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Glomerular Losartan (DuP 753)-Sensitive Angiotensin II Receptor Density Is Increased in Young Spontaneously Hypertensive Rats[1]

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ABSTRACT. There is increasing evidence that an activated intrarenal renin-angiotensin system (RAS) alters renal hemodynamics and fluid balance and that such events may lead to the development of hypertension. To examine the role of the glomerular RAS in the development of hypertension in the spontaneously hypertensive (SHR) rat, we studied angiotensin (ANG) II receptors in isolated glomeruli from young (4- to 5-wk-old) and adult (10- to 12-wk-old) SHR and from age-matched, normotensive Wistar-Kyoto (WKY) rats. Glomerular ANG II receptor density in young SHR is 3-fold higher than in age-matched WKY rats (2033 ± 154 *versus* 742 ± 151 receptors/μm^2; $p < 0.05$) and 1.5-fold higher than in adult SHR and WKY rats (1128 ± 85 and 1198 ± 181 receptors/μm^2, respectively; $p < 0.05$). Additional studies demonstrated that the differences in receptor density are not related to disparity in receptor occupancy and that they are also independent of systemic ANG levels. Suppression of RAS by ANG converting enzyme inhibitors resulted in a 3-fold increase in receptor density in young SHR rats and a 4.5-fold increase in young WKY rats; receptor density remained greater in young SHR rats (5915 ± 318 *versus* 3358 ± 234 receptors/μm^2, $p < 0.05$). Furthermore, competitive binding experiments using the nonpeptide ANG II antagonists losartan (AT_1) and PD 123319 (AT_2) indicate that the greater ANG II receptor density in the young SHR rats represents an increase in the number of a single population of AT_1 receptors. We postulate that increased glomerular ANG II receptor density in the young SHR rats may contribute to the initiation of hypertension in this animal model. (*Pediatr Res* 35: 671–676, 1994)

Abbreviations

ANG, angiotensin
SHR, spontaneously hypertensive, (Aoki-Okamoto) strain of rats
WKY, Wistar-Kyoto strain of rats
RAS, renin-angiotensin system
GFR, glomerular filtration rate
IC_{50}, 50% inhibiting concentration

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It is has long been postulated that the kidney plays a major role in the pathogenesis of genetic hypertension in rats (1–3). Among the many mechanisms proposed for the role of the kidney in the initiation of primary hypertension, several pertain to alterations in glomerular hemodynamics. Events resulting in the reduction of glomerular filtration surface area lead to decreased GFR and impaired sodium excretion (1, 3) with subsequent extracellular fluid volume expansion that could lead to the development of systemic hypertension (4). An observation consistent with this premise is that GFR (5) and water excretion (1) are decreased in SHR compared with age-matched WKY rats before the onset of hypertension (5).

ANG II is a major regulator of systemic and renal hemodynamics as well as glomerular function (6, 7). ANG II activates receptors and induces mesangial contraction (8), thereby reducing glomerular surface area, ultrafiltration coefficient, and GFR (6). Despite similar circulating ANG II concentrations in SHR and age-matched WKY rats (9), prior treatment with the ANG II receptor antagonist losartan attenuates the rise in blood pressure that is observed normally with age (10). We postulate that activated glomerular ANG II receptors may account for altered glomerular function, as reported in prehypertensive rats (1, 3–5). Although many studies have examined glomerular ANG II receptors in rats, only two have investigated the role of glomerular ANG II receptors in the initiation of hypertension in SHR rats (11, 12). These studies have shown that receptor density and affinity are similar in the young SHR rats and their age-matched controls. These investigations, however, did not characterize glomerular ANG II receptor subtype during the development of hypertension. Recent availability of specific nonpeptide antagonists has suggested that ANG II receptor subtype changes with development (13).

The purpose of this study was to measure glomerular ANG II receptor density and binding affinity in the young SHR rats, evaluate their response to pharmacologic and biochemical stimuli, and characterize ANG II receptor subtype during the development of hypertension. We found that the density of glomerular ANG II receptors, which are primarily losartan (AT_1 subtype) sensitive, is increased in 4-wk-old SHR compared with age-matched WKY and adult SHR rats. The difference in receptor density is not related to alterations in receptor occupancy or circulating ANG concentration.

