Role of angiotensin II in arterial pressure and renal hemodynamics in rats with altered renal development: age- and sex-dependent differences

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Reverte V, Tapia A, Baile G, Gambini J, Gíménez I, Llinas MT, Salazar FJ. Role of angiotensin II in arterial pressure and renal hemodynamics in rats with altered renal development. Am J Physiol Renal Physiol 304: F33–F40, 2013. First published October 24, 2012; doi:10.1152/ajprenal.00424.2012—Numerous studies have demonstrated that angiotensin II (ANG II) is involved in hypertension and renal changes occurring as a consequence of an adverse event during renal development. However, it was unknown whether this involvement is sex and age dependent. This study examines whether the increments in arterial pressure (AP) and in the renal sensitivity to ANG II are sex and age dependent in rats with altered renal development. It also evaluates whether the ANG II effects are accompanied by increments in AT1 receptors and oxidative stress. Experiments were performed in 3- to 4- and 10- to 11-mo-old rats treated with vehicle or an AT1 receptor antagonist (ARA) during the nephrogenic period. ARAnp-treated rats were hypertensive, but an age-dependent rise in AP was only found in males. Three days of treatment with candesartan (7 mg·kg−1·day−1) led to a fall of AP that was greater (P < 0.05) in male than in female 10- to 11-mo-old ARAnp-treated rats. Oxidated proteins were elevated (P < 0.05), and the decrease in AP elicited by candesartan was reduced (P < 0.05) when these rats are also treated with tempol (18 mg·kg−1·day−1). Hypertension was not maintained by an elevation of AT1 receptors in kidneys and mesenteric arteries. The acute renal hemodynamic response to ANG II (30 ng·kg−1·min−1) was similarly enhanced (P < 0.05) in both sexes of ARAnp-treated rats at 3–4 but not at 10–11 mo of age. Our results suggest that an adverse event during the nephrogenic period induces an ANG II-dependent increase in AP that is aggravated only in males during aging and that oxidative stress but not an increase in AT1 receptor contributes to the rise in AP. This study also shows that the renal sensitivity to ANG II is transitorily enhanced in both sexes of rats with altered renal development.

angiotensin II; sex- and age-dependent changes; fetal programming; hypertension; renal function; oxidative stress

IT IS WELL ACCEPTED THAT AN adverse event during the perinatal period predisposes an individual to the development of hypertension and renal disease later in life. The mechanisms involved in the development of hypertension and renal disease have been examined in several models of fetal programmed hypertension. Some of these models, such as those induced by the administration of glucocorticoids or a low protein intake to the pregnant mother, or by the reduction in uterine perfusion, have in common a decrease in renin-angiotensin system (RAS) activity during renal development (7, 11, 47). The importance of this decrease in the development of hypertension and renal disease later in life is supported by studies in which a converting enzyme inhibitor (CEI) or an AT1 receptor antagonist (ARA) is administered during the nephrogenic period (7, 17–19, 32, 34, 38). The development of hypertension and renal disease in most of these experimental models is clearly sex dependent (7, 10, 22, 35, 44).

Although other mechanisms seem to be also involved (7, 10, 27, 29), the RAS plays an important role in the rise of arterial pressure (AP) and in the alterations of renal function that occur at an adult age in the fetal programming models currently used (2, 7, 14, 16, 20, 38). This involvement has been reported in studies showing that AP decreases to normal levels when a CEI or an ARA is administered (2, 7, 14, 38) and that several components of the RAS are activated (2, 15, 20, 37). One hypothesis tested in this study was that the importance of ANG II in maintaining hypertension is sex and age dependent in rats with an adverse event during renal development. It was also expected that the possible greater involvement of ANG II on AP elevation in males is accompanied by an increase in AT1 receptor expression in resistance vessels, and by a greater increment in oxidative stress. Our hypothesis was based on studies showing that the ANG II effect increases with age (4), are modulated by sex hormones (5, 9, 26–28, 30, 48), and are mediated by changes in oxidative stress (31, 49). An increase in the renal sensitivity to ANG II effects has also been demonstrated in male animals with fetal programmed hypertension (28, 37). However, it was unknown whether this increase in the renal sensitivity to ANG II is sex dependent and changes similarly in both sexes during ageing. It was also unknown whether the renal hemodynamic effects of ANG II are accompanied by changes in renal AT1 receptor expression. Thus one aim of this study was to examine whether there are sex- and age-dependent differences in the renal sensitivity to ANG II in rats with an altered renal development. It was also evaluated whether these possible differences in the renal hemodynamic response to ANG II are associated with changes in renal AT1 receptor expression or in oxidative stress levels.

