glomeruli and tubuli, and a sex-dependent increment in proteinuria (35). However, the mechanism that is involved in the hypertension secondary to a decrease in COX2 activity during nephrogenic period remains unknown, as well as whether this decrease in COX2 activity induces sex- and aging-dependent alterations in the renal response to different stimuli.

One hypothesis tested in this study is that the renin-angiotensin system (RAS) plays an important role in the hypertension secondary to a reduced COX2 activity during the nephrogenic period. We also examine whether the modest reduction in nephron endowment elicited by this decrease in COX2 activity leads to a significant impairment in the renal ability to modify renal hemodynamics and to increase sodium excretion in response to appropriate stimuli, such as an increase in plasma amino acids (AA) levels or an acute volume expansion (VE). Whether the possible attenuation in the renal excretory ability is sex-dependent during aging and leads to the development of a sodium-sensitive hypertension is also examined. Another hypothesis tested is that renal sensitivity to ANG II is enhanced in hypertensive rats with a decrease in COX2 activity during renal development, being that this increment in renal ANG II sensitivity is sex-dependent during aging. Studies to examine whether the previous hypotheses are correct have been performed at 3–4 and/or 9–11 mo of age in rats treated with a COX2 inhibitor during the nephrogenic period (COX2np).

MATERIALS AND METHODS

Sprague Dawley (SD) rats were purchased from the University of Murcia Animal Research Laboratory. Protocols were designed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of the University of Murcia. Food (Harlan Teklad) and water were supplied ad libitum. Female SD rats (~230 g body wt) were placed with a male, taking day 0 of pregnancy the morning that sperm was found in the vaginal smear. Vehicle or a COX2 inhibitor (rofecoxib, 2 and 4 mg·kg⁻¹·day⁻¹) was given to dams from embryonic day 16 until delivery and to newborn pups during first three postnatal weeks, since nephrogenesis in rats takes place from midgestation until the second to third postnatal week (10). Solutions with and without rofecoxib were orally administered to pups at a rate of 0.96 μl/g body wt. At postnatal day 0, litter size was fixed (8–10) to ensure similar nourishment during the suckling period. Litters with <8 pups were excluded.

Arterial pressure measurement. Systolic blood pressure (SBP) was measured in conscious rats at 3–4 and 9–11 mo of age by the tail-cuff method (23–26,29) using a CODA 2 noninvasive system (Kent Scientific). To reduce stress and to obtain an accurate reading, rats were first habituated to the measurement device and to a temperature of 30°C for 10–15 min. Definitive measurements began when rats remained unperturbed into the chamber throughout the inflation-deflation cycles. The SBP value in each rat is the mean of at least 10 measurements taken during 2–3 days. The SBP values obtained with this method are correlated to those found in conscious freely moving rats through a femoral artery catheter (29).

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Renal function studies. After overnight fasting, rats were anesthetized with 0.1 ml of ketamine (Ketolar, Parke Davis; 100 mg/ml im) and 0.1 ml/100 g of pentobarbital (Pentothal, Abbott; 50 mg/ml ip). After tracheotomy, catheters were inserted into the bladder for collection of urine samples, as well as into the left femoral artery to measure mean arterial pressure (MAP) (PowerLab, ADInstruments) and for blood withdrawal. Another catheter was implanted into the left femoral vein for intravenous infusions. Then, rats were placed on a temperature-regulated surgical table to maintain a stable body temperature. To stabilize hematocrit level 1 ml/100 g body wt of 6% of BSA (Sigma) was infused. [3H]-inulin (2 μCi/ml; American Radiolabeled Chemicals) was given as an intravenous bolus (1 ml iv) and as an infusion (1.5 μCi/ml) dissolved in isotonic saline (1 ml/100 g body wt −1·h −1). A transit-time flow probe (Transonic Systems) was implanted on the left renal artery for renal blood flow (RBF) measurement. Renal plasma flow (RPF) changes were calculated considering RBF and hematocrit values. Urine samples were collected for measurements of urine flow rate (UV), [3H]-inulin, and urinary sodium excretion. Plasma samples were collected in heparinized capillaries 5 min before the end of each clearance period to measure [3H]-inulin and electrolyte concentrations. A 70-min stabilization period was allowed before experiments began.

