Changes in renal hemodynamics and excretory function induced by a reduction of ANG II effects during renal development

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Changes in renal hemodynamics and excretory function induced by a reduction of ANG II effects during renal development. Am J Physiol Regul Integr Comp Physiol 293: R695–R700, 2007. First published May 9, 2007; doi:10.1152/ajpregu.00191.2007.—The aim was to evaluate whether blockade of ANG II effects during renal development modifies the renal response to an increment of plasma amino acid concentration. It was also examined in anesthetized rats whether the reduction of the renal ability to eliminate an acute volume expansion (VE), elicited by blockade of ANG II during renal development, is sex and/or age dependent. Newborn Sprague-Dawley rats were treated with vehicle or an AT1-receptor antagonist (ARA) during postnatal nephrogenesis. Amino acid infusion induced increments (P < 0.05) of glomerular filtration rate (31 ± 6%) and renal plasma flow (26 ± 5%) in male but not in female vehicle-treated rats. Natriuretic and diuretic responses to amino acid infusion were similar in male and female vehicle-treated rats. These renal hemodynamics and excretory responses to amino acid infusion were abolished in ARA-treated rats. Renal responses to VE were evaluated at 3–4 and 9–10 mo of age in vehicle and ARA-treated rats. VE-induced natriuresis and diuresis were reduced by more than 38% (P < 0.05) in 3- to 4-mo-old male and female ARA-treated rats. An age-dependent reduction (P < 0.05) in the renal ability to eliminate VE was found in male but not in female rats treated with ARA. Our results demonstrate that the renal effects induced by an increment in amino acids are abolished when ANG II effects have been reduced during nephrogenesis. In addition, this reduction of ANG II effects elicits an impairment of the renal ability to eliminate an acute VE in males and females, which is aggravated by age only in male rats.

amino acid; filtration rate; nephrogenesis; renal vasodilatation; volume expansion

The role of ANG II in the regulation of renal development has been demonstrated in many studies (6, 10, 11, 14, 23). Recently, our group (14, 23) has shown important sex differences in the renal response to the administration of an AA solution were examined in anesthetized rats whether females treated with an ARA during nephrogenic period were treated with either ARA or vehicle during renal development. Blockade of ANG II effects during renal development also reduces the renal ability to eliminate an acute sodium load in male rats at adult age (18). However, it is also unknown whether females treated with an ARA during nephrogenic period have a reduced ability to eliminate a sodium load. The hypothesis is that the renal excretory ability will be less altered in female than in male animals because the blockade of ANG II effects induces a significant papillary atrophy only in males (23), and it is well known that the renal papilla is involved in the regulation of sodium excretion (9, 22). Other hypothesis to be examined in this study is whether blockade of ANG II effects during nephrogenic period accelerates the normal age-related decline in the renal excretory ability that occurs in male rats (15, 16).

Materials and Methods

Sprague-Dawley (SD) rats were purchased from the Animal Research Laboratory of the University of Murcia. Protocols were designed according to the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society. Experimental protocols, were approved by the Bioetica Committee of the University of Murcia. All rats were housed in rooms with controlled temperature (23–24°C) and 12:12-h dark-light cycles. Food 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 evidence was found in the vaginal smear. At postnatal day 0, litter size was fixed between 8 and 10 to ensure a similar nourishment during suckling period. Litters with less than eight pups were excluded. Newborn rats were treated from postnatal day 1 to postnatal day 14 with vehicle (isotonic saline) or an ARA (L-158,809; Merck Sharp & Dohme) at an oral dose of 7 mg·kg−1·day−1. Rats at 3–4 and 9–10 mo of age were used in this study.

