Changes in angiotensin receptors expression play a pivotal role in the renal damage observed in spontaneously hypertensive rats

Sharon L. Landgraf, Mira Wengert, Jaqueline S. Silva, Gisele Zapata-Sudo, Roberto T. Sudo, Christina Maeda Takiya, Ana Acacia S. Pinheiro, and Celso Caruso-Neves

The renal renin-angiotensin system plays a central role in the development of hypertension. The aim of this work was to verify the expression of angiotensin II receptors AT1R and AT2R in the microsomal fraction of renal cortex and correlate this with the development of hypertension and renal damage in spontaneously hypertensive rats (SHR) using Wistar-Kyoto rats (WKY) as controls. AT1R expression increased (126%) and AT2R expression decreased (66%) in 4-wk-old SHR; AT2 expression decreased in 14-wk-old SHR (61%) compared with respective age-matched WKY. These modifications were correlated to the increase in protein kinase C activity and decrease in protein kinase A activity.

Four-week-old SHR showed large accumulations of macrophages in kidney glomerulus and the tubulointerstitial area, dense cortical collagen deposition, and arterial proliferative changes in the walls of arterioles and medium-sized vessels. Similar modifications were also observed in 14-wk-old SHR. Four-week-old SHR treated with losartan (30 mg·kg⁻¹·day⁻¹) or hydralazine (15 and 30 mg·kg⁻¹·day⁻¹) by gavage for 10 wk did not develop hypertension. The decrease in AT1R expression and renal damage observed in SHR remained even after treatment with hydralazine. On the other hand, losartan treatment prevented the modifications observed in 14-wk-old SHR, indicating that renal injuries are caused specifically by AT1 rather than an increase in blood pressure. Our results indicate that the imbalance in AT1R and AT2R expression is associated with an inflammatory process that contributes to renal injury in adult SHR and to the development of hypertension.

\[ \text{renin-angiotensin system; kidney; angiotensin II receptors; inflammation}\]

\[ \text{THE KEY ROLE OF THE KIDNEY in primary hypertension pathogenesis has been proposed based on measurements of renal function and renal transplantation studies in spontaneously hypertensive rats (SHR) and human patients (10, 14). Renal damage is commonly caused by hypertension, which is a key to morbidity and mortality (23). On the other hand, renal damage could be involved in the genesis of hypertension, showing the dangerous loop in this correlation. Some studies showed functional alterations in tissues that precede the development of hypertension (17, 27, 32). Based on these observations, one attractive hypothesis is that asymptomatic alterations in renal function at an early stage could be contributed to development of the primary hypertension. Identification of the mechanisms responsible for these events becomes very attractive as an early marker of primary hypertension development and in prevention of hypertension. One candidate could be the renin-angiotensin system (RAS), which plays an important role in renal function and regulation of blood pressure.}\]

The effects of angiotensin II (ANG II) are mediated mainly by two receptors: angiotensin type 1 receptor (AT1R) or angiotensin type 2 receptor (AT2R) (11). AT1R activation is involved in vasoconstriction and proinflammatory action; AT2R counteracts the effects triggered by AT1R that are crucial to the final action of ANG II (11). Kobori et al. (20) observed that the content of ANG II in the renal cortex was increased in hypertensive 14-wk-old SHR, but it was not changed in prehypertensive young SHR. On the other hand, an increase in AT1R mRNA was observed in young SHR but not in adult SHR (15). In some tissues such as the vascular bed, in particular in the aorta and mesenteric artery, a decrease in AT2R expression was observed (9, 35). These results suggest that the intrarenal RAS may be inappropriately activated in primary hypertension. Overactivity of ANG II could be observed when AT1R expression or the AT1R/AT2R ratio increased.