Experimental protocols: blood pressure response to candesartan. This ANG II receptor antagonist (ARA) was administered by gavage (7 mg·kg −1·day −1) during 3 days to conscious 9–11-mo-old rats. Systolic BP was measured during renal function studies. This administration of candesartan reduced SBP (27 ± 2 mmHg) induced by an ANG II infusion (30 ng/kg iv) (n = 5).

Changes in plasma renin activity. Catheters were inserted into the femoral artery in anesthetized rats (isoflurane; Abbott) to obtain blood samples to analyze plasma renin activity (PRA) by using a commercial RIA kit for ANG I (Diasorin).

Renal response to AA infusion was examined in 3–4-mo-old rats. Two 20-min baseline clearance collections were followed by an intravenous infusion of a mixed 10% AA solution (3 ml/h). Five minutes after AA infusion was initiated, two 20-min clearances were obtained.

Renal response to acute VE was examined in 3–4- and 9–11-mo-old rats. Two 20-min baseline clearances were followed by a VE (6% body wt during 55 min) elicited by isotonic saline infusion. Two consecutive 20-min clearances were obtained during the last 40 min of VE.

Response to an increment in sodium intake. Three- to four- and nine- to eleven-mo-old rats were kept in individual metabolic cages to evaluate the changes in UV, glomerular filtration rate (GFR), and proteinuria during 24-h periods. After 2 days of adaptation, rats were maintained with a normal sodium diet (NSD) (0.4% Panlab) during 3 days. Then, the sodium diet was increased (8%) (Panlab) by seven consecutive days and decreased again to normal levels (0.4%) during 4 days (recovery period). Blood samples from the tail were obtained during renal function studies. This administration of AA increased SBP to similar levels in COX2-treated and control rats, this fall being greater (P < 0.05) vs. control group of the same sex. Candesartan administration decreased SBP to similar levels in COX2-treated and control rats, this fall being greater (P < 0.05) vs. control group of the same sex.

RESULTS

Blood pressure response to candesartan. SBP was higher (P < 0.05) in male (135 ± 2 mmHg) and female (131 ± 2 mmHg) COX2-treated rats than in male (117 ± 1 mmHg) and female (119 ± 1 mmHg) control rats at 9–11 mo of age (Fig. 1). Candesartan administration decreased SBP to similar levels in COX2-treated and control rats, this fall being greater (P < 0.05) in COX2-treated (male: 36 ± 4 mmHg; female: 31 ± 4 mmHg) than in control (male: 16 ± 1 mmHg; female: 20 ± 2 mmHg) rats.

Changes in PRA. PRA (in ng ANG I·ml −1·h −1) (n = 6–8 rats/group) was similar in control (males: 7.0 ± 1.5; females:

\[ \text{Candesartan (7 mg/kg/day)} \]

\[ \text{COX2np} \]

\[ \text{Basal} \]

\[ \text{1} \]

\[ \text{2} \]

\[ \text{3} \]

\[ \text{Days} \]

\[ \text{SBP (mmHg)} \]

\[ \text{90} \]

\[ \text{120} \]

\[ \text{150} \]

\[ \text{180} \]

\[ \text{210} \]

\[ \text{240} \]

\[ \text{Control} \]

\[ \text{•} \]

\[ \text{COX2np} \]

\[ \text{*} \]

\[ \text{*} \]

\[ \text{*} \]

\[ \text{*} \]

Fig. 1. Systolic blood pressure (SBP) changes elicited by three consecutive days’ administration of an AT1 receptor antagonist to conscious 9–11-mo-old rats treated with vehicle (control) or a COX2 inhibitor during nephrogenic period (COX2np). Top: SBP changes in males (n = 6 control; n = 8 COX2np). Bottom: SBP changes in females (n = 6 control; n = 6 COX2np). *P < 0.05 vs. basal period. #P < 0.05 vs. control group of the same sex.
Renal effects induced by perinatal COX2 inhibition

Females

COX2np males: 8.7

fractional excretion of sodium (FeNa) (1.16 ± 0.52%; 6.6 ± 0.10 to 2.86 ± 0.26%, P < 0.05) in control females. Figure 2 also shows that renal hemodynamic was unaffected by AA infusion in COX2np-treated females. As occurred in COX2np-treated males, UV and FeNa were also similar before (0.03 ± 0.01 μl·min⁻¹·g⁻¹ and 1.72 ± 0.29%, respectively) and after (0.04 ± 0.00 μl·min⁻¹·g⁻¹ and 1.86 ± 0.23%, respectively) AA infusion in COX2np-treated females.

