Sex and age differences of renal function in rats with reduced ANG II activity during the nephrogenic period

Analia Loria, Virginia Reverte, Francisco Salazar, Fara Saez, M. Teresa Llinas, and F. Javier Salazar

Department of Physiology, School of Medicine, University of Murcia, Murcia, Spain

Submitted 12 February 2007; accepted in final form 12 April 2007

The role of renin-angiotensin-system (RAS) in renal development has been proposed in studies demonstrating an elevation of all RAS components during the nephrogenic period (7, 8, 12). This role of RAS is also supported by studies showing that blockade of angiotensin II (ANG II) effects during the nephrogenesis period leads to important renal abnormalities (13, 28). The importance of ANG II in renal development and the fact that neonatal administration of AT1-receptor antagonist (ARA) produces irreversible changes in renal morphology have been confirmed by our group (25). This study also demonstrated significant sex differences in the renal response to ARA administration during the nephrogenic period. It was found that glomeruli number decreased similarly in males and females, and that the subsequent increase in glomerular volume, glomerulosclerosis, and interstitial fibrosis was greater in adult males than in females (25). It was also observed that only males have a papillary volume reduction in response to ARA administration.

It is known that aging is associated with changes in renal function that are sex dependent (2, 3, 22). However, it remains to be examined whether the age-related changes in renal function are accentuated when nephron number decreases during renal development. It is also unknown whether these changes are sex dependent. It is expected that the effects induced by ARA blockade during renal development will lead to a time-dependent deterioration of renal hemodynamics and to an increment in proteinuria that will be more important in males than in females.

The papillary atrophy observed in males but not in females, and the greatest medullary fibrosis found in ARA male-treated rats (25), most probably will also lead to an impairment of the urinary concentrating ability in response to prolonged dehydration only in males. Since it is expected that medullary interstitial fibrosis will increase during aging in ARA-treated rats, it is possible that the renal ability to concentrate urine will be more deteriorated only in treated males as they age.

The main objective of this study was to determine whether the blockade of ANG II effects during the nephrogenic period accelerates the age-related changes in renal hemodynamics and proteinuria, and to examine if these changes are sex dependent. It was also examined whether the deterioration of the renal response to prolonged water dehydration in ARA-treated rats is sex and/or age dependent.

MATERIALS AND METHODS

Sprague-Dawley (SD) rats were purchased from the Experimental 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. All rats were housed in rooms with controlled temperature (24°C) and 12:12-h dark-light cycle. Food and water were supplied ad libitum. Female SD rats (230–240 g body wt) were placed with a fertile male, taking day 0 of pregnancy as the morning that sperm evidence was found. At postnatal day 0, litter size was fixed between 8 and 10. 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, 7 mg·kg⁻¹·day⁻¹) administered by gavage. Blockade of AT1 receptors was performed during the first 14 days of life because AT1 receptors were present from postnatal day 14 (25), most probably will also lead to an impairment of the urinary concentrating ability in response to prolonged dehydration only in males. Since it is expected that medullary interstitial fibrosis will increase during aging in ARA-treated rats, it is possible that the renal ability to concentrate urine will be more deteriorated only in treated males as they age.

The main objective of this study was to determine whether the blockade of ANG II effects during the nephrogenic period accelerates the age-related changes in renal hemodynamics and proteinuria, and to examine if these changes are sex dependent. It was also examined whether the deterioration of the renal response to prolonged water dehydration in ARA-treated rats is sex and/or age dependent.

MATERIALS AND METHODS

Sprague-Dawley (SD) rats were purchased from the Experimental 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. All rats were housed in rooms with controlled temperature (24°C) and 12:12-h dark-light cycle. Food and water were supplied ad libitum. Female SD rats (230–240 g body wt) were placed with a fertile male, taking day 0 of pregnancy as the morning that sperm evidence was found. At postnatal day 0, litter size was fixed between 8 and 10. 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, 7 mg·kg⁻¹·day⁻¹) administered by gavage. Blockade of AT1 receptors was performed during the first 14 days of life because nephrogenesis is still active in this time frame (13).