MATERIALS AND METHODS

Sprague-Dawley (SD) rats were purchased from the University of Murcia Animal Research Laboratory. The study was approved by the University review committee, and experimental protocols were designed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Food (Harlan Teklad) and water were supplied ad libitum. Female SD rats (~230 g body wt) were placed with males, taking as day 0 of pregnancy the morning that sperm evidence was found in the vaginal smear. At postnatal day 0, litter size was fixed (8–10) to ensure similar nourishment during the

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Experimental Protocols

Arterial pressure response to candesartan (with and without tempol). This ARA was administered by gavage (7 mg·kg⁻¹·day⁻¹) for 3 days to conscious rats. Systolic arterial pressure (SAP) was measured during the basal period (3 days) and 3 h after each ARA administration. The numbers of rats at 3–4 mo of age were as follows: five control males, five control females, five ARA antagonist (ARAnp)-treated males, and five ARAnp-treated females. The numbers of rats at 10–11 mo of age were as follows: seven control males, seven control females, six ARAnp-treated males, and seven ARAnp-treated females. To examine whether the fall in SAP elicited by candesartan was related to the ANG II effects on oxidative stress, another set of experiments was performed in which candesartan was administered by gavage to rats already treated with tempol, a scavenger of superoxide anions (39). After a basal period of 3 days, tempol was administered in the drinking water (18 mg·kg⁻¹·day⁻¹) for 7 consecutive days to 10–11 mo-old conscious rats. Four days after the initiation of tempol administration, candesartan was simultaneously given (7 mg·kg⁻¹·day⁻¹) for 3 days. SAP levels were measured during the basal period (3 days) and each day that tempol was given. The numbers of rats in each group were the following: eight control males, eight control females, eight ARAnp-treated males, and seven ARAnp-treated females.

Arterial pressure was measured in conscious rats by the tail-cuff method as described (17, 18, 34, 35, 38) using a CODA 2 noninvasive system (Kent Scientific). To reduce the stress and to obtain an accurate reading, rats were first habituated during several days to the measurement device and to an ambient temperature of 30°C for 10–15 min. Definitive measurements began when rats remained unperturbed in the chamber throughout the inflation-deflation cycles. The SAP values in each rat are the mean value of at least 10 measurements taken over 2–3 days. In previous studies (3, 38), it was found that the SAP values obtained using the tail-cuff method are highly correlated with those obtained in conscious freely moving animals using other methods (radiotelemetry and intra-arterial devices).

Changes in plasma renin activity, AT₁ receptor expression, and oxidized protein levels. Catheters were inserted into the femoral artery (isoflurane, Abbott) to make catheters were inserted into the bladder for collection of urine samples and into the left femoral artery to measure mean arterial pressure (MAP, PowerLab, ADInstruments) throughout the experiment and for blood withdrawal. Then, one catheter was implanted in the left femoral vein for intravenous (iv) infusions. Rats were placed on a temperature-regulated surgical table to maintain a stable body temperature. To stabilize hematocrit level after surgical stress, 1 ml/100 g body wt of 6% of bovine serum albumin (Sigma) was infused.

\[ \text{[H]inulin (2 µCi/ml)} \]