Renal response to acute VE. Table 1 shows the renal response to a VE in control and COX2np-treated rats at 3–4 mo of age. MAP was similar in both sexes of vehicle- and COX2np-treated rats and did not change during VE. Basal GFR and RPF were similar in both sexes of control and COX2np-treated rats and did not change in response to the acute VE. No differences in FeNa and UV were found between groups during the basal period. VE led to an increment (P < 0.05) of FeNa and UV in control and COX2np-treated rats, but these increments of FeNa and UV were lower (P < 0.05) in male (37% and 46%, respectively) and female (39% and 41%, respectively) COX2np-treated than in their respective control group. The reduction in the excretory response to VE was similar in both sexes of COX2np-treated rats.

Renal responses to VE in 9–11-mo-old rats are shown in Table 2. As occurred at the younger age, MAP was similar before and after VE in each group of rats at 9–11 mo of age. Basal GFR and RPF were similar in each group of rats and did not change during VE. Both FeNa and UV were also similar during basal period and increased during VE in both groups of male and female rats at the older age. However, the renal excretory ability to eliminate the sodium load was impaired (P < 0.05) in both sexes of COX2np-treated rats with respect to that found in their age-matched control rats (Table 2).

Response to an increment in sodium intake. Fig. 3 shows the SBP and proteinuria during normal and high sodium intake in control and COX2np-treated rats at 3–4 mo of age. It can be observed that basal SBP was elevated (P < 0.05), but basal proteinuria was unchanged in both sexes of COX2np-treated rats with respect to the values in their respective control group. SBP and proteinuria did not change in response to a HSD at 3–4 mo of age in both groups of control and COX2np-treated rats (Fig. 2). Creatinine clearance was also similar during NSD and HSD in control males (0.71 ± 0.02 and 0.80 ± 0.02 ml/min, respectively), control females (0.81 ± 0.04 and 0.80 ± 0.07 ml/min, respectively), COX2np-treated males (0.76 ± 0.04 and 0.71 ± 0.07 ml/min, respectively) and COX2np-treated females (0.78 ± 0.03 and 0.67 ± 0.01 ml/min, respectively). SBP, proteinuria, and creatinine clearance remained during the recovery period at similar levels to those found during basal period. Food intake was similar in both sexes of control and COX2np-treated rats during NSD, HSD, and when sodium intake was again reduced to normal levels.

7.7 ± 2.4) and COX2np-treated (males: 9.0 ± 1.5; females: 6.6 ± 0.8) rats at 3–4 mo of age. Also, no significant differences in PRA were found between groups at 9–11 mo of age (control males: 5.8 ± 1.1; control females: 8.4 ± 1.4; COX2np males: 8.7 ± 1.3; COX2np females: 9.5 ± 2.8).

Renal response to AA infusion. This infusion did not modify MAP in any experimental group. Figure 2 shows the renal hemodynamic responses to AA infusion in both groups of control and COX2np-treated rats. Basal renal hemodynamic and excretory functions were not different in control and COX2np-treated rats. The AA infusion led to a renal vasodilation and hyperfiltration in control male rats since it induced an elevation in GFR (1.32 ± 0.10 to 1.93 ± 0.16 ml·min⁻¹·g⁻¹; P < 0.05) and RPF (3.92 ± 0.29 to 5.01 ± 0.36 ml·min⁻¹·g⁻¹; P < 0.05). The AA infusion also elicited an increment in UV (0.03 ± 0.01 to 0.05 ± 0.01 μl·min⁻¹·g⁻¹; P < 0.05) and fractional excretion of sodium (FeNa) (1.16 ± 0.39 to 1.69 ± 0.52%; P < 0.05) in these male rats. Contrary to that found in control male rats, AA infusion to COX2np-treated male rats did not induce significant changes in GFR and RPF (Fig. 2). Both UV and FeNa were also not significantly different before and after (0.05 ± 0.01 μl·min⁻¹·g⁻¹ and 1.50 ± 0.17%, respectively) AA infusion in COX2np-treated males. In contrast to the renal hemodynamic response to AA infusion in control males, this infusion did not elicit changes in GFR and RPF in control females (Fig. 2). Nevertheless, AA infusion did induce an increment in both UV (0.03 ± 0.00 to 0.05 ± 0.00 μl·min⁻¹·g⁻¹; P < 0.05) and FeNa (1.48 ± 0.13 to 2.86 ± 0.26%, P < 0.05) in control females. Figure 2 also shows that renal hemodynamic was unaffected by AA infusion in COX2np-treated females. As occurred in COX2np-treated males, UV and FeNa were also similar before (0.03 ± 0.01 μl·min⁻¹·g⁻¹ and 1.72 ± 0.29%, respectively) and after (0.04 ± 0.00 μl·min⁻¹·g⁻¹ and 1.86 ± 0.23%, respectively) AA infusion in COX2np-treated females.
Table 1. Renal response to an acute volume expansion at 3–4 mo of age in rats treated with vehicle (control) or a COX2 inhibitor during nephrogenic period (COX2np)