Rats at 3 or 11 mo of age were included in eight experimental groups. The number of rats in each group at 3 mo of age was as follows: control males (n = 9), control females (n = 8), ARA-treated males (n = 6), and ARA-treated females (n = 6). The number of 11-mo-old rats included in each group was as follows: control males (n = 7), control females (n = 7), ARA-treated males (n = 9), and ARA-treated females (n = 8).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: F. J. Salazar, Dept. of Physiology, School of Medicine, Univ. of Murcia, 30100 Murcia, SPAIN (e-mail: salazar@um.es).
Systolic blood pressure (BP) was measured at 3 and 11 mo of age in conscious rats by a tail-cuff method using a LE 5002 Storage Pressure Meter (Letica, Barcelona, Spain). To obtain an accurate BP reading, rats were first habituated to the BP measurement device. Definitive measurements began when rats remained unperturbed into the chamber throughout the inflation-deflation cycles. The BP value in each rat is the mean value of 10 measurements performed during 3 days.

To test the fluid handling and urinary concentrating ability, rats were kept individually in metabolic cages. After 2 days of adaptation, measurements of water intake and urine volume were taken during two consecutive 24-h periods. A basal blood sample from the tail was also obtained. Osmolality and creatinine concentrations in plasma and urine and proteinuria were measured. Thereafter, rats were deprived of food and water for 40 h, and urine was collected during the last 24 h of dehydration. Another blood sample from the tail was taken at the end of the experiment. Osmolality was determined in plasma and urine after water dehydration.

Analytic methods. Glomerular filtration rate (GFR) was determined by the endogenous creatinine clearance. This method has been used previously by our group in conscious animals (9, 23), and the values of GFR found were similar to those obtained in anesthetized rats using the [3H]inulin clearance (10, 20). Proteinuria was measured by the micro Lowry method. This procedure is based on Peterson’s modification (19). An alkaline cupric tartrate reagent complexes with the peptide bonds and forms a purple color whose absorbance is read at 543 nm. The standard and reactive of Lowry and Folin were from Sigma (St. Louis, MO). Plasma and urine osmolality was determined by the freezing point depression method (Vapro Osmometer model 5520, Wescor, Milan, Italy). Tubular solute free water reabsorption by the freezing point depression method (Vapro Osmometer model Sigma (St. Louis, MO). Plasma and urine osmolality was determined by the freezing point depression method (Vapro Osmometer model 5520, Wescor, Milan, Italy). Tubular solute free water reabsorption (\( T_{fW} \)) was calculated as \( C_{OSM} \) – UV, where \( C_{OSM} = (U_{OSM}/P_{OSM}) \times UV, \) where \( C_{OSM} \) is osmolar clearance; \( UV \) is urine flow rate; \( U_{OSM} \) is urine osmolality, and \( P_{OSM} \) is plasma osmolality.

Statistical analysis. Data in table, 1 and 2, and Figs. 1 and 2 are expressed as means \( \pm \) SE. One-way ANOVA and the Fisher test were used to evaluate the differences between and within groups (GB Stat, Dynamic Microsystems, 1996). \( P < 0.05 \) was considered statistically significant.

RESULTS

As shown in Table 1, BP and GFR were similar in male and female vehicle-treated rats, and both parameters did not change with age in these control groups. Table 1 also shows that BP increased similarly \( (P < 0.05) \) in males and females at 3 and 11 mo of age in rats treated with an ARA during the first 14 days of age. It was found that this elevation in BP was gradual and greater \( (P < 0.05) \) in older rats. The BP increment was confirmed in new groups of ARA-treated rats \((n = 9)\) in which BP was measured monthly during the first 12 mo of life (results not shown). At 3 mo of age, GFR was not affected by ARA treatment in males and females (Table 1). However, GFR was decreased \((P < 0.05)\) in the oldest ARA-treated male rats compared with the control group and ARA-treated female rats at the same age (Table 1). Water intake was similar in male and female vehicle-treated animals at both ages. This parameter increased \((P < 0.05)\) to the same extent at 3 mo of age in male and female ARA-treated rats. Daily water consumption remained elevated \((P < 0.05)\) at 11 mo in both groups treated with the ARA, but the increment was greater \((P < 0.05)\) in females than in males (Table 1).

Proteinuria increased in ARA-treated males at 3 mo of age \((95 \pm 8 \text{ mg/day}, P < 0.05)\) compared with the control group \((46 \pm 2 \text{ mg/day})\) (Fig. 1). This elevation was not observed in female ARA-treated rats, since their protein excretion \((48 \pm 2 \text{ mg/day})\) was similar to that found in their control group \((33 \pm 2 \text{ mg/day})\). At 11 mo of age, proteinuria was accentuated \((P < 0.05)\) in ARA-treated males \((251 \pm 10 \text{ mg/day})\), and male control rats showed higher values \((73 \pm 6 \text{ mg/day}, P < 0.05)\) than at 3 mo of age (Fig. 1). Female ARA-treated rats began to show proteinuria at 11 mo \((95 \pm 7 \text{ mg/day}, P < 0.05)\), but this parameter was not affected by age in the corresponding control group.