Despite the evidence indicating that there could be an imbalance in angiotensin receptors during the development of hypertension, little is known about the correlation between changes in AT1R and AT2R expression and the consequences on renal function and renal injury. Some questions still remain to be answered: 1) Is there any correlation between AT1R and AT2R expression in SHR? 2) Is the imbalance between angiotensin receptors responsible for renal injuries? 3) Is there renal injury before hypertension is established? The aim of this work was to verify the expression of renal cortex AT1R and AT2R and correlate this with the development of primary hypertension and renal damage in prehypertensive and hypertensive SHR.

\[ \text{METHODS}\]

Animals and experimental protocol. Male Wistar-Kyoto (WKY) and SHR aged newborn, 4 (prehypertensive young), or 14 (hypertensive adult) wk were used in the experiments. All the animal procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Ethics Committee of Federal University of Rio de Janeiro (number IBCCF004).
Animals were randomly divided into five groups according to the treatment: group 1, WKY-V, used as normotensive controls, received vehicle (water); group 2, SHR-V, SHR treated with vehicle; group 3, SHR-L, SHR treated with 30 mg/kg losartan; group 4, SHR-H15, SHR treated with 15 mg/kg hydralazine; and group 5, SHR-H30, SHR treated with 30 mg/kg hydralazine. We chose hydralazine 15 and 30 mg·kg⁻¹·day⁻¹ because these doses have been found to reduce blood pressure levels in SHR (16, 35). The treatments lasted 10 wk and were administered by gavage once daily. The body weight was measured daily for adjustment of the losartan and hydralazine doses. Blood pressure levels in SHR (16, 35) were administered by gavage once daily. The body weight was measured daily for adjustment of the losartan and hydralazine doses. Blood pressure was monitored weekly in conscious rats using tail-cuff plethysmography for the duration of the treatment. To validate the results obtained by the tail-cuff method, a plastic catheter (PE-50) was introduced into the right carotid artery and connected to a pressure transducer (Spectramed model P23XL; Johannesburg, South Africa) for measuring arterial blood pressure in conscious rats. Data were recorded on a polygraph (Grass Technologies, model 7400, West Warwick, RI). After the treatment, the animals were housed in metabolic cages as described below. The animals were anesthetized with 100 mg/kg of ketamine and 20 mg/kg of xylazine and then a blood sample was obtained from the vena cava and the kidneys were removed. One kidney was used for immunohistological studies and the other kidney was used to prepare the microsomal fraction of the kidney cortex as described below.

Measurement of renal function. To determine renal function the animals, SHR and WKY were housed in individual metabolic cages before and after treatment. The cages were placed in a temperature-controlled room regulated on a 12:12-h light-dark cycle, with free access to standard chow and tap water. After an adaptation period of 48 h, urine volume and water were measured for the next 24 h. Urine samples were centrifuged at 3,000 g for 5 min and the supernatant was separated and stored at −20°C until assayed for total protein, sodium, and creatinine concentrations. Blood samples were collected and centrifuged at 2,000 g for 10 min to obtain plasma to measure sodium and creatinine concentrations. Sodium levels were measured by the Iselab clinical testing apparatus (DRAKE, São Paulo, SP, Brazil); creatinine and urinary protein concentrations were determined by colorimetric kits (Gold Analisa, Belo Horizonte, MG, Brazil). Urinary fractional excretion of sodium (FENa) and creatinine clearance were calculated. The glomerular filtration rate (GFR) was obtained by creatinine clearance in conscious animals.

Preparation of the microsomal fraction. The microsomal fraction of renal cortex was obtained as described previously (33). Briefly, the kidneys were removed and homogenized in a cold solution containing (mmol/l) 250 sucrose, 10 HEPES-Tris (pH 7.6), 2 EDTA, and 1 PMSF. The homogenate was centrifuged at 7,112 g at 4°C for 10 min and the final supernatant was ultracentrifuged at 223,171 g at 4°C for 1 h. The pellet was resuspended and homogenized in 250 mmol/l sucrose stored at −20°C. Protein concentrations were determined by the Folin phenol method (22) using bovine serum albumin as standard.

Cell culture. LLC-PK1 cells, a well-characterized porcine proximal tubule cell line (American Type Culture Collection, Rockville, MD), were maintained in DMEM (GIBCO-BRL) with 10% fetal bovine serum and 1% penicillin and streptomycin (37°C and 5% CO2). Before use, the cells were kept overnight in medium depleted of serum and incubated with 10⁻¹² and 10⁻¹⁰ mol/l phorbol 12-myristate 13-acetate (PMA) overnight.