MATERIALS AND METHODS

Animals. Male SHR and WKY control rats were obtained at least 3 d before study from Harlan Farms, Indianapolis, IN. The animals were maintained on natural ingredient 4% fat rat food (TD 170705, Tek-lad, Madison, WI) with 0.44% (0.2 mEq/g) sodium, 0.97% potassium, and 1.85% calcium and had free access to tap water. The four study groups consisted of young SHR and age-matched WKY rats (4–5 wk old, 55–80 g) and

adult SHR and WKY rats (10–12 wk old, 195–250 g). In each experiment, five to seven young or two to four adult animals were used. A total of 96 young SHR, 98 young WKY, 52 adult SHR, and 61 adult WKY rats were killed. All studies outlined were approved by the Institutional Review Board at University of Texas Southwestern Medical Center.

Direct blood pressure was measured in one rat selected randomly from each group. Blood pressure was measured within 2 d of the assay. The mean of at least six measurements recorded 5 min apart was obtained. Animals were anesthetized with an intraperitoneal injection of 50–75 mg of pentobarbital (Abbott Labs, North Chicago, IL) per kg of body weight, and blood pressure measurements were obtained from a carotid artery catheter connected to a blood pressure transducer (G23db, Statham Instruments Inc., Oxnard, CA) and a recorder (R611, Beckman Instruments Inc., Schiller Park, IL). Blood pressure was assessed in 12 young SHR, 12 young WKY, 10 adult SHR, and 10 adult WKY rats.

Preparation of isolated glomeruli. Glomeruli were isolated by a modification of the mechanical sieving technique described by Fong and Drummond (14). Briefly, the animals were killed by rapid decapitation and their excised kidneys were chilled immediately in ice-cold oxygenated Krebs solution, which was used throughout the procedure as a rinsing buffer. The renal cortex was bisected and minced into a pastelike consistency. This material was pressed through a 150-μm bronze sieve and onto successive sieves (180-, 106-, and 75-μm openings for adults and 180-, 106-, and 53-μm openings for young rats). The material retained on the 75-μm (adult) or 53-μm (young) sieve was placed in a 50-mL siliconized conical tube and centrifuged four times at 120 × *g* for 3 min. The glomeruli obtained were resuspended in incubation medium. The incubation medium contained 20 mM sodium phosphate, 125 mM sodium chloride, 5 mM magnesium chloride (pH 7.4), 125 μg/mL 1–24 ACTH, and 2 g/dL BSA. ACTH was added to prevent ANG II degradation (15, 16). Recent studies in rat brains showed that ANG II receptors (AT_1) increased 2.3-fold when peptidase inhibitors were used during incubation (17). Aliquots of the final glomerular preparation were examined by light microscopy to verify purity (>90%). The average time needed for glomerular isolation was 45 min. The suspension of isolated glomeruli from each experiment was adjusted to a standard amount of protein (10–50 μg/mL) using the method of Lowry *et al.* (18).

Glomerular morphometrics. Aliquots of glomerular suspension were obtained from each experiment for measurement of planar surface area. Forty μL of glomerular suspension, containing around 200 glomeruli, were placed on glass slides with constructed walls to create a well to prevent distortion when covered with 22 × 22-mm coverslips. Glomerular diameter was measured in the horizontal plane using a 400× light microscope fitted with a micrometer. An average of three measurements of the diameter were obtained. Glomerular surface area (SA) was calculated using the following formula: $SA = 4\pi \cdot r^2$, where r = mean glomerular radius. Glomerular specimens were blinded to avoid sizing bias.