Changes in UV, \( U_{OSM}, P_{OSM}, \) and \( C_{OSM} \) in response to water dehydration are shown in Table 2. It can be seen that basal UV was lower \((P < 0.05)\) in control than in ARA-treated rats at 3 mo of age. This difference remained at 11 mo, but UV was higher in females than in males treated with the ARA \((P < 0.05)\). After water deprivation, UV decreased \((P < 0.05)\) in each experimental group but remained elevated \((P < 0.05)\) in male and female ARA-treated rats (Table 2).

Table 2 shows that basal \( U_{OSM} \) was similar in male and female vehicle-treated young rats and decreased \((P < 0.05)\) in each ARA-treated group compared with its corresponding control group. Water deprivation elicited elevations of \( U_{OSM} \) \((P < 0.05)\) in all experimental groups. However, the increment

---

**Table 1.** Body weight and baseline measurements of BP, GFR, and water intake at 3 and 11 mo of age in male and female rats treated with vehicle or ARA during nephrogenic period

<table>
<thead>
<tr>
<th></th>
<th>3 mo Old</th>
<th>11 mo Old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>ARA</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>360±9</td>
<td>352±8</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>115±0.4</td>
<td>127±0.4*</td>
</tr>
<tr>
<td>GFR, ml min⁻¹ g⁻¹</td>
<td>0.96±0.17</td>
<td>1.10±0.08</td>
</tr>
<tr>
<td>Water intake, ml/day</td>
<td>23±1</td>
<td>35±2*</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, g</td>
<td>239±4</td>
<td>243±8</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>114±1.1</td>
<td>127±0.4*</td>
</tr>
<tr>
<td>GFR, ml min⁻¹ g⁻¹</td>
<td>0.87±0.10</td>
<td>1.07±0.08</td>
</tr>
<tr>
<td>Water intake, ml/day</td>
<td>21±2</td>
<td>40±5*</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE. ARA, AT₁-receptor antagonist; BP, blood pressure; GFR, glomerular filtration rate. \* \( P < 0.05 \) vs. vehicle; † \( P < 0.05 \) vs. 3 mo of age.
of U_{OSM} was greater \((P < 0.05)\) in vehicle than in ARA-treated male rats at 3 \((505 \pm 89 vs. 336 \pm 35 \text{ mosmol/kgH}_2\text{O})\) and at 11 mo of age \((702 \pm 155 vs. 359 \pm 35 \text{ mosmol/kgH}_2\text{O})\) (Table 2).

The increment of U_{OSM} in response to dehydration was similar at 3 mo of age in both female groups (vehicle: 392 \pm 70 mosmol/kgH\(_2\)O; ARA: 502 \pm 68 mosmol/kgH\(_2\)O) (Table 2). The increments of U_{OSM} were higher \((P < 0.05)\) at 11 mo of age in ARA-treated females \((691 \pm 106 \text{ mosmol/kgH}_2\text{O})\) than in ARA-treated males \((359 \pm 35 \text{ mosmol/kgH}_2\text{O})\).

It also can be observed in Table 2 that P_{OSM} increased \((P < 0.05)\) similarly in all experimental groups at 3 mo of age. However, at 11 mo of age, water deprivation induced an increment in P_{OSM} that was greater in ARA-treated male rats than in the other three experimental groups \((P < 0.05)\). At 3 mo of age, water deprivation elicited a significant decrease of C_{OSM} in each experimental group, but no sex-associated differences of C_{OSM} were found in both vehicle and ARA-treated rats, before and after dehydration (Table 2). C_{OSM} was unchanged from 3 to 11 mo of age in both groups of male rats. However, basal C_{OSM} was significantly higher in older ARA-treated than in control female rats (Table 2).