Immunoblotting. AT₁R and AT₂R were immunodetected in the microsomal fraction of renal cortex with specific primary antibodies [1:500; Santa Cruz Biotechnology (AT; SC-579 and AT₂; SC-9040), Santa Cruz, CA]. Proteins were resolved on SDS-PAGE gels and transferred to polyvinylidene difluoride membranes (Amersham Biosciences, Piscataway, NJ) according to the manufacturer’s instructions. After antibody labeling, detection was performed with ECL Plus (Amersham Biosciences). Densitometry determinations were calculated as the ratio between the receptors and actin expression [goat polyclonal anti-actin; 1:500; Santa Cruz Biotechnology (1:500). *Statistically significant compared with age-matched WKY (P < 0.05). †Statistically significant compared with SHR-V (P < 0.05). ‡Statistically significant compared with SHR-L (P < 0.05). §Statistically significant compared with SHR-H30 (P < 0.05).

Table 1. SBP and renal function parameters in SHR

<table>
<thead>
<tr>
<th>Age, wk</th>
<th>Animal</th>
<th>Body Wt, g</th>
<th>Daily Urine Volume, ml</th>
<th>SBP, mmHg</th>
<th>GFR, ml/min</th>
<th>GFR, ml·min⁻¹·100 g⁻¹</th>
<th>UPCR</th>
<th>FENa⁺, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>WKY</td>
<td>75.1 ± 3.93</td>
<td>2.5 ± 0.31</td>
<td>114 ± 5.10</td>
<td>0.43 ± 0.04</td>
<td>0.58 ± 0.03</td>
<td>0.19 ± 0.02</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>SHR</td>
<td>58.9 ± 2.81*</td>
<td>1.3 ± 0.15*</td>
<td>115 ± 2.51</td>
<td>0.21 ± 0.03*</td>
<td>0.32 ± 0.015*</td>
<td>0.32 ± 0.03</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>14</td>
<td>WKY-V</td>
<td>334.5 ± 10.10</td>
<td>9.5 ± 0.67</td>
<td>125 ± 4.83</td>
<td>2.95 ± 0.15</td>
<td>0.91 ± 0.06</td>
<td>0.52 ± 0.03</td>
<td>0.22 ± 0.018</td>
</tr>
<tr>
<td>14</td>
<td>SHR-V</td>
<td>241.6 ± 6.47*</td>
<td>5.5 ± 0.47*</td>
<td>196 ± 9.62*</td>
<td>2.24 ± 0.23*</td>
<td>0.95 ± 0.09</td>
<td>1.88 ± 0.18*</td>
<td>0.10 ± 0.008*</td>
</tr>
<tr>
<td>14</td>
<td>SHR-L</td>
<td>251.2 ± 15.44*</td>
<td>15.2 ± 2.16*</td>
<td>127 ± 1.70†</td>
<td>1.42 ± 0.05†</td>
<td>0.55 ± 0.05*</td>
<td>1.12 ± 0.08*</td>
<td>0.57 ± 0.081†</td>
</tr>
<tr>
<td>14</td>
<td>SHR-H15</td>
<td>273.3 ± 15.53*</td>
<td>9.3 ± 1.66 ***§</td>
<td>124 ± 3.91†</td>
<td>1.20 ± 0.11***§</td>
<td>0.42 ± 0.03***§</td>
<td>2.13 ± 0.18*</td>
<td>0.36 ± 0.038***§</td>
</tr>
<tr>
<td>14</td>
<td>SHR-H30</td>
<td>276.6 ± 21.21†</td>
<td>13.1 ± 1.29†</td>
<td>127 ± 1.53†</td>
<td>0.90 ± 0.06†</td>
<td>0.31 ± 0.01***§</td>
<td>2.52 ± 0.21†</td>
<td>0.72 ± 0.021†</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. SBP, systolic blood pressure monitored by tail-cuff plethysmography; GFR, glomerular filtration rate; UPCr, ratio between urinary protein and creatinine; FEna⁺, fractional excretion of sodium; Wistar-Kyoto rats treated with vehicle (water); SHR-V, spontaneously hypertensive rats treated with vehicle; SHR-L, SHR treated with 30 mg·kg⁻¹·day⁻¹; SHR-H15, SHR treated with 15 mg·kg⁻¹·hydralazine ·day⁻¹; and SHR-H30, SHR treated with 30 mg·kg⁻¹·hydralazine ·day⁻¹. All animals were treated as described in METHODS. The values of SBP measured by invasive method (WKY-V = 132 ± 2.84; SHR-V = 198 ± 12.82; SHR-L = 122 ± 6.50; SHR-H15 = 125 ± 1.53; SHR-H30 = 118 ± 3.30) were similar to noninvasive methods, as indicated in the table. *Statistically significant compared with age-matched WKY (P < 0.05). †Statistically significant compared with SHR-V (P < 0.05). ‡Statistically significant compared with SHR-L (P < 0.05). §Statistically significant compared with SHR-H30 (P < 0.05).
lamina elastica externa. Therefore, these parameters were considered to select and measure the medium-sized arteries. To discriminate them from the other arteries, the following aspects were considered. Small-sized arteries (e.g., the interlobular arteries) are only located in the cortex and have no lamina elastica externa. The interlobar arteries are located in the medulla. Immunohistochemistry was performed by the avidin-biotin-peroxidase method using the LSAB-HRP kit from Dako (Carpinteria, CA) according to the manufacturer’s instructions. The chromogen substrate was diaminobenzidine.