Systemic ANG II concentration in unrestrained awake rats. With the rats under ether anesthesia, a silicone elastomer medical-grade tube (0.05 cm inner diameter, 0.09 cm outer diameter) was implanted into the right external jugular vein as previously described by Harms and Ojeda (17). After surgery, the catheter was filled with heparinized saline (250 U/mL). Three to 4 d later, and 2 h before the time of blood sampling, a 15-cm long segment of PE-50 tubing was attached by a 23-gauge stainless steel connector to the free end of the cannula and was allowed to hang outside the cage, thus creating enough slack on the tubing to prevent the animal from damaging the catheter. Without disturbing the animal, 0.5 mL of blood was withdrawn into a chilled plastic syringe and was placed in a cold, siliconized, polypropylene tube containing 7.5 μL of 125 mM disodium EDTA and 7.5 μL of 25 μM chymostatin. The blood was centrifuged immediately at 4°C, and the plasma was separated and stored at −70°C. ANG peptides were separated by HPLC, and each fragment was assayed by RIA using polyclonal antibody as previously described (19).

ANG II radioligand binding assay. The binding of ANG II to isolated glomeruli was measured using the technique described by Wilkes (16) with minor modifications. The total incubation volume was 200 μL, consisting of glomeruli (10–50 μg of protein), 3×10^{-10} M [^{125}I]ANG II and increasing concentrations from 10^{-10} M to 10^{-4} M of the following unlabeled competitors: [Sar^1,Val^5,Ala^8]ANG II (saralasin), ANG II, Des,Asp^1-ANG II (ANG III), ANG I, losartan (specific AT_1, receptor antagonist), PD 123319 (specific AT_2 receptor antagonist), or [Arg^8]vasopressin acetate. The incubations were performed at 22°C and were terminated by the addition of 4 mL of ice-cold PBS. Bound and free radioligand were separated by filtration through 45-μm nitrocellulose Millipore filters (Millipore Corp., Bedford, MA), which were then rinsed three times with 4 mL of ice-cold PBS. The radioactivity associated with the filters was determined in a gamma counter (Compugamma 1282, LKB-Wallac, Turku, Finland) with 64% counting efficiency. Nonspecific binding was determined in each experiment by incubating glomeruli with 3×10^{-10} M [^{125}I]ANG II in the presence of 10^{-6} M unlabeled ANG II. Specific binding was calculated by subtracting nonspecific from total binding. Nonspecific binding represented approximately 0.1% of total radioactivity per assay tube, and it was always <10% of specific binding. Degradation of radiolabeled ANG II was assessed by incubation of glomeruli in the presence of 3×10^{-10} M [^{125}I]ANG II for 2 h at 22°C. Free radiolabeled ANG II and its fragments were then separated from the glomeruli by vacuum filtration through nitrocellulose Millipore filters and quantization by HPLC, using methodology we have described previously (19).

Receptor occupancy. To assess receptor occupancy by endogenous ANG II, isolated glomeruli from young SHR and WKY rats were incubated with $MgCl_2$ to dissociate bound ANG II from the receptor as previously described (20). In one set of experiments, glomeruli were preincubated with 3 M $MgCl_2$ for 3 min at 4°C before incubation for radioligand binding in the presence of $MgCl_2$. This resulted in a reduction in glomerular [^{125}I]ANG II binding to the level of nonspecific binding. In a second set of experiments, glomeruli were preincubated with $MgCl_2$ and then washed four times with ice-cold Krebs solution. The glomeruli were then resuspended in the incubation medium containing 25 μM chymostatin to inhibit glomerular production of ANG II (24), and radioligand binding studies were performed using the methods described above.

ANG II suppression. To study the effect of local ANG II production on glomerular receptor density, we pharmacologically inhibited glomerular ANG II production with enalaprilat (Merck Sharp and Dohme, West Point, PA). Enalaprilat (0.5 mg/kg body weight) was administered intraperitoneally in 10 doses over 5 d (21). This regimen suppressed systemic ANG II concentration in anesthetized rats from 11.6 to 3.6 pg/mL (69%).