Figure 2 shows that blockade of ANG II effects during the nephrogenic period induced a significant change in the basal \(T_{H2O}^C\) \((-33 \pm 1 \text{ vs. } -41 \pm 2 \text{ ml/day in the control group})\) at 3 mo of age. Increment of \(T_{H2O}^C\) elicited by dehydration was lower \((P < 0.05)\) in ARA-treated than in vehicle-treated males \((16 \pm 1 \text{ vs. } 27 \pm 2 \text{ ml/day})\). Figure 2 also shows that basal \(T_{H2O}^C\) and the increment of \(T_{H2O}^C\) during dehydration were different \((P < 0.05)\) in male ARA-treated rats \((-28 \pm 3 \text{ and } 17 \pm 3 \text{ ml/day, respectively})\) and in the control group \((-44 \pm 3 \text{ and } 24 \pm 4 \text{ ml/day, respectively})\) at 11 mo of age. Renal defect aggravation in urinary concentrating ability seems to be age dependent in ARA-treated males, since \(T_{H2O}^C\) during dehydration was greater \((P < 0.05)\) at 11 \((-11 \pm 1 \text{ ml/day})\) than at 3 mo of age \((-16 \pm 1 \text{ ml/day})\).

In contrast to the results found in males, blockade of ANG II effects during the nephrogenic period did not reduce the ability to concentrate urine in response to prolonged water deprivation in females (Fig. 2). \(T_{H2O}^C\) was not different before and after dehydration in both groups of 3-mo-old female rats. When \(T_{H2O}^C\) is compared at different ages in females, it can be observed that the renal ability to concentrate urine increases with age, since \(T_{H2O}^C\) was lower in the oldest rats (Fig. 2).

**DISCUSSION**

This study demonstrates the existence of important sex differences in the renal changes elicited by the blockade of ANG II effects during the late phase of the nephrogenic period, and that these sex differences are accentuated during aging. It was found that GFR only decreases in ARA-treated males, since \(T_{H2O}^C\) during dehydration was greater \((P < 0.05)\) at 11 \((-11 \pm 1 \text{ ml/day})\) than at 3 mo of age \((-16 \pm 1 \text{ ml/day})\).

The role of ANG II in renal development has been demonstrated in several studies \((6, 11, 13, 28)\). Recently, our group has confirmed these results previously reported and demonstrated that the renal alterations elicited by ANG II blockade during the nephrogenic period were more significant in males.
ARA-treated rats were similar to those found in control rats. Further studies are required to examine the mechanisms involved in the hypertension induced by the reduction of ANG II and single-nephron GFR, since nephron number decreased by 37% in these rats (25). Glomerular hyperfiltration was expected because it occurs after renal ablation in the remnant nephrons (16). Since glomerular hyperfiltration may be secondary to an elevation in glomerular capillary pressure after a reduction in nephron number (15), and single-nephron GFR seems to be greater in male than in female ARA-treated rats (25), it may be hypothesized that capillary pressure increased more in ARA-treated males than in ARA-treated females. The elevated capillary pressure would contribute to the greater increment in proteinuria and to the age-dependent decline in GFR found in male ARA-treated rats. The greater capillary pressure in males would hasten the injury to functioning glomeruli and would perpetuate the vicious circle of ongoing nephron loss. It is possible that compensatory adjustments to the decrease in nephron number were necessary for the preservation of a normal renal function, but in the long run were central for the progressive nature of renal disease. The elevation in proteinuria induced by an increase in glomerular capillary hydraulic pressure has been found in several forms of renal injury associated with permanent nephron loss (4).

The present study is the first one demonstrating that males are more susceptible than females to the time-dependent decline in renal function and elevation in proteinuria in response to a similar decrease in nephron number. Further studies are needed to examine the mechanisms responsible for these sex differences. However, it is speculated that sex hormones are probably involved, since it is known that there is a pronounced sexual dimorphism in the age-dependent renal injury and decline in renal function, with females protected due to both the protective estrogens and the lack of damaging androgens (1, 2, 21, 22).

As already mentioned, blockade of ANG II effects during the nephrogenic period only induces in males a reduction in papillary volume and an important increment in medullary interstitial fibrosis (25). With these results, one may envisage that medullary circulation and its function are hampered only in males, and that the renal ability to concentrate urine in response to a prolonged dehydration is deteriorated in males but not in females treated with an ARA during the nephrogenic period. Since it is well known that the urinary concentrating ability changes during aging (26, 27), it was also anticipated that the renal adaptation to a prolonged dehydration is more affected by aging in male than in female ARA-treated rats. The decrease in the renal ability to enhance urinary osmolality in response to a dehydration in ARA-treated males was expected and may be explained by the papillary atrophy (6, 14) and by

than in females. Furthermore, only treated males had a papillary atrophy (25). These effects occurred, despite the fact that ARA administration elicited similar changes of BP and nephron number in males and females (25). This study was designed to evaluate whether blockade of ANG II effects during renal development leads to age-dependent changes in GFR, proteinuria, and in the renal adaptation to a prolonged dehydration. Our aim was also to examine whether these changes are sex dependent. It was expected that kidneys with reduced nephron number would be more susceptible to subsequent injuries, and that the age-dependent functional decline would be greater in males.