American Radiolabeled Chemicals was given as an iv bolus (1 ml) and as a continuous infusion (1.5 µCi/ml) dissolved in isotonic saline (1 ml/100 g body wt·h⁻¹). A transistime flow probe (Transonic Systems) was implanted in the left renal artery for renal blood flow (RBF) measurement. Renal plasma flow (RPF) changes were calculated by considering RBF and hematocrit values. A 70-min stabilization period was allowed before experiments began. Two 20-min basal clearance periods were followed by an iv infusion of captopril (10 ng·kg⁻¹·min⁻¹). In previous experiments, it was found that this infusion does not modify the glomerular filtration rate (GFR) but leads to an increment (P < 0.05) of RPF in control (17.4 ± 1.5%) and ARAnp-treated (14.7 ± 2.0%) rats. An iv ANG II infusion (30 ng·kg⁻¹·min⁻¹) was started 30 min after captopril administration began. Fifteen minutes after initiation of ANG II infusion, two more 20-min clearances were obtained. Renal ANG II effects were examined in captopril-treated rats to exclude that the differences found in these effects were secondary to differences in endogenous ANG II levels. GFR was measured by clearances of [³H] inulin. Urine samples were collected into preweighed vials for [³H] inulin measurements. Urine flow rate (UV) was determined gravimetrically. Blood samples were collected in heparinized capillaries 5 min before the end of each clearance period to measure plasma [³H]inulin. The numbers of rats at 3–4 mo of age were as follows: nine control males, eight control females, eight ARAnp-treated males, and eight ARAnp-treated females. The numbers of rats at 10–11 mo of age were as follows: seven control males, eight control females, nine ARAnp-treated males, and nine ARAnp-treated females.

Statistical Analysis

Data in the text, table, and figures are given as means ± SE. Data from both clearances during each period were averaged for comparison. Differences between experimental periods within one group were evaluated using ANOVA for repeated measures and Fisher’s test. Differences between groups were examined with the use of ANOVA and Fisher’s test.

RESULTS

Arterial Pressure Response to Candesartan (With and Without Tempol)

SAP pressure was higher (P < 0.05) in male (135 ± 2 mmHg) and female (133 ± 1 mmHg) ARAnp-treated than in male (121 ± 1 mmHg) and female (115 ± 1 mmHg) control

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rats at 3–4 mo of age. At this age, candesartan reduced \((P < 0.05)\) SAP to similar levels in ARAnp-treated and control rats, with the fall in SAP greater \((P < 0.05)\) in ARAnp-treated than in control rats. Arterial pressure increased significantly between 3–4 and 10–11 mo of age in male \((135 \pm 2 \text{ to } 167 \pm 8 \text{ mmHg})\) but not in female \((133 \pm 1 \text{ to } 139 \pm 1 \text{ mmHg})\) ARAnp-treated rats. Figure 1 shows the response of SAP to candesartan alone (7 mg·kg\(^{-1}\)) and with the simultaneous infusion (30 ng/kg). It was also found that SAP does not change between days 4 and 7 when tempol is administered alone (18 mg·kg\(^{-1}·\text{day}^{-1}\)) in the drinking water for 7 consecutive days \((n = 4 \text{ in each group})\).

Changes in PRA, AT\(_1\) Receptor Expression, and Oxidated Protein Levels

PRA (in ng ANG I·ml\(^{-1}·\text{h}^{-1}\)) was similar in control (males: \(7.5 \pm 1.6\); females: \(7.7 \pm 2.2\) and ARAnp-treated (males: \(7.5 \pm 2.0\); females: \(7.1 \pm 1.6\)) rats at 3–4 mo of age. No significant differences in PRA were also found between groups at 10–11 mo of age (control males: \(6.3 \pm 1.1\); control females: \(6.9 \pm 2.9\); ARAnp males: \(5.0 \pm 1.0\); ARAnp females: \(6.3 \pm 1.0\)).