Materials. 2-n-Butyl-4-chloro-5-hydroxymethy-1-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl] imidazole potassium salt (losartan, previously DuP 753) was supplied courtesy of E.I. duPont de Nemours and Company, Wilmington, DE. 1-{[4-(dimethylamino)-3-methylphenyl]methyl}-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c] pyridine-6-carboxylic acid, ditrifluoroacetate, monohydrate (PD 123319) was supplied courtesy of Parke-Davis, Ann Arbor, MI, and enalaprilat was provided courtesy of Merck Sharp and Dohme. Human [^{125}I]Tyr^4-ANG II (2200 Ci/mmol) was obtained from New England Nuclear, Boston, MA. All other reagents were obtained from Sigma Chemical Co., St. Louis, MO.

Data analysis. Radioligand binding studies were analyzed by nonlinear regression analysis of the data using the LIGAND program of Munson and Rodbard (22) adapted for microcomputers (Elsevier Biosoft, Cambridge, UK). Hill coefficients were

also calculated from the same program. Receptor density (B_{max}) was calculated by the following equation:

$$\text{receptors}/\mu m^2 = \frac{B_{max}\ (\text{fmol/mg}) \times 6.023 \times 10^8\ (\text{receptors/fmol})}{\text{glomeruli/mg} \times \text{glomerular surface area}\ (\mu m^2)}$$

Analysis of variance was used to compare mean values between groups. Significance was accepted if p was <0.05. Data are presented as mean ± 1 SEM.

RESULTS

Mean arterial pressure was not different between young SHR and WKY rats (86 ± 8 and 75 ± 5 mm Hg, respectively). However, mean arterial pressure was higher in adult SHR compared with adult WKY rats (156 ± 2 and 86 ± 5 mm Hg, respectively, $p < 0.01$). Planar glomerular surface area for young SHR, young WKY, adult SHR, and adult WKY rats was 5542 ± 148, 5226 ± 150, 11 936 ± 180, and 11 310 ± 202 μm^2, respectively. Glomerular surface area was significantly higher in adult compared with young rats ($p < 0.01$), but there was no difference between SHR and WKY rats in either age group. Systemic ANG II concentrations were similar in young SHR and WKY rats (8.4 ± 1.3 and 7.1 ± 2.6 pg/mL, respectively) (Table 1). In adult SHR and WKY rats, systemic ANG II concentrations were also similar (4.2 ± 0.77 and 3.7 ± 1.3 pg/mL, respectively) (Table 1). Systemic ANG II concentrations were higher in young compared with adult SHR or WKY rats ($p < 0.05$).

Under the incubation conditions used in our study, specific radioligand binding achieved equilibrium by 105 min and was stable for up to 4 h. We selected incubation periods for radioligand binding of 120 min. Specific radioligand binding was linear with glomerular protein concentration within the range used in all assays. After 2 h of incubation in the presence of glomeruli, ACTH, and albumin, 88 ± 1% ($n = 4$) of radiolabeled ANG II eluted in a single HPLC peak with a retention time identical with intact [^{125}I]ANG II. Without ACTH in the incubation medium, radiolabeled ANG II was degraded into multiple peptide fragments. Only 5 ± 2% of the radioactivity eluted at the same retention time as intact [^{125}I]ANG II. The mechanisms by which ACTH prevent ANG II degradation is not certain. We were also able to prevent ANG II degradation by the addition of 25 μM chymostatin, a protease inhibitor (21).

LIGAND analysis of ANG II binding in all four study groups indicated a one-site model (22) of high-affinity receptors as demonstrated by the linearity of the Scatchard plots k_d in the nanomolar range. Hill coefficients ranged between 0.91 and 1.05, indicating a lack of cooperativeness of receptor binding (22).

Changes in the three-dimensional structure of the glomerulus observed during development may alter the number of glomerular ANG II receptors (16). This variable was taken into account by calculating the number of receptor sites per unit surface area. When reported in this manner, receptor density in the young SHR rats was 3-fold higher compared with that in age-matched WKY rats (2033 ± 154 *versus* 742 ± 151 receptors/μm^2) and 1.7-fold higher (1198 ± 181 *versus* 1128 ± 85 receptors/μm^2) than that in adult WKY and SHR rats (Fig. 1). Receptor density increased 2-fold during maturation in the WKY rats ($p < 0.05$) but decreased during maturation in the SHR rats ($p < 0.05$).