The cortical collagen content was achieved using image analysis software (Image-Pro Plus software). Twenty-five microscopic fields of interstitial areas per animal were randomly captured (high-quality images 2,048 x 1,536 pixels), avoiding glomeruli and vessels to obtain the percentage area of collagen deposited.

For the quantification of ED1-positive cells, 25 consecutive microscopic fields of glomeruli and the tubulointerstitial region were captured for each rat, and ED1-positive cells were counted. Data were expressed as macrophages per glomeruli (means ± SE) and tubulointerstitial macrophages per mm² (means ± SE). The thickness of arteriolar and medium-sized arterial vessel walls was also measured using Image-Pro Plus software, in the fields examined for each rat, and the average thickness of the vessel walls was obtained for each rat. Data are expressed as means ± SE.

Statistical analysis. The means were compared by one-way ANOVA taking into account the treatment in the experimental groups. The magnitude of the differences was evaluated using the multiple comparative Bonferroni test. The correlation between AT₁/AT₂ ratio and PKC activity was determined by the Durbin-Watson statistic. The values of all parameters were considered significantly different at P < 0.05.

RESULTS

Systolic blood pressure and renal function parameters. The results in Table 1 show that the 24-h urine flow of 4- and 14-wk-old SHR was lower than their respective age-matched WKY. However, after 10 wk of treatment with losartan and hydralazine, SHR-L and SHR-H30 showed a higher urine flow compared with WKY-V and SHR-V. The SHR-H15 exhibited
a higher urine flow only when compared with SHR-V. Water intake was unchanged in all groups. The reduced urinary volume observed in SHR-V was accompanied by a decrease in FENa/H11001. On the other hand, the increased urinary volume observed in animals treated with losartan and hydralazine was accompanied by an increase in FENa/H11001 compared with WKY-V and SHR-V. No changes in FENa/H11001 were detected in young SHR compared with age-matched WKY. These results are in agreement with a decrease in proximal tubule Na+/H11001-ATPase activity in SHR treated with losartan (29).

Systolic blood pressure (SBP) was similar in 4-wk-old WKY and SHR. The hypertensive animals showed a progressive increase in SBP up to an average of 196 ± 9.62 mmHg at 14 wk, whereas age-matched WKY maintained their SBP at basal levels. Losartan and hydralazine treatment for 10 wk prevented the development of hypertension in SHR. The GFR, estimated by the creatinine clearance, was lower in young and adult SHR than their respective age-matched controls. However, there was no difference in this parameter between SHR-V and WKY-V when the GFR was corrected by body weight. Both losartan (SHR-L) and hydralazine (SHR-H15 and SHR-H30) treatments decreased the GFR compared with SHR-V. The SBP measured by the tail-cuff method was confirmed using an invasive method with a plastic catheter (PE-50) introduced into the right carotid artery and connected to a pressure transducer (Spectramed model P23xL).