<table>
<thead>
<tr>
<th></th>
<th>Control n = 7</th>
<th>COX2np n = 8</th>
<th>Control n = 9</th>
<th>COX2np n = 10</th>
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<tr>
<td><strong>GFR, ml·min⁻¹·g⁻¹ kw</strong></td>
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<tr>
<td>Basal</td>
<td>1.51 ± 0.11</td>
<td>1.57 ± 0.08</td>
<td>1.42 ± 0.07</td>
<td>1.60 ± 0.09</td>
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<td>Vol. expansion</td>
<td>1.58 ± 0.10</td>
<td>1.63 ± 0.07</td>
<td>1.47 ± 0.06</td>
<td>1.59 ± 0.07</td>
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<tr>
<td><strong>RPF, ml·min⁻¹·g⁻¹ kw</strong></td>
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<tr>
<td>Basal</td>
<td>4.39 ± 0.22</td>
<td>4.27 ± 0.22</td>
<td>4.05 ± 0.09</td>
<td>4.03 ± 0.29</td>
</tr>
<tr>
<td>Vol. expansion</td>
<td>4.35 ± 0.24</td>
<td>4.40 ± 0.21</td>
<td>4.10 ± 0.15</td>
<td>3.98 ± 0.19</td>
</tr>
<tr>
<td>FE(\text{Na}, %)</td>
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</tr>
<tr>
<td>Basal</td>
<td>1.38 ± 0.15</td>
<td>1.27 ± 0.31</td>
<td>2.08 ± 0.43</td>
<td>1.98 ± 0.22</td>
</tr>
<tr>
<td>Vol. expansion</td>
<td>8.02 ± 0.28*</td>
<td>5.47 ± 0.81*#</td>
<td>9.17 ± 0.61*</td>
<td>6.27 ± 0.47*#</td>
</tr>
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<td>UV, µl·min⁻¹·g⁻¹ bw</td>
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<td></td>
<td></td>
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<tr>
<td>Basal</td>
<td>0.18 ± 0.04</td>
<td>0.10 ± 0.02</td>
<td>0.20 ± 0.05</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>Vol. expansion</td>
<td>0.81 ± 0.03*</td>
<td>0.44 ± 0.06*#</td>
<td>0.89 ± 0.06*</td>
<td>0.56 ± 0.05*#</td>
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</table>

GFR, glomerular filtration rate; RPF, renal plasma flow; Fe\(\text{Na}\), fractional excretion of sodium; UV, Urinary flow rate; kw, kidney weight; bw, body weight; Vol., volume. *\(P < 0.05\) vs. basal. #\(P < 0.05\) vs. control.