AT\(_1\) receptor expression in mesenteric arteries and kidneys in each group of rats is shown in Fig. 3. It shows that AT\(_1\) receptor expression in mesenteric arteries is independent of sex, age, and treatment since no significant differences were found between groups. No significant differences in renal AT\(_1\) receptor expression were also found between males and females, and between control and ARAnp-treated rats, at 3–4 mo of age. Contrary to what was found in mesenteric arteries, age- and sex-dependent differences were found in renal AT\(_1\) receptor expression. This reninal expression only increased \((P < 0.05)\) in male rats between 3–4 and 10–11 mo of age and was greater \((P < 0.05)\) in control than in ARAnp-treated male rats at 10–11 mo of age. Renal AT\(_1\) receptor expression was also greater \((P < 0.05)\) in control than in ARAnp-treated female rats at this age. The sex-dependent difference at 10–11 mo of age in AT\(_1\) receptor expression was found in control \([\text{males: } 6.1 \pm 0.6 \text{ arbitrary units (a.u.)}; \text{females: } 1.0 \pm 0.1 \text{ a.u.}]\) and ARAnp-treated \([\text{males: } 2.7 \pm 0.4 \text{ a.u.}; \text{females: } 0.6 \pm 0.0 \text{ a.u.}]\) rats (Fig. 3).

Oxidated protein levels in plasma and renal tissue in 10- to 11-mo-old rats are shown in Fig. 4. Plasma oxidated proteins levels were greater \((P < 0.05)\) in male than in female rats. These levels were also elevated \((P < 0.05)\) in both sexes of ARAnp-treated rats with respect to those found in their respective control group. Oxidated protein levels in renal tissue were also enhanced \((P < 0.05)\) in male and female ARAnp-treated rats. A significant sex-dependent difference in the renal oxidated protein levels was found in hypertensive but not in normotensive rats (Fig. 4).
to ANG II at 3–4 mo of age is also evident when renal vascular resistance (RVR) changes are examined (Fig. 5). The ANG II-induced increment in RVR was greater \( (P < 0.05) \) in ARAnp-treated (males: 19 ± 2; females: 17 ± 2) than in control (males: 8 ± 1; females: 11 ± 2 mmHg·ml\(^{-1}\)·min\(^{-1}\)) rats. The acute ANG II infusion also elicited a fall \( (P < 0.05) \) in RPF and GFR in each group of rats at 10–11 mo of age, but, contrary to what was found at the youngest age, the hemodynamic response was similar in ARAnp-treated and control rats in the oldest rats (Table 1). The fact that renal vasoinhibition elicited by ANG II was similar in these rats is also evident when the changes in RVR are examined. It can be observed in Fig. 5 that the ANG II-induced increment in RVR was similar in these rats (control males: 14 ± 3; ARAnp males: 13 ± 2; control females: 11 ± 1; ARAnp females: 9 ± 2 mmHg·ml\(^{-1}\)·min\(^{-1}\)) at 10–11 mo of age.

**DISCUSSION**

The importance of ANG II in maintaining the hypertension and renal changes secondary to an adverse event during renal development has been examined in several previous studies (2, 7, 14–16, 20, 37, 38). However, this is the first study examining whether there are sex and age differences in the role that ANG II plays in the hypertension secondary to an adverse event during renal development and whether this hypertension is associated with changes in AT1 receptor expression in resistance vessels and renal tissue and changes in oxidative stress. This is also the first study that has evaluated whether there are sex- and age-dependent differences in the renal sensitivity to an acute ANG II infusion in animals with a decrease in ANG II effects during renal development. The role of ANG II in mediating the hypertension and renal changes secondary to an alteration in renal development has been examined in adult and middle-age rats by evaluating 1) the response to 3 days of candesartan administration; 2) the changes in PRA and AT1 receptor expression in mesenteric arteries and renal tissue; and 3) the renal hemodynamic response to an acute ANG II infusion. The importance of oxidative stress was examined by measuring the levels of one oxidative stress marker and by comparing the AP response to