The ratio between urinary protein and creatinine (UPCr) was higher in both 4- and 14-wk-old SHR than their age-matched WKY. Losartan treatment partially reversed the UPCR ratio compared with WKY-V. On the other hand, the administration of hydralazine did not modify the increase in UPCR ratio observed in SHR-V compared with WKY-V. The UPCR ratio observed in SHR treated with hydralazine (SHR-H15 and SHR-H30) was higher than that observed in SHR treated with losartan (SHR-L).

Differential expression of AT1R and AT2R between SHR and WKY. Initially, the expression of ANG II receptor subtypes in the microsomal fraction of the renal cortex of WKY and SHR was analyzed by immunoblotting (Fig. 1). AT1R and AT2R expression was unaltered in prehypertensive newborn (2 days old) SHR compared with age-matched WKY (Fig. 1, A and B).
In prehypertensive 4-wk-old SHR, AT$_1$R expression increased (126%) and AT$_2$R expression decreased (66%) compared with age-matched WKY rats (Fig. 1, C and D). In hypertensive 14-wk-old SHR, AT$_2$R expression still decreased 61% compared with 14-wk-old WKY, whereas there was no change in AT$_1$R expression (Fig. 1, E and F).

Signaling-coupled pathways to AT$_1$R and AT$_2$R. In a previous paper we observed that, in the proximal tubule, PKC activity is associated with AT$_1$R activation, and PKA activity is associated with AT$_2$R activation (12, 30). The activity of both protein kinases could be a good parameter to measure the functionality of each receptor. PKC activity was increased 9 and 2 times in 4- and 14-wk-old SHR, respectively, compared with their age-matched WKY (Fig. 2A). On the other hand, PKA activity was decreased in SHR at both ages (Fig. 2A). Furthermore, there is a significant correlation between the increase in PKC activity and the AT$_1$R/AT$_2$R ratio ($r$/$H_1^{0.751}$; $P$/$H_1^{0.0005}$; Fig. 2B).

So far, the scenario indicates that in hypertensive animals there is an imbalance between AT$_1$R and AT$_2$R and, consequently, between the cellular pathways associated with them, PKC and PKA, respectively. Furthermore, the observation that AT$_1$R expression is enhanced only in 4-wk-old SHR could suggest that AT$_1$R promotes some changes involved in inhibition of AT$_2$R expression. To clarify this issue, LLC-PK$_1$ cells, a well-known model of proximal tubule cells, were treated with a phorbol ester, PMA ($10^{-12}$ and $10^{-10}$ mol/l), PKC activator, and coupled to AT$_1$R. The AT$_1$R expression was not different from controls for both PMA concentrations (Fig. 3A); however, AT$_2$R expression decreased 46% with both PMA concentrations (Fig. 3B).

To investigate the role of AT$_1$R and AT$_2$R expression in vivo, we treated 4-wk-old SHR with 30 mg·kg$^{-1}$·day$^{-1}$ losartan (SHR-L) or vehicle (SHR-V) for 10 wk by gavage. AT$_1$R expression was not modified in the different groups (Fig. 4A). On the other hand, AT$_2$R expression decreased 48% in SHR-V compared with age-matched WKY-V (Fig. 4B). Losartan treatment restored AT$_2$R levels to control values (WKY-V). Under these conditions, the high blood pressure was also reduced to the control value (see Table 1). These results indicate that the
effect of losartan could be due to 1) inhibition of cellular response coupled to AT$_1$R or 2) the decrease in blood pressure induced by losartan. To solve this question, we used another antihypertensive drug, hydralazine, which is not related to RAS. Hydralazine treatment with 15 (SHR-H15) or 30 mg·kg$^{-1}$·day$^{-1}$ (SHR-H30) decreased blood pressure (see Table 1). On the other hand, the decrease in AT$_2$R expression observed in SHR-V remained even after treatment with both doses of hydralazine (SHR-H15 and SHR-H30) compared with control WKY-V (Fig. 4D). AT$_1$R expression was unchanged by hydralazine treatment (SHR-H15 and SHR-H30; Fig. 4C). These results show that the effect of losartan in AT$_1$R and AT$_2$R expression is not due to the decrease in the blood pressure.