Surgical Preparation for Acute Renal Function Studies

After an overnight fast, male and female rats were anesthetized with 0.1 ml im of ketamine (100 mg/ml Ketolar; Parke Davis) and 0.1 ml im of xylazine (10 mg/ml; Zentiva) in a volume of 0.1 ml. ARA-treated females were treated with vehicle or an AT1-receptor antagonist (ARA) during the nephrogenic period. Amino acid infusion induced increments (P < 0.05) of glomerular filtration rate (31 ± 6%) and renal plasma flow (26 ± 5%) in male but not in female vehicle-treated rats. Natriuretic and diuretic responses to amino acid infusion were abolished in ARA-treated rats. Renal responses to VE were evaluated at 3–4 and 9–10 mo of age in vehicle and ARA-treated rats. VE-induced natriuresis and diuresis were reduced by more than 38% (P < 0.05) in 3- to 4-mo-old male and female ARA-treated rats. An age-dependent reduction (P < 0.05) in the renal ability to eliminate VE was found in male but not in female rats treated with ARA. Our results demonstrate that the renal effects induced by an increment in amino acids are abolished when ANG II effects have been reduced during nephrogenesis. In addition, this reduction of ANG II effects elicits an impairment of the renal ability to eliminate an acute VE in males and females, which is aggravated by age only in male rats.

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ml/100 g ip of pentobarbital sodium (50 mg/ml Pentothal; Abbott). After tracheotomy, catheters were inserted into the bladder for urine collections and into the left femoral artery to measure mean arterial pressure (MAP) (PowerLab, ADInstruments) and for blood withdrawal. A blood sample was collected for basal hematocrit measurement. One catheter was then implanted into the left femoral vein for intravenous infusions. Rats were placed on a temperature-regulated surgical table to maintain a stable body temperature throughout the experiment. To stabilize hematocrit levels after surgical stress, one solution of 0.5 ml/100 g BSA (6%; Sigma) was administered. [3H]inulin (2 μCi/ml; American Radiolabeled Chemicals) and 12 mg/ml sodium p-aminohippurate (PAH; Sigma) were given as an intravenous bolus (1 ml). A continuous intravenous infusion of [3H]inulin (1.5 μCi/ml) and PAH (12 mg/ml) dissolved in isotonic saline, or in a mixed 10% AA solution (Aminoplasmat L-10; Braun Medical), was maintained throughout the experiment (3 ml/h). A 60-min stabilization period was allowed before experimental maneuvers were started.

**Experimental Protocols**

*Amino acid infusion studies.* Two 20-min basal clearance collections were followed by a continuous intravenous infusion of a mixed 10% AA solution (3 ml/h). Five minutes after AA infusion was initiated, two 20-min clearances were obtained. MAP was continuously recorded, and data were averaged for each clearance period. Urine samples were collected into preweighed vials for measurements previously recorded, and data were averaged for each clearance period. Urine samples were collected into preweighed vials for measurements of urinary flow rate (UV), [3H]inulin, PAH, and urinary sodium excretion (UNaV). Plasma samples were collected in heparinized capillaries 5 min before the end of each clearance period to measure [3H]inulin, PAH, and electrolyte concentrations. Kidneys were removed and weighed when the experiments were finished. Response to AA infusion was evaluated at 3–4 mo of age in male (n = 9) and female (n = 9) animals treated with vehicle and in male (n = 9) and female (n = 8) animals treated with ARA during the nephrogenic period.

*Acute volume expansion studies.* Two 20-min basal clearances were followed by a volume expansion (VE; 6% body wt, during 55 min) elicited by the infusion of isotonic saline. Two consecutive 20-min clearances were obtained during the last 40 min of VE. The same parameters described for the AA infusion studies were measured in the VE studies. Renal response to VE was examined in rats ages 3–4 and 9–10 mo old. The number of rats in each group at 3–4 mo of age were as follows: eight vehicle-treated males, nine vehicle-treated females, eight ARA-treated males, and nine ARA-treated females. The number of 9- to 10-mo-old rats included in each group were as follows: seven vehicle-treated males, nine vehicle-treated females, six ARA-treated males, and six ARA-treated females.