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**Renal Hemodynamic Response to an Acute ANG II Infusion**

ANG II led to an increment \( (P < 0.05) \) in MAP that was not statistically different in both groups of male (control: 11 ± 1 mmHg; ARAnp-treated: 15 ± 2 mmHg) and female (control: 8 ± 2 mmHg; ARAnp-treated: 13 ± 3 mmHg) rats at 3–4 mo of age. Similar changes in MAP were found after ANG II infusion at 10–11 mo of age. Renal hemodynamic parameters during the basal period were similar in each group of female rats (Table 1). However, basal RPF and GFR were reduced at 3–4 and 10–11 mo of age in ARAnp-treated male rats compared with the values found in control males. An age-dependent decrease \( (P < 0.05) \) in basal GFR was observed in both groups of ARAnp-treated rats. ANG II infusion induced a fall in RPF and GFR in each group at both age ranges (Table 1). This acute infusion led to renal vasoinhibition that was greater in ARAnp-treated than in control rats at 3–4 mo of age. The decrease in RPF and GFR was greater in ARAnp-treated (−45 ± 3 and −43 ± 9\%, respectively) than in control (−28 ± 3 and −21 ± 3\%, respectively) male rats. The fall in GFR and RPF was also greater in ARAnp-treated (−42 ± 2 and −44 ± 8\%, respectively) than in control (−28 ± 4 and −21 ± 5\%, respectively) female rats. The difference in the renal response to ANG II at 3–4 mo of age is also evident when renal vascular

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**Fig. 3.** AT1 receptor expression (arbitrary units; a.u.) in mesenteric arteries and kidneys of control and ARAnp-treated rats of both sexes and at 3–4 and 10–11 mo of age. *\( P < 0.05 \) vs. female rats. +\( P < 0.05 \) vs. control group.

**Fig. 4.** Oxidized proteins levels (a.u.) in plasma and kidneys of 10-to 11-mo-old control and ARAnp-treated rats. *\( P < 0.05 \) vs. female rats. #\( P < 0.05 \) vs. control group.
Table 1. Renal hemodynamic response to ANG II infusion in 3- to 4- and 10- to 11-mo-old rats treated with vehicle (control) or ARA (ARAnp) during nephrogenic period

<table>
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<tr>
<th></th>
<th>3–4 Mo Old</th>
<th>10–11 Mo Old</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>ARAnp</td>
</tr>
<tr>
<td>Renal plasma flow, ml·min⁻¹·g kidney wt⁻¹</td>
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<tr>
<td>Basal</td>
<td>4.72 ± 0.31</td>
<td>3.07 ± 0.23*</td>
</tr>
<tr>
<td>ANG II</td>
<td>3.39 ± 0.18†</td>
<td>1.70 ± 0.16†</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml·min⁻¹·g kidney wt⁻¹</td>
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<tr>
<td>Basal</td>
<td>1.30 ± 0.17</td>
<td>0.89 ± 0.08*</td>
</tr>
<tr>
<td>ANG II</td>
<td>0.99 ± 0.09†</td>
<td>0.52 ± 0.10†</td>
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Values are means ± SE. ARAnp, AT₁ receptor antagonist. *P < 0.05 vs. control group. †P < 0.05 vs. basal period. ‡P < 0.05 vs. 3–4 mo of age.

candesartan with and without the simultaneous administration of tempol.