Figure 4 shows the effects of the prolonged increment in sodium intake on SBP and proteinuria at 9–11 mo of age. Basal SBP was elevated in male and female COX2np-treated rats with respect to the values found in control rats, being the increment similar to that found at 3–4 mo of age. As occurred at the younger age (Fig. 3), the prolonged HSD did not elicit a further elevation of SBP in any group of the 9–11-mo-old rats (Fig. 4). During NSD, proteinuria was slightly greater in COX2np-treated than in control male rats and similar in both groups of female rats. The HSD led to an elevation in proteinuria in each group at the oldest age examined in this study. Proteinuria during HSD, and the % elevation of proteinuria with respect to the basal period, were greater (\(P < 0.05\)) in male COX2np-treated rats (114 ± 12 µg/min and 59%, respectively) than in female COX2np-treated rats (72 ± 8 µg/min and 33%, respectively) and greater than in male (68 ± 3 µg/min and 24%, respectively) and female (57 ± 8 µg/min and 25%, respectively) control rats. When sodium intake was reduced to normal levels, proteinuria returned to levels not significantly different to those found during the basal period. Creatinine clearance was similar during NSD and HSD in control males (1.10 ± 0.12 and 1.20 ± 0.015 ml/min, respectively), control females (0.71 ± 0.07 and 0.76 ± 0.09 ml/min, respectively), COX2np-treated males (0.94 ± 0.12 and 1.18 ± 0.12 ml/min, respectively), and COX2np-treated females (0.84 ± 0.04 and 0.78 ± 0.03 ml/min, respectively) at 9–11 mo of age. Creatinine clearance remained unchanged in each group when sodium intake decreased to normal levels. Food intake was similar in both sexes of control and COX2np-treated rats throughout the experiment.

Renal hemodynamic response to acute ANG II infusion.

ANG II infusion led to an increment (\(P < 0.05\)) in MAP that was not statistically different in both groups of male (control: 10 ± 1 mmHg; COX2np: 8 ± 2 mmHg) and female (control: 8 ± 2 mmHg; COX2np: 12 ± 4 mmHg) rats at 3–4 mo of age. Similar changes in MAP were found after ANG II infusion at 9–11 mo of age. Renal hemodynamic parameters during basal period were similar in both sexes of control and COX2np-treated rats at 3–4 mo and did not change between the ages examined (Table 3). The ANG II infusion induced a significant decrease of RPF and GFR in each experimental group. No significant differences between control and COX2np in both sexes and at both ages were found in the renal hemodynamic response to ANG II (Table 3).

Table 2. Renal response to an acute volume expansion at 9–11 mo of age in rats treated with vehicle (control) or a COX2 inhibitor during nephrogenic period

<table>
<thead>
<tr>
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<th>Control n = 7</th>
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<th>Control n = 9</th>
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<tr>
<td>Basal</td>
<td>1.43 ± 0.08</td>
<td>1.28 ± 0.07</td>
<td>1.48 ± 0.09</td>
<td>1.47 ± 0.13</td>
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<tr>
<td>Vol. expansion</td>
<td>1.45 ± 0.06</td>
<td>1.32 ± 0.07</td>
<td>1.49 ± 0.09</td>
<td>1.43 ± 0.15</td>
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<tr>
<td><strong>RPF, ml·min⁻¹·g⁻¹ kw</strong></td>
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</tr>
<tr>
<td>Basal</td>
<td>4.43 ± 0.21</td>
<td>3.95 ± 0.35</td>
<td>4.16 ± 0.15</td>
<td>4.64 ± 0.35</td>
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<tr>
<td>Vol. expansion</td>
<td>4.63 ± 0.26</td>
<td>4.19 ± 0.52</td>
<td>4.29 ± 0.15</td>
<td>5.12 ± 0.31</td>
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<tr>
<td>FE(\text{Na}, %)</td>
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</tr>
<tr>
<td>Basal</td>
<td>0.69 ± 0.15</td>
<td>0.78 ± 0.10</td>
<td>1.22 ± 0.33</td>
<td>1.20 ± 0.20</td>
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<tr>
<td>Vol. expansion</td>
<td>6.76 ± 0.12*</td>
<td>5.34 ± 0.60*#</td>
<td>7.10 ± 0.76*</td>
<td>4.74 ± 0.25*#</td>
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<td>UV, µl·min⁻¹·g⁻¹ bw</td>
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<tr>
<td>Basal</td>
<td>0.07 ± 0.02</td>
<td>0.05 ± 0.01</td>
<td>0.10 ± 0.02</td>
<td>0.08 ± 0.01</td>
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<tr>
<td>Vol. expansion</td>
<td>0.65 ± 0.07*</td>
<td>0.42 ± 0.10*#</td>
<td>0.62 ± 0.07*</td>
<td>0.33 ± 0.05*#</td>
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COX2np, COX2 inhibitor during the nephrogenic period. *\(P < 0.05\) vs. basal. #\(P < 0.05\) vs. control.
This study reveals that the reduction of COX2 activity during nephrogenic period programs the development of an ANG II-dependent hypertension and reduces renal functional reserve since the renal vasodilatory and excretory responses to increments in plasma AA concentration are abolished and the renal ability to eliminate an acute sodium load is blunted.