To confirm that the effect of losartan is correlated to cellular response coupled to AT$_1$R and AT$_2$R, we measured PKC and PKA activities. Losartan treatment restored PKC and PKA activities (Fig. 4E). After the losartan treatment, PKC activity was significantly lower in SHR-L than in the control (WKY-V).

We then investigated the level of renal damage. At this point, the question was whether the renal inflammatory process was a consequence of hypertension or renal injury was already present before the development of hypertension. To address this question, we used 4-wk-old SHR in which the hypertension was not established and 14-wk-old SHR in which the hypertension was already established.

**Smooth muscle proliferation of arteriole and medium-sized vessel walls.** The magnitude of smooth muscle proliferation was evaluated by measuring the thickness of arteriolar and medium-sized vessel walls stained with monoclonal mouse anti-α-SMA antibody. Four-week-old SHR already showed significant increase in smooth muscle thickness on both arteriole and medium-sized vessel walls compared with age-matched WKY (Fig. 5, B and D, respectively). Similar results were obtained in 14-wk-old SHR compared with adult WKY (Fig. 6, B and G). Losartan treatment for 10 wk completely reversed the increase in smooth muscle thickness in arterioles (Fig. 6C) but did not change the thickness in medium-sized vessels in adult SHR compared with WKY-V (Fig. 6H). The renal damage was also analyzed in SHR treated with 15 and 30 mg·kg$^{-1}$·day$^{-1}$ hydralazine to verify whether hypertension or the renal AT$_1$/AT$_2$ ratio per se was responsible for renal injury. Both hydralazine treatments decreased blood pressure (see Table 1), but did not show any improvement in the magnitude of smooth muscle proliferation in arterioles (Fig. 6, D and E) and medium-sized vessels of adult SHR (Fig. 6, J and H). These results suggest that losartan reverses the increase in smooth muscle thickness in arterioles independent of blood pressure normalization.

**Glomerular and tubulointerstitial infiltration.** The extent of the glomerular and tubulointerstitial infiltration was quantified by the number of ED1-positive macrophages in glomeruli and in the tubulointerstitial area, respectively. Large accumulations of ED1-positive cells were observed in both glomeruli and the tubulointerstitial area in 4- and 14-wk-old SHR (Figs. 7 and 8). Losartan treatment partially reversed the glomerular macrophage infiltration observed in 14-wk-old SHR compared with age-matched WKY (Fig. 7E). Furthermore, losartan treatment completely reversed tubulointerstitial macrophage infiltration in hypertensive adult SHR compared with WKY-V (Fig. 8E). On the other hand, hydralazine treatment did not modify the macrophage infiltration in glomeruli and tubulointerstitium observed in 14-wk-old SHR (Figs. 7, F and G, and 8, F and G, respectively), suggesting that losartan-mediated renal injury attenuation was independent of blood pressure.

**Cortical interstitial fibrosis.** The degree of fibrosis was visualized by Picosirisu Red staining for collagen fibers. Collagen deposition was enhanced in 4- and 14-wk-old SHR compared with age-matched WKY (Fig. 9, B and D, respectively). Losartan treatment for 10 wk reduced fibrosis completely in 14-wk-old SHR compared with age-matched WKY (Fig. 9E). Both hydralazine treatments failed to reverse the increase in collagen deposition observed in hypertensive 14-wk-old SHR (Fig. 9, F and G). Furthermore, collagen deposition in SHR-H15 was higher than in SHR-V (Fig. 9F).