**Calculations**

Standard clearance formulas were used to calculate GFR and effective renal plasma flow (ERPF) using the renal clearances of inulin and PAH, respectively. These clearances were normalized by kidney weight. UV was determined gravimetrically, and UNaV was calculated by multiplying UV by the urine sodium concentration, as measured by a flame photometer (Instrumentation Laboratory 943).

**Statistical Analysis**

The data for the two clearances obtained during basal periods, AA infusion, and acute VE were averaged for statistical comparisons because the fluid and solute excretions were measured in steady-state conditions. Data are expressed as means ± SE. For each group, differences between values obtained during basal and experimental periods were evaluated by one-way ANOVA and Fisher test (GB Stat, Dynamic Microsystems, 1996). P < 0.05 was considered significant.

**RESULTS**

**Renal Response to a Mixed AA Infusion**

The AA infusion did not modify MAP in any experimental group (Table 1). The AA infusion led to a renal vasodilatation and hyperfiltration in vehicle-treated male rats because it induced an elevation in GFR (1.47 ± 0.12 to 1.91 ± 0.11 ml/min·100 g−1; P < 0.05) and ERPF (4.28 ± 0.17 to 5.39 ± 0.24 ml/min·100 g−1; P < 0.05) (Fig. 1) and a decrease in renal vascular resistance (RVR) (Table 1). The AA infusion also elicited an increment in UV (23 ± 7 to 45 ± 9 µl/min; P < 0.05) and UNaV (3.2 ± 0.9 to 7.8 ± 1.6 µeq/min; P < 0.05) in these male rats. Basal renal hemodynamic and excretory functions in ARA-treated males were similar to those found in vehicle-treated males (Table 1 and Fig. 1). Contrary to that found in vehicle-treated male rats, AA infusion to ARA-treated male rats did not induce changes in GFR, effective renal blood flow (ERBF), ERPF, RVR, UV, and UNaV (Table 1 and Fig. 1).

In contrast to the already mentioned response found in vehicle-treated males, the AA infusion did not elicit changes in GFR, ERBF, ERPF, and RVR in vehicle-treated female rats (Table 1 and Fig. 2). Nevertheless, this infusion did induce an increment in both UV (32 ± 3 to 58 ± 3 µl/min; P < 0.05) and UNaV (5.8 ± 0.6 to 10.4 ± 0.8 µeq/min; P < 0.05) in vehicle-treated females. Basal renal hemodynamics and UNaV were similar in vehicle- and ARA-treated females. As occurred in ARA-treated males (Fig. 1), the AA infusion did not modify renal hemodynamic and excretory function in ARA-treated females (Table 1 and Fig. 2).

**Renal Response to an Acute Extracellular VE**

Table 2 shows the renal response to VE in rats 3–4 mo old treated with vehicle or ARA during the nephrogenic period. MAP was similar in vehicle- and ARA-treated rats of both sexes and did not change during VE. This VE did not modify renal hemodynamics but elicited a significant increment of UV and fractional sodium excretion (FENa) in vehicle-treated males (Table 2). As shown in Table 2, basal GFR and ERPF were reduced in male ARA-treated rats compared with the values found in the control group (P < 0.05). However, basal UV and FENa were similar in vehicle- and ARA-treated males.

**Table 1. Changes in MAP, ERBF, and RVR in response to an amino acid infusion in 3-mo-old rats treated with vehicle or ARA during nephrogenic period**

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>ARA</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>115±4</td>
<td>111±3</td>
</tr>
<tr>
<td>Amino acids, eq/min</td>
<td>18.5±1.1</td>
<td>16.5±1.0</td>
</tr>
<tr>
<td>ERBF, ml/min</td>
<td>6.6±0.5</td>
<td>6.9±0.4</td>
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</table>

Values are means ± SE. ARA, AT1-receptor antagonist; MAP, mean arterial pressure; ERBF, effective renal blood flow; RVR, renal vascular resistance. *P < 0.05 vs. basal.
Acute VE also led to an elevation of UV and FENa in ARA-treated males, but the increments in these parameters were lower (44% and 38%, respectively) than those found in vehicle-treated males ($P < 0.05$).