Contrary to what has been shown in studies in which renal development is altered by affecting other mechanisms (23, 29), the impairment in renal function secondary to COX2np is not associated with an increase in the renal sensitivity to ANG II or in the development of an aging-dependent sodium-sensitive hypertension. New evidence is given supporting that renal injury is greater during aging in males than in females as a consequence of a reduction in COX2 activity during the nephrogenic period since proteinuria, a hallmark of renal damage, increases more in males than in females when sodium intake is enhanced.

The BP increases in COX2np-treated rats are similar to those reported in a previous study (26), which also shows important aging-dependent deterioration in renal function and renal structure in male, but not in female, COX2np-treated rats. However, the mechanism involved in this BP increase was unknown. Considering the well-known inverse relationship between nephron endowment and the risk to develop hypertension (2, 36), it can be suggested that the higher BP in COX2np-treated rats is mainly secondary to a 17% decrease in nephron number (26) but could also be a consequence of other renal changes elicited by COX2 inhibition during renal development (21). The hypothesis that ANG II is involved in maintaining this increment in BP was supported by studies showing that RAS plays an important role in the hypertension found in several models with a reduced nephron endowment (7, 10, 19, 25, 27, 29). Our findings are consistent with the hypothesis that ANG II contributes to maintaining BP elevated, even when there is a modest alteration in renal development. It was also examined whether this involvement was greater in males than in females.
because ANG II effects are modulated by sex hormones (22). A previous study in another experimental model with an altered renal development has shown that the role of ANG II in maintaining BP elevated is greater in males than in females (23). The absence of differences between both sexes of COX2np-treated rats in the decrease of BP elicited by candesartan could be explained by a small increment of ANG II effects in these rats.

This study also investigates whether the involvement of the RAS in maintaining BP elevated in COX2np-treated rats is secondary to an increase in PRA. Contradictory data have been reported with respect to changes in PRA and other components of the RAS in the hypertension secondary to different “insults” during renal development (4, 10, 13). These different results may be explained by the multiplicity of models employed and the sex and age at which the studies were performed. Only two studies have examined whether there are changes in PRA during aging in animals with an altered renal development and whether these changes are sex-dependent, but the results reported are also contradictory (13, 23). One possibility tested was that PRA is greater in males than in females, since it has been shown that PRA levels are modulated by sex hormones (22). PRA results were obtained in samples collected from anesthetized animals, as in other previous studies (4, 13, 23) and show that PRA is not elevated in COX2np-treated rats. Our data suggest that the regulation of PRA is altered in these rats because an increased BP per se would be expected to reduce renin release (16). The involvement of ANG II in maintaining BP elevated in the absence of changes in PRA could be explained by changes in other components of the RAS in resistance vessels and/or in renal tubules, such as AT1 and AT2 receptors and angiotensin-converting enzyme (ACE) activity (4, 6, 7, 14, 23, 27, 30). Further studies are needed to assess whether the involvement of ANG II in the BP increase in COX2np-treated rats is related to changes in oxidative stress and/or in renal sympathetic activity, as occurs in other experimental models of developmental programming (15, 23).

The absence of renal hemodynamic changes in both sexes of COX2np-treated rats at 3–4 mo of age was expected since the results shown are similar to those previously reported (26). Total GFR and RBF are not different to those found in control rats, but glomerular pressures and flows are most probably elevated since these rats have a 17% reduction in nephron endowment (26). This study examines whether these possible changes in glomerular pressures and flows modify renal functional reserve to an extent important enough to reduce the renal responses to stimuli that induce a vasodilatory response and/or an increase in renal excretory ability. A previous study has shown that a 37% reduction in nephron endowment elicited by a decrease in ANG II effects prevents the renal changes elicited by an increase in plasma AA levels (12). The hypothesis was that a small alteration in nephron endowment would not be enough to reduce renal functional reserve or that this reduction in renal reserve would be evident in males but not in females. This latter possibility was supported by studies showing that the effects elicited by a severe maternal protein restriction leads to similar changes in adult male and female offspring (13), but the effects elicited by a modest maternal protein restriction are only evident in male offspring (34).