**DISCUSSION.**

In this study, we found changes in AT$_1$R and AT$_2$R expression in the renal cortex of both young and adult SHR leading to an increase in the AT$_1$R/AT$_2$R ratio and, consequently, an overactivation of the PKC-signaling pathway coupled to AT$_1$R. This overactivation of AT$_1$R is involved in renal damage and development of hypertension. Furthermore, it was observed that renal damage preceded the development of hypertension in SHR, indicating that renal injuries are likely a causative factor rather than only a consequence of the establishment of hypertension. Our results open new perspectives to understand the role of the kidney in the development of hypertension.
Fig. 6. Effects of losartan treatment on the thickness of arteriole in SHR. Animals were treated as described in methods. The conditions are described in Table 1 legend. A–E: representative photomicrographs of arterioles: WKY-V (A), SHR-V (B), SHR-L (C), SHR-H15 (D), and SHR-H30 (E). F–J: representative photomicrographs of medium-sized vessels: WKY-V (F), SHR-V (G), SHR-L (H), SHR-H15 (I), and SHR-H30 (J). Bar = 20 μm. Quantitative analysis of the thickness of arteriole (L) and medium-sized vessel walls (M; n = 6 per group). Values are expressed as means ± SE. Arrows indicate arterioles (A–E) and medium-sized vessels (F–J). *Statistically significant compared with WKY-V (P < 0.05). #Statistically significant compared with SHR-V (P < 0.05). +Statistically significant compared with SHR-L (P < 0.05).
primary hypertension, with AT1R playing a pivotal role in this process.

Here, we observed that AT1R expression is enhanced in prehypertensive rats, which leads to an increase in PKC activity and this is followed by a decrease in AT2R expression. In agreement, it has been shown that AT1R mRNA expression in prehypertensive 4-wk-old SHRs is increased (15). The change in the AT1R/AT2R ratio is correlated with renal damage observed in adult SHRs because the treatment with losartan for 10 wk completely reversed the decrease in AT2R expression and most of the renal injuries observed in nontreated adult SHRs. This effect of losartan seems to be due to its action on AT1R-coupled pathways because hydralazine treatment at low and high doses, which decreases blood pressure similarly to losartan, did not reverse the renal injury and the decrease in AT2R expression observed in SHR. The AT2R differential expression observed in SHRs does not seem to be specific for renal tissue. A decrease in AT2R expression has also been reported in the mesenteric artery and aorta of SHRs (9, 35). These data indicate that imbalance in the expression of angiotensin receptors could be responsible, at least in part, for injuries observed in different tissues before the development of hypertension.

Our results point to a crucial role of the AT1R and AT2R ratio in the renal damage observed in SHR and, consequently, in the development of hypertension. In addition, the observation that losartan treatment reversed inhibition of AT2R expression and renal damage indicates that modifications in AT1R expression could be a causative factor more than a consequence of hypertension development. This hypothesis is supported by the observation that the increase in AT1R expression occurs in 4-wk-old SHR when hypertension is not yet established. It is possible to postulate that the absence of a regulatory effect of AT2R on the action of AT1R plays an important role during renal injury in SHRs (5, 26). This hypothesis is strengthened by the observation that the overexpression of AT2R ameliorates renal injury in a 5/6 nephrectomy model (26). In addition, Benndorf et al. (5) showed that AT2R deficiency in mice aggravates renal injury in chronic kidney disease. More recently, the crucial role of AT2R in the beneficial effects of AT1R blocker in the treatment of glomerular injury was shown (25).
Contrary to the view that AT2R counteracts the actions of AT1R, some evidence shows that AT2R could trigger a cellular response leading to proinflammatory effects in various cell lines, such as PC12 cells and rat glomerular endothelial cells (34). In agreement with this view, it was observed that an antagonist of AT2R has an anti-inflammatory effect in models of renal disease (6). One possible explanation for the apparent contradictory effects of AT2R on renal disease could be due to the mutual expression and modulation between AT1R and AT2R. When AT2R is blocked, the effect observed could be due to changes in AT1R expression rather than the block on AT2R.

The observation that AT1R expression is not changed in newborn SHRs and increases in 4-wk-old SHRs compared with their respective age-matched control WKY indicates that some error in the regulation of this receptor expression occurs between birth and the fourth week in SHR. Previous studies demonstrated that ANG II increases AT1R expression in proximal tubules (8). However, a possible role of ANG II in the increase in AT1R expression in prehypertensive SHRs is unlikely, because it has been observed that the intrarenal ANG II level is not changed in these animals (20). It has also been shown that the expression of AT1R can be regulated by several substances such as growth factors, mineralocorticoids, and free radicals, which open several possibilities for the increase in AT1R expression in prehypertensive SHRs (2, 3). Further studies are necessary to clarify this issue.