As shown in Table 2, basal GFR and ERPF were similar and did not change during VE in both groups of 3-mo-old female rats. Acute VE elicited an elevation ($P < 0.05$) of UV and FENa in these groups of female rats, but the increments were 54% lower ($P < 0.05$) in ARA-treated than in vehicle-treated females. The reduction of the renal excretory ability in the young rats induced by ARA treatment during renal development was similar in males and females (Table 2).

Renal hemodynamics and excretory responses to VE in 9- to 10-mo-old rats are shown in Table 3. As occurred in the younger rats, MAP was similar before and after VE in each group of rats at 9–10 mo of age. During VE, GFR and ERPF did not change but UV and FENa increased ($P < 0.05$) in vehicle-treated male animals (Table 3). Compared with the values found in the control group, GFR and ERPF were reduced ($P < 0.05$) by 27% and 22%, respectively, in ARA-treated males. The renal excretory ability to eliminate the sodium load was attenuated in these males because the VE-induced increments in UV and FENa were diminished by >40% compared with the changes found in 9- to 10-mo-old vehicle-treated males (Table 3).

GFR and ERPF were similar in both groups of the older female rats before and after VE. However, the increments of UV and FENa elicited by VE were 33% lower in ARA-treated than in vehicle-treated females (Table 3).

Figure 3 shows that the renal ability to eliminate an acute sodium load decreases with age in ARA-treated males but not in ARA-treated females. It can be observed that the VE-induced increments in $U_{NaV}$ and UV were lower in ARA-treated male rats age 9–10 mo ($36.4 \pm 4.9 \text{ neq} \cdot \text{min}^{-1} \cdot \text{g body wt}^{-1}$ and $0.19 \pm 0.03 \mu\text{l} \cdot \text{min}^{-1} \cdot \text{g body wt}^{-1}$, respectively) than in ARA-treated male rats age 3–4 mo ($53.6 \pm 3.2 \text{ neq} \cdot \text{min}^{-1} \cdot \text{g body wt}^{-1}$ and $0.35 \pm 0.08 \mu\text{l} \cdot \text{min}^{-1} \cdot \text{g body wt}^{-1}$, respectively). However, the increments in $U_{NaV}$ and UV were similar in ARA-treated female rats age 9–10 mo ($48.0 \pm 5.1 \text{ neq} \cdot \text{min}^{-1} \cdot \text{g body wt}^{-1}$ and $0.35 \pm 0.08 \mu\text{l} \cdot \text{min}^{-1} \cdot \text{g body wt}^{-1}$, respectively) and in ARA-treated female rats age 3–4 mo ($46.9 \pm 4.4 \text{ neq} \cdot \text{min}^{-1} \cdot \text{g body wt}^{-1}$ and $0.30 \pm 0.04 \mu\text{l} \cdot \text{min}^{-1} \cdot \text{g body wt}^{-1}$, respectively) (Fig. 3).

**DISCUSSION**

This study presents new evidences demonstrating that renal hemodynamics and excretory changes elicited by an increment in plasma AA levels are abolished when ANG II effects have been blocked during renal development. Another novel finding is that this blockade of ANG II effects reduces the renal excretory response to an acute VE similarly in young male and female rats. Finally, new evidences are reported suggesting that the age-dependent deterioration of the renal ability to eliminate...
Table 3. Renal responses to an acute volume expansion in 9-mo-old rats treated with vehicle or ARA during nephrogenic period