The BP increment elicited by ARA administration during the nephrogenic period was expected, because it was similar to that found in other studies (25, 28). Our results extend these previous observations by showing that the elevation in BP was accentuated by aging. The hypertension found is in close agreement with clinical studies reporting that it occurs in virtually all subjects with reduced glomeruli number (4, 15). Further studies are required to examine the mechanisms involved in the hypertension induced by the reduction of ANG II effects during the nephrogenic period. Since hypertension is a major risk factor for a progressive renal disease (17), it is possible that the BP increment contributes to the same extent in both sexes to the time-dependent deterioration of renal function.

This study shows that GFR values in conscious 3-mo-old ARA-treated rats were similar to those found in control rats. These results are in disagreement with those showing that anesthetized ARA-treated male rats had a 12% decrease in GFR (25). One possible explanation for the discrepancy is that GFR was examined in anesthetized rats in the previous study (25). Renal hemodynamics would be more sensitive in ARA-treated male rats to the greater vasoconstrictor levels in anesthetized than in conscious animals (23, 24). Despite the discrepancy in the GFR values obtained, the results of both studies of our group support the notion that a decrease in nephron number during renal development leads to an earlier age deterioration of renal hemodynamics in males than in females.

Fig. 2. Tubular solute free water reabsorption ($T_{\text{osm}}^H$) changes in response to dehydration (DH) at 3 and 11 mo of age in rats treated with vehicle or ARA during nephrogenic period. $P < 0.05$ vs. *vehicle and +3 mo of age.
an alteration in medullary tubulogenesis (18). However, as far as we know, our study is the first showing that the RAS blockade during the nephrogenic period does not reduce in females the renal ability to concentrate urine in response to a prolonged dehydration. This is also the first study demonstrating that the impairment of renal ability to concentrate urine is age dependent in males. This notion is supported by the fact that both $T_{\text{H,O}}$ and $P_{\text{Osm}}$ were greater during water deprivation at 11 than at 3 mo of age in ARA-treated male rats.

The decrease of basal $U_{\text{Osm}}$ in ARA-treated males may not be attributable only to the reduction in papillary volume, since it was also found in ARA-treated females, and these females have a normal papillary volume (25). The lower basal $U_{\text{Osm}}$ in ARA-treated rats seems also to be secondary to an elevation in water intake. Further studies are required to determine the mechanisms responsible for the important activation of water intake when ANG II effects are blocked during the nephrogenic period. However, it has been proposed that this greater water intake is secondary to an increment in the number and affinity of central AT$_1$ receptors in adult life (5).

In summary, the results of this study present new evidence showing that a reduction in functional glomeruli during renal development leads to a greater decline in renal function and to a greater increment in proteinuria in males than in females that hasten the decline normally occurring with advancing age. It is also proposed that the decrease of ANG II effects during the nephrogenic period reduces only in males the urinary concentrating ability in response to a prolonged dehydration, and that this reduction is aggravated during aging.

ACKNOWLEDGMENTS

The authors thank Merck Sharp & Dohme for the generous supply of L-158,809.

GRANTS

This study was supported by a grant from Direccio `n General de Investigaci `on de Ministerio de Educaci `on y Ciencia (Spain) (SAF2003-08429). F. Salazar was supported by the same grant. A. Loria and V. Reverte were supported by a grant of Ministerio de Educacion y Ciencia (Spain) (SAF2003-08429). F. Salazar was supported by a grant from Direccio ´n General de Investigacion de Ministerio de Educacion y Ciencia (Spain) (SAF2003-08429). F. Salazar was supported by the same grant. A. Loria and V. Reverte were supported by a grant from the Consejeria de Sanidad de la Comunidad Autonoma de Murcia (Spain). F. Saez was supported by a predoctoral grant from the Ministerio de Educacion y Ciencia (Spain) (BES-2004-05369).

REFERENCES


5. Butler DG, Pak SH, Midgley A, Nemati B. AT1 receptor blockade with losartan during gestation in Wistar rats leads to an increase in thirst and sodium appetite in their adult female offspring. Regul Pept 105: 47–57, 2002.