The AP and renal hemodynamic results obtained in ARAnp-treated rats before any experimental maneuver are similar to those previously reported (18, 32, 38) and confirm that there are important sex differences in the age-dependent increment in AP and in the renal hemodynamic changes when there is an adverse event during renal development. These changes found in ARAnp-treated rats may be secondary to a 37% decrease in nephron number that is similar in males and females (34), but also to other renal changes elicited by the reduction of ANG II effects during the nephrogenic period (19). Sex-dependent differences in the cardiovascular response to an adverse fetal environment have been reported using different models of fetal programming of hypertension (7, 10, 22, 34, 35, 44). However, so far it was unknown whether the importance of ANG II in maintaining hypertension and in the regulation of renal hemodynamic is sex dependent during aging. One hypothesis tested in this study was that this role of ANG II would be age and sex dependent. This hypothesis was based on studies showing that ANG II is involved in maintaining the hypertension secondary to an adverse event during renal development (2, 7, 12, 14, 16, 20, 38), and on studies demonstrating that the role of ANG II in the regulation of cardiovascular and renal function increases with age (4). It was also known that vasoconstrictor ANG II effects are modulated by sex hormones (31, 52). The results obtained confirmed our hypothesis and present new evidence suggesting that the ANG II–AT₁ receptor axis is progressively activated in male ARAnp-treated rats and that it is involved in the greater age-dependent increment of AP found in males. Only one previous study had examined the AP changes elicited by reducing the ANG II effects in both sexes of adult animals with a fetal programming model of hypertension (20). However, Manning and Vehaskari (20) did not examine whether the importance of RAS is sex dependent at different ages because a CEI was continuously administered from the second to the tenth month of life.

This is the first study testing the hypothesis that the greater ANG II dependence of AP in males is related to a different ANG II-dependent activation of oxidative stress in both sexes of animals with an adverse event during renal development. This hypothesis was based on studies showing that renal oxidative stress is enhanced in young offspring of mothers consuming a low-protein diet during pregnancy (42) and that renal oxidative stress is enhanced in male but not in female adult rats submitted to an intrauterine growth restriction (29). It was also known that the ANG II effect on oxidative stress is enhanced in adult sheep exposed to glucocorticoids in utero (12). Our hypothesis was also supported by studies demonstrating that oxidative stress mediates the vasoconstrictor effects of ANG II (49) and that the ANG II-dependent activation of NADPH oxidase is reduced by estrogens (9) and stimulated by androgens (31). The oxidative status in our study was examined by measuring the levels of one marker of oxidative stress and the AP response to a scavenger of superoxide anions such as tempol. With the results obtained taken together (Figs. 2 and 4), it is proposed that an increment in oxidative stress
plays an important role in maintaining hypertension secondary to an alteration of nephrogenesis during the late nephrogenic period. The effect of oxidative stress on AP seems to be ANG II dependent since the fall in AP elicited by candesartan is similar with and without the prolonged administration of tempol (Fig. 2). However, and contrary to what was expected, the fall in AP elicited by tempol was similar in both sexes of ARAnp-treated rats. Therefore, despite the different levels of oxidized proteins found in male and female ARAnp-treated rats (Fig. 4), oxidative stress does not seem to contribute to the sex differences in the ANG II-dependent hypertension found in ARAnp-treated rats. The hypothesis that oxidative stress does not account for the sex differences in the AP response to ANG II is supported by the results obtained by Schneider et al. (43) in mice during a 15-s infusion of ANG II.

In this study, whether the role of the RAS in maintaining hypertension is secondary to an increase in PRA has been examined. No significant changes and up- or downregulation of several components of the RAS have been reported in other experimental models in which hypertension is secondary to manipulations that reduce RAS activity during nephrogenic period (2, 14, 46). Contradictory data have also been reported when the age-dependent changes in PRA have been examined in male animals with fetal programmed hypertension (13, 20). The contradictory data may be explained by the multiplicity of models employed and the sex and age at which the studies were performed. 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 (30). The results reported in this study were obtained in samples collected from anesthetized animals, as in other previous studies (2, 11, 20, 46), and show that PRA is not elevated in ARAnp-treated rats compared with the PRA values found in control rats. However, our results suggest that the regulation of PRA is affected in these ARAnp-treated rats because PRA levels are inappropriately elevated, since the elevation in AP should be accompanied by a decrease in renin release (25).