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>ARA</th>
<th>Vehicle</th>
<th>ARA</th>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td></td>
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</tr>
<tr>
<td>Basal</td>
<td>118±4</td>
<td>110±3</td>
<td>115±3</td>
<td>114±5</td>
</tr>
<tr>
<td>Volume expansion</td>
<td>116±4</td>
<td>109±2</td>
<td>114±4</td>
<td>114±4</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·g kidney wt⁻¹</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.52±0.10</td>
<td>1.24±0.07†</td>
<td>1.44±0.06</td>
<td>1.35±0.03</td>
</tr>
<tr>
<td>Volume expansion</td>
<td>1.58±0.08</td>
<td>1.28±0.08†</td>
<td>1.50±0.05</td>
<td>1.39±0.04</td>
</tr>
<tr>
<td>ERPF, ml·min⁻¹·g kidney wt⁻¹</td>
<td></td>
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</tr>
<tr>
<td>Basal</td>
<td>4.5±0.2</td>
<td>3.6±0.2†</td>
<td>4.0±0.1</td>
<td>3.8±0.1</td>
</tr>
<tr>
<td>Volume expansion</td>
<td>4.4±0.2</td>
<td>3.9±0.3</td>
<td>4.1±0.1</td>
<td>3.9±0.3</td>
</tr>
<tr>
<td>UV, µl·min⁻¹·g body wt⁻¹</td>
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<td></td>
<td></td>
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<tr>
<td>Basal</td>
<td>0.17±0.04</td>
<td>0.18±0.02</td>
<td>0.20±0.04</td>
<td>0.20±0.04</td>
</tr>
<tr>
<td>Volume expansion</td>
<td>0.79±0.03*</td>
<td>0.53±0.04*†</td>
<td>0.86±0.05*</td>
<td>0.50±0.07**†</td>
</tr>
<tr>
<td>FENa, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.42±0.13</td>
<td>1.91±0.24</td>
<td>2.23±0.37</td>
<td>1.99±0.43</td>
</tr>
<tr>
<td>Volume expansion</td>
<td>7.95±0.29*</td>
<td>5.94±0.32†</td>
<td>9.18±0.52*</td>
<td>5.17±0.64†</td>
</tr>
</tbody>
</table>

Values are means ± SE. GFR, glomerular filtration rate; ERPF, effective renal plasma flow; UV, urinary flow rate; FENa, fractional excretion of sodium. *P < 0.05 vs. vehicle. †P < 0.05 vs. vehicle.

an acute VE is accelerated in male but not in female rats in which ANG II effects have been reduced during renal development.

The results of this study show that anesthetized ARA-treated rats have an arterial pressure not significantly different from that found in rats treated with vehicle during the nephrogenic period. These results contradict those reported previously by our group (14, 23) showing that arterial pressure is enhanced in ARA-treated rats. The discrepancy may be explained by the fact that, in these previous studies (14, 23), arterial pressure was measured in conscious rats. This hypothesis is based on evidences showing that arterial pressure regulation is different in conscious and in anesthetized rats (12, 19). Our study also confirms the results of a previous study (14) that demonstrated that basal renal hemodynamics is altered in male but not in female animals treated with ARA during the nephrogenic period and that this alteration increases with age.

Blockade of ANG II effects leads to a reduction in nephron number that is similar in male and female rats, but the associated increments in calculated single nephron GFRs are greater in male than in female rats (23). It was expected that the decrease in glomeruli number would lead to a functional overload of the existing units and that the loss of “renal functional reserve” would render the kidney with lower nephron number susceptible to failure when other stimuli that induce renal vasodilatation and hyperfiltration are superimposed. One of these stimuli is an elevation in plasma AA concentration (2, 3, 5, 25–28). The hypothesis that the functional reserve capacity is reduced in the ARA-treated males seems to be confirmed by our results. One possible explanation of why GFR and renal blood flow did not increase in response to AA in these male ARA-treated rats is that glomerular pressures and flows were at maximal, and therefore potentially harmful, levels. Blockade of the renal hemodynamic response to an increment in plasma AA concentration could also be secondary to an alteration of the mechanisms involved in regulating this response (5, 25, 27, 28).
The absence of renal hemodynamic changes in female rats treated with vehicle was clearly unexpected because of the well-accepted notion that an increment in plasma AA levels elicits a fall in RVR and an elevation in GFR (2, 3, 5, 25–28). Furthermore, it is known that AA elicits a significant renal vasodilation in normotensive women (26). However, after reviewing the scientific literature, we realized that this is the first study in which the renal response to the infusion of a mixed AA solution has been examined in normotensive female rats. As far as we know, only the renal response to glycine has been evaluated in female rats (2, 17), and it was found that glycine infusion induced significant rises in GFR and ERPF. Although the dose of AA infused was able to induce in males a change in GFR and ERPF that is similar to the maximal effect found in other studies (5, 27), it is possible that this dose was below the threshold for female SD rats; therefore, a higher dose of AA is needed to elicit a renal vasodilation. Further studies are needed to evaluate the mechanisms involved in the sex differences found in the renal response to an increase in AA.