The current study reveals that a modest decrease in nephron endowment reduces renal functional reserve to the extent that the renal hemodynamic and excretory responses to an increase in the plasma AA levels are abolished. The renal responses to the increment of plasma AA in both sexes of control rats are similar to those reported (12). Further studies are needed to evaluate why the increase in plasma AA elicits an increase of RBF and GFR in male but not in female normotensive rats. One possible explanation of why GFR and RBF do not change in response to an increase in plasma AA levels in COX2np-treated male rats is that glomerular pressures and flows are already at a level that cannot increase more when submitted to a vasodilatory stimulus. Blockade of the renal hemodynamic response to an increase in plasma AA could also be secondary to the elevated glomerulosclerosis index and glomerular volumes in these rats (26) and to an alteration of the mechanisms involved in regulating this response (28, 33). The mechanisms responsible for the blockade of the excretory response to the increment in AA in both sexes of COX2np-treated rats are unknown, but it may be speculated that this blockade is a consequence of the tubular effects elicited by reducing COX2 activity during the nephrogenic period (35). The renal excretory response to AA in COX2np-treated rats may also be

### Table 3. Renal hemodynamic response to ANG II infusion in 3–4- and 9–11-mo-old rats treated with vehicle (control) or COX2np

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>ANG II</th>
<th>Basal</th>
<th>ANG II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal plasma flow, ml·min⁻¹·g⁻¹ kw</td>
<td>4.72 ± 0.31</td>
<td>4.44 ± 0.20</td>
<td>4.89 ± 0.60</td>
<td>4.93 ± 0.64</td>
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<td>Glomerular filtration rate, ml·min⁻¹·g⁻¹ kw</td>
<td>3.59 ± 0.18</td>
<td>3.02 ± 0.17</td>
<td>2.51 ± 0.28</td>
<td>2.73 ± 0.22</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal plasma flow, ml·min⁻¹·g⁻¹ kw</td>
<td>1.30 ± 0.17</td>
<td>1.51 ± 0.20</td>
<td>1.30 ± 0.16</td>
<td>1.16 ± 0.11</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml·min⁻¹·g⁻¹ kw</td>
<td>0.99 ± 0.09</td>
<td>0.98 ± 0.17</td>
<td>0.99 ± 0.12</td>
<td>0.92 ± 0.09</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal plasma flow, ml·min⁻¹·g⁻¹ kw</td>
<td>4.47 ± 0.76</td>
<td>4.35 ± 0.54</td>
<td>4.59 ± 0.35</td>
<td>4.16 ± 0.49</td>
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<tr>
<td>Glomerular filtration rate, ml·min⁻¹·g⁻¹ kw</td>
<td>3.21 ± 0.52</td>
<td>3.09 ± 0.34</td>
<td>3.10 ± 0.21</td>
<td>3.17 ± 0.35</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. basal period.
secondary to an increase in ANG II since the hypertension is ANG II-dependent. It is also known that a mild elevation in ANG II reduces renal excretory ability by increasing proximal sodium reabsorption (20) and that proximal reabsorption is elevated in different models with a reduced nephron endowment (3).

The results of this study indicate that a moderate alteration in renal development also reduces the natriuretic and diuretic response to an acute VE in young adult rats of both sexes and to a similar extent that the decrease in the renal excretory ability observed when renal development is altered by reducing ANG II effects (12, 18). We predicted that this attenuation in the renal excretory ability would be enhanced in male but not in female COX2np-treated rats at 9–10 mo of age. This hypothesis was supported by results showing that tubulointerstitial damage increase between both ages in male but not in female COX2np-treated rats (26) and by results showing that an alteration in renal development elicited by decreasing ANG II leads to an accelerated age-dependent impairment of renal excretory ability only in male rats (12). The results obtained did not support our hypothesis since the reduced excretory ability to eliminate the acute VE at 3–4 mo of age was maintained but not enhanced at 9–11 mo of age. Our results do not allow us to determine the mechanisms involved in the reduced renal excretory ability, but it is speculated that it may be secondary to an elevation of ANG II effects since the hypertension is ANG II-dependent and ANG II plays an important role in modulating the renal response to an acute VE (20).