The results showing that there is an association between prehypertension and inflammatory markers besides endothelial dysfunction have led some researchers to propose that previous intervention could avoid the establishment of hypertension (7). Norrelund et al. (27) showed that a narrowed lumen in renal afferent arterioles in 7-wk-old SHRs is involved in the pathogenesis of hypertension in this animal model. Here, we showed, besides the increase in the thickness of the arterioles, an increase in medium-sized vessels, glomerular and interstitial inflammation, and collagen deposition in 4-wk-old SHRs. These modifications were followed by an increase in UPCr in 4-wk-old SHRs. These results reinforce the result obtained by Lopes de Faria et al. (21) that 4-wk-old SHRs have higher growth in mesangial cells. These changes are followed by a change in the GFR similar to the results obtained by other
These data indicate that renal damage precedes the development of hypertension and that some genetic renal disorder occurs that could be responsible for the development of hypertension. In contrast with these results, Kobori et al. (20) did not observe interstitial infiltration, glomerular sclerotic, and arteriolar thickness in 7-wk-old SHRs. These contradictory effects could be due to differences in the techniques used in the studies.

In the present work, we observed that hydralazine increases $\text{FENa}^{+}$, which was unexpected for an antihypertensive drug not related to RAS. Similar results were observed by Kline and McLennan (19) in 7- to 9-wk-old SHR. They showed that chronic hydralazine treatment (20 mg·kg$^{-1}$·day$^{-1}$) was able to increase the $\text{FENa}^{+}$. These authors proposed that this effect could be correlated to changes in the acute pressure-natriuresis curve. In a previous work, Green (13) observed that rats treated with hydralazine (20 mg·kg$^{-1}$·day$^{-1}$) have increased $\text{FENa}^{+}$ and that this effect was prevented by pretreatment with indomethacin. This result indicates that prostaglandins mediate the effect of hydralazine on the $\text{FENa}^{+}$. However, the mechanism involved in the increase of $\text{FENa}^{+}$ by hydralazine has still to be determined.

Several studies show that ANG II is central in inflammatory processes (31). The treatment of the prehypertensive SHRs for 10 wk with losartan abolished or significantly ameliorated renal injury indicating a pivotal role in renal damage observed in SHRs. Similar results were obtained in the Trial of Preventing Hypertension (TROPHY) study. In this study, it was shown that pharmacological treatment of prehypertensive patients with the AT$_1$R antagonist, candesartan, can postpone the development of hypertension (18). In a previous paper, we observed that treatment of 4-wk-old SHRs up to 10 wk of age prevents the development of hypertension up to 14 wk of age (29). Furthermore, Baumann et al. (4) showed that prehypertensive treatment with losartan postpones the development of hypertension until 48 wk of age and had cardioprotective effects up to 72 wk of age.

Despite the prevention of hypertension in SHRs treated with losartan, we observed that the increase in thickness of medium-sized vessel walls was unchanged. Other factors besides ANG...
II and AT1R could be involved in the inflammatory renal process in prehypertensive hypertension. In renal tissues, the mechanism of AT1R action in the tissue inflammation during the hypertension is not known. Tanabe et al. (31) demonstrated that residual aldosterone has a significant effect on target organ damage in hypertension, even during chronic administration of an AT1R blocker in SHR. In agreement with this hypothesis, Baumann et al. (3) observed that treatment with spironolactone reduced blood pressure in SHR up to 36 wk of age, and this was associated with a reduction in cardiac and renal collagen deposition. It is known that cAMP has an inhibitory effect on the production of inflammatory mediators such as TNF-α (1) and reactive oxygen intermediate generation. Furthermore, Preiss et al. (28) showed that PKC is critical for PLA-LDK-induced Akt/PKB phosphorylation and survival in thp-1 monocytic cells. The observation that prehypertensive and hypertensive SHR have renal inflammatory processes could be associated with the observation that these animals have higher PKC and lower PKA activities. Our results show that changes in renal AT1R expression lead to a decrease in AT1R expression, increase in PKC activity, decrease in PKA activity, an inflammatory process, and development of hypertension.

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No conflicts of interest, financial or otherwise, are declared by the author(s).

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