The infusion of the mixed AA solution induced a natriuretic and diuretic response that was similar in male and female rats treated with vehicle during the nephrogenic period. This is also the first study showing that the excretory response to an increment in plasma AA levels is abolished when ANG II effects have been reduced during renal development. Although our results do not propose the mechanisms responsible for the blockade of this renal excretory response, it may be speculated that an enhanced proximal tubule reabsorption may be partly responsible for the lower sodium and water excretion found in ARA-treated rats. This hypothesis is based on the fact that proximal sodium reabsorption is elevated in different experimental models with reduced nephron number (4).

The blunted natriuretic and diuretic responses to an acute VE in 3-mo-old ARA-treated male SD rats confirms the results reported by Nilsson et al. (18). These authors proposed that the reduced excretory response to a VE may be secondary to a lower ability to increase renal interstitial hydrostatic pressure (RIHP). Other new finding in this study is that the blockade of ANG II during the nephrogenic period also reduced in female animals the renal ability to eliminate an acute sodium load. The fact that this reduction is similar in males and females treated with ARA during the nephrogenic period does not confirm our hypothesis that the excretory ability would be more affected in male than in female rats. The hypothesis was based on studies showing that the increments in RIHP are partly explained by an elevation in papillary blood flow (9, 22) and showing that ARA-treated male but not ARA-treated female rats have a papillary atrophy (23). Our results do not allow us to determine the mechanisms involved in the reduced excretory response to VE observed in young male and female ARA-treated rats. However, it is speculated that this lower excretory ability may be secondary to an elevation of intrarenal ANG II effects. This hypothesis is supported by studies showing that the renin-angiotensin system activity is enhanced in young animals that have been submitted during renal development to stimuli that reduce ANG II effects (7, 24, 29) and demonstrating that ANG II plays an important role in the regulation of RIHP and sodium excretion during an acute VE (13, 20).

As far as we know, this is also the first study showing that the reduction of ANG II effects during renal development leads to an accelerated age-dependent impairment of the renal ability to eliminate an acute sodium load in male but not in female rats. The mechanisms responsible for this further deterioration are unknown, but it may be speculated that a decrease in nitric oxide production (3, 8) and the different sexual hormones (3, 21) might be involved. The importance of sexual hormones in the greater reduction of renal excretory ability in male rats is supported by studies showing that the ratio of nitric oxide to angiotensin II is enhanced by estrogens and reduced by androgens (21). The hypothesis that a fall in nitric oxide may be involved is supported by studies showing that nitric oxide synthase expression decreases from 3–5 to 11–13 mo of age in male but not in female SD rats (8) and by studies demonstrating that renal excretory response to an acute sodium load is impaired when intrarenal nitric oxide synthesis is reduced (1, 13).

In summary, the results reported in this study suggest that the renal abilities to decrease RVR and to increase GFR and urinary sodium and water excretion are significantly deteriorated when ANG II effects have been reduced during renal development. In addition, this reduction of ANG II effects...
leads to an impairment of the renal ability to eliminate an acute sodium load that seems to be further deteriorated by aging in male but not in female rats.

GRANTS

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