It was also expected that a HSD would exaggerate the hypertension in COX2np-treated rats because an increase in BP would be needed to maintain fluid and electrolyte balance at an advanced age (8, 32). In support of this hypothesis, it has also been shown that an alteration in renal development elicited by a decrease of ANG II effects during nephrogenic period leads to the development of an aging-dependent sodium-sensitive hypertension (29). Conversely, we found that the prolonged HSD did not elicit a further increment of BP in COX2np-treated rats, but our data suggest that even a modest reduction of renal reserve renders the aged kidney more susceptible to failure when other “secondary insults” are superimposed. The increment in proteinuria during HSD was greater in male than in female COX2np-treated rats at an advanced age (Fig. 4), and it is well accepted that proteinuria accelerates kidney disease progression to end-stage renal failure through multiple pathways (1). The sex-dependent progression of renal damage found in this study confirms results of our group showing that tubulointerstitial damage is greater in male than in female COX2np-treated rats at 9–11 mo of age (26). Our results provide new evidence that a sex-dependent mechanism is protecting females from the aging-dependent progression of renal damage that occurs as a consequence of a modest alteration in renal development. This mechanism is probably related to the greater glomerular hypertrophy and greater tubular damage found in male rats (26) and to a sex-dependent increment in the production of COX2-derived metabolites (24). Sex hormones may also be involved (22), but future studies need to examine their importance because it is unknown whether cycling is altered in animals with a developmental programming of hypertension and renal disease. Sex-dependent differences reported in this and other studies may also be secondary to mechanisms independent of sex steroids. This hypothesis is supported by studies showing that sex-dependent differences are evident long before sex hormones differ (6, 9).

The renal sensitivity to acute increments of ANG II in COX2np-treated rats and whether a possible greater renal sensitivity is sex- and/or age-dependent in these rats has also been examined. It is important since an increase in the renal sensitivity to ANG II may be involved in maintaining elevated BP when systemic RAS is not activated (16). Previous studies have shown that the renal hemodynamic effects elicited by ANG II are enhanced in animals with an adverse renal development (19, 23, 27), but only one of these studies has examined whether this sensitivity is different in both sexes and whether this sensitivity changes during aging (23). That previous study showed that renal AT1 receptor sensitivity is similarly enhanced in both sexes at an adult and an advanced age (23). However, it was unknown whether the renal sensitivity to acute increments in ANG II is enhanced when renal development is altered by a reduced COX2 activity. One possibility was that the renal ANG II effects would be sex- and/or age-dependent, since these effects are modulated by sex hormones (17, 19), and the role of ANG II in the regulation of cardiovascular and renal function increases with age (5). Renal sensitivity was examined in rats pretreated with captopril since ACE activity may be elevated in animals with reduced nephron endowment (30). The renal hemodynamic response observed during ANG II infusion to normotensive rats is similar to that reported in the only previous study that has examined whether this renal response is sex-dependent during aging (23). Contrary to what was expected, the renal response to ANG II infusion was not enhanced in both sexes of 3–4-mo-old rats with an altered renal development elicited by a decreased COX2 activity. It was also found that the renal hemodynamic response remained similar to that observed in normotensive rats when examined at an advanced age (9–11 mo of age). This study presents the novel finding that, contrary to what occurs when renal development is altered by affecting other mechanisms (19, 23, 27), the renal sensitivity to increments in ANG II is not enhanced at an adult or advanced age when renal development is altered by decreasing COX2 activity. However, we cannot rule out the possibility that a reduced COX2 activity early in life leads to changes in some components of the intrarenal RAS, such as ACE or AT1 and AT2 receptors, and in the postreceptor mechanisms involved in the renal response to ANG II.

Considering that human kidney development is completed before birth (3, 6), the results of this study suggest that an insult inducing a reduction of COX2 activity during the third trimester of pregnancy in humans would lead to the development of an ANG II-dependent hypertension, to the reduction of renal functional reserve even at a young adult age, and to progressive renal damage that would be greater in males than in females. The current study gives substantial support to the notion that stimuli eliciting a moderate alteration in renal development will induce a significant deterioration of renal function and an increment in arterial pressure during aging. These observations also emphasize the importance of not affecting the mechanisms involved in the regulation of renal development.
REFERENCES