To the best of our knowledge, this is the first study that has investigated whether the increment in AP found in models of fetal programming of hypertension can be explained by changes of AT1 receptor expression in extrarenal resistance vessels and whether this expression is sex and age dependent in normotensive animals. The results obtained show that AT1 receptor expression in mesenteric arteries is similar in control and ARAnp-treated rats of both sexes and at both ages examined. Therefore, these results suggest that the age- and sex-dependent increment in AP that occurs as a consequence of a decrease of ANG II effects during renal development is not secondary to an AT1 receptor increment. The hypertension found in ARAnp-treated rats is most probably secondary to an age- and sex-dependent increment of ANG II sensitivity in resistance vessels. Sex differences in the AP response to the AT1 receptor antagonist at 10–11 but not at 3–4 mo of age could also be explained by different levels of AT2 receptor expression in both sexes of ARAnp-treated rats (21, 22).

This study has examined whether the renal sensitivity to an acute increment in ANG II is similarly enhanced in both sexes of adult animals with an adverse renal development. It has been reported that the renal hemodynamic effects elicited by ANG II are enhanced in these adult male animals (28, 37), but it was unknown whether this increase in renal ANG II sensitivity is sex dependent. The hypothesis was that there is a sex dependency in the greater renal sensitivity to ANG II when there is an alteration in renal development, since it has been shown that testosterone mediates the greater renal sensitivity to ANG II in young male rats subjected to growth restriction during fetal development (28). It is also known that the ANG II-induced vasoconstriction is modulated by estrogens (26).

The results obtained suggest that renal sensitivity to ANG II is similar in both sexes of adult normotensive rats. Only one previous study has examined whether there are sex-dependent differences in the renal response to ANG II in normotensive animals (40). That study showed that the ANG II-induced increment in RVR was greater in male than in female mice. The discrepancy between the results found in both studies may be related to the fact that ANG II was infused in the previous study during only 15 s in mice not pretreated with a CEI. The absence in the renal vessels of a greater vasoconstrictor effect of ANG II in males has also been demonstrated in studies performed in humans (6, 23). In fact, these studies showed that the renal vasoconstrictor effects of ANG II are greater in women than in men. Our study also reports novel findings suggesting that the renal hemodynamic response to an acute ANG II infusion does not change in both sexes between adulthood and middle age in normotensive rats. The possible age-dependent change in the renal response to ANG II has only been examined in male normotensive rats (1, 45, 53). Some of these studies also reported that the magnitude of the renal vasoconstrictor response to ANG II is not affected by age (1, 53). The renal hemodynamic responses to ANG II found in our study (Table 1) do not fit well with the evidence that renal AT1 receptor expression increases with age in male and does not change in female normotensive rats (Fig. 3). The observed renal AT1 receptor expression confirms the results obtained in adult male and female normotensive mice (40) and in normotensive male rats at different ages (41, 50). However, this is the first study that has examined the age-dependent changes in renal AT1 receptor expression in females. A sex difference in the renal activation of signaling pathways downstream of the ANG receptors (43) could contribute to the observed renal response to ANG II. The different evolution of renal AT1 receptor expression in both sexes was expected, since it has been shown that estrogens and androgens modulate renal AT1 receptor expression (5, 26, 33). The sex-dependent difference in renal AT1 receptors may be related to the fact that female rats exhibit less age-related renal disease (1).

The results of this study demonstrated that the renal hemodynamic response to ANG II is enhanced in both sexes of adult rats with an altered nephrogenesis (Table 1, Fig. 5). However, and contrary to what was expected, the renal response was similarly enhanced in both sexes of ARAnp-treated rats. This study also examined whether the greater renal sensitivity to ANG II infusion in ARAnp-treated rats can be explained by an increment in renin response to ANG II. The different evolution of renal AT1 receptor expression in both sexes was expected, since it has been shown that estrogens and androgens modulate renal AT1 receptor expression (5, 26, 33). The sex-dependent difference in renal AT1 receptors may be related to the fact that female rats exhibit less age-related renal disease (1).
there are sex-dependent differences in renal AT_1 receptor expression in animals with fetal programmed hypertension (22). These results suggest that the enhanced renal hemodynamic effects are not secondary to an increase in total AT_1 receptor expression. However, the possibility cannot be excluded that receptor number is enhanced in each glomeruli since total renal AT_1 receptor expression is normal but the number of glomeruli is reduced by 37% in ARAnp-treated rats (34). In support of the hypothesis that glomerular AT_1 receptor expression is enhanced in ARAnp-treated rats, it has been shown that these rats have glomerular hypertrophy (34) and that larger glomeruli express more surface receptors (50). It is also possible that the greater renal hemodynamic response to the acute ANG II infusion is secondary to an enhanced affinity and/or activity of glomerular AT_1 receptors to ANG II (37).

Finally, it is possible that the enhanced renal vasoconstrictor response to ANG II is secondary to a fall in AT_2 receptors. In this regard, it has been reported that AT_2 receptor expression is reduced and the AT_1/AT_2 ratio is enhanced in male rats in which RAS activity decreased during the nephrogenic period (21, 37).

As previously mentioned, there are no studies evaluating whether the enhanced renal sensitivity to ANG II is maintained during aging in both sexes of animals with fetal programmed hypertension and whether this possible increment in the renal response to ANG II is accompanied by similar changes in renal AT_1 receptor expression. The proposed hypothesis was that renal ANG II sensitivity would be even greater at 9–11 than at 3–4 mo of age in ARAnp-treated rats and that renal AT_1 receptor expression would also be greater at the oldest age. The results obtained only partly confirm our hypothesis since they suggest that renal AT_1 receptor sensitivity seems to be enhanced in these hypertensive rats at 10–11 mo of age. It was found that the acute ANG II infusion led to a similar renal vasoconstriction in normotensive and hypertensive rats (Table 1, Fig. 5) despite that renal AT_1 receptor expression was lower in hypertensive than in normotensive rats (Fig. 3). It is obvious that the renal hemodynamic responses to ANG II cannot be explained by the observed changes in AT_1 receptor expression since this expression was lower in hypertensive than in control rats and greater in male than in female ARAnp-treated rats. A postreceptor mechanism could be involved in the observed sex-dependent differences in renal ANG II effects (43). Further studies are needed investigating the sex-dependent mechanisms involved in the renal response to ANG II during aging. It also remains to be investigated why renal AT_1 receptor expression is lower in hypertensive rats with an adverse event during renal development than in control rats. One possibility could be the renal AT_1 receptor expression decrease in response to a high local ANG II concentration (51).

However, high local ANG II concentration does not seem to be the explanation for the decrease in renal AT_1 receptors in ARAnp-treated rats because the renal vasodilatation in response to a CEI in our study was similar to that found in control rats. Another possible explanation for lower renal AT_1 receptor expression in ARAnp-treated rats is the long-term increment of renal perfusion pressure in hypertensive rats.

In summary, this study presents novel findings suggesting that a decrease in ANG II effects during the nephrogenic period induces an ANG II-dependent increment in arterial pressure later in life that is significantly greater in males than in females.

This hypertension is secondary to an increase in oxidative stress but not in AT_1 receptor expression in resistance vessels or renal tissue. In addition, this study reveals for the first time that the renal hemodynamic response to ANG II is similarly enhanced during adulthood in males and females with an adverse event during renal development, despite that renal AT_1 receptor expression is similar to that found in the control group. Novel findings are also reported showing that the renal hemodynamic response to ANG II is only transitorily enhanced in animals with an adverse event during renal development and that renal AT_1 receptor expression is lower in hypertensive than in normotensive animals during aging. The results reported in this study may have pathophysiological implications since RAS activity decreases during renal development as a consequence of the administration of glucocorticoids or a low protein intake by the pregnant female, or as a consequence of placental insufficiency (7, 11, 47).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

AGE- AND SEX-DEPENDENT EFFECTS OF ANG II ON FETAL PROGRAMMING