Table 1. *Systemic ANG II concentrations of ANG II*

Animals	Systemic ANG II (pg/mL)
Young SHR rats	8.4 ± 1.3*
Young WKY rats	7.1 ± 2.7*
Adult SHR rats	4.2 ± 0.8
Adult WKY rats	3.7 ± 1.3

* $p < 0.05$ *vs* adult SHR and adult WKY rats.

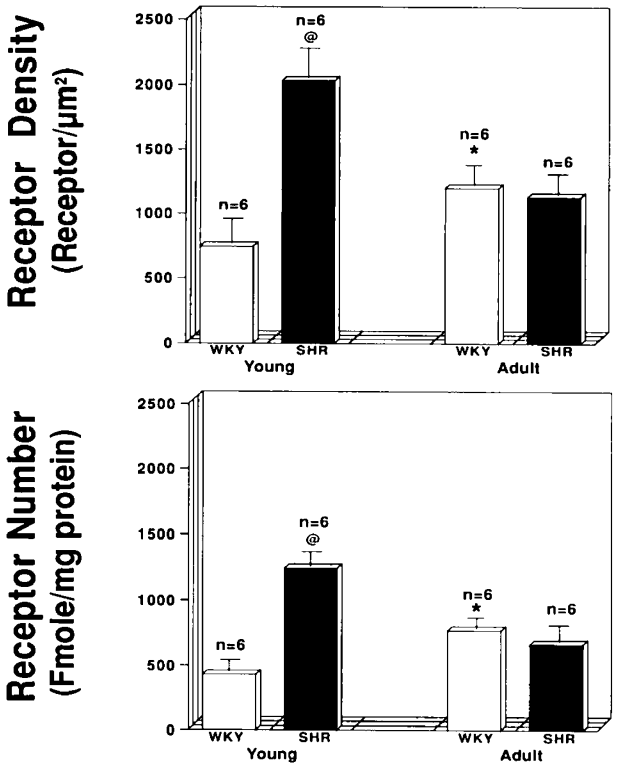

Fig. 1. Comparison of glomerular ANG II receptor density (receptors/μm^2 planar glomerular surface area, *upper panel*) and receptor number (fmol/mg protein, *lower panel*) in WKY and SHR rats at 4–5 wk of age (young) and 10–12 wk of age (adult). Receptor density and number in young SHR rats are increased compared with young WKY, adult SHR, and WKY rats (@, $p < 0.05$). Receptor density and number are increased in adult WKY rats compared with young WKY rats (*, $p < 0.05$). Values are mean ± SEM.

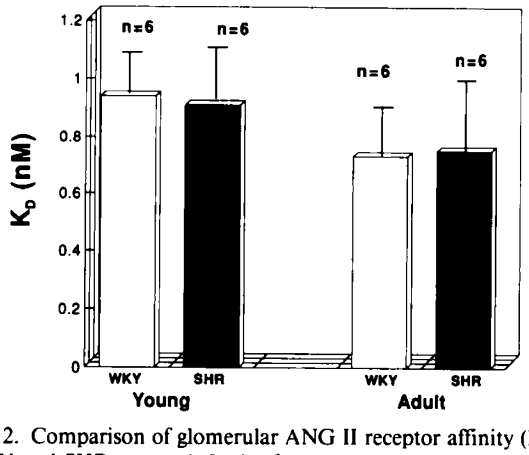

Fig. 2. Comparison of glomerular ANG II receptor affinity (k_d, nM) in WKY and SHR rats at 4–5 wk of age (young) and 10–12 wk of age (adult). Values are mean ± SEM.

Receptor number reported in fmol/mg glomerular protein in the young SHR was also 3-fold higher (1245 ± 30 *versus* 430 ± 42 fmol/mg protein) compared with that in age-matched WKY rats and 1.7-fold higher than that in adult WKY and SHR rats (768 ± 81 *versus* 663 ± 50 fmol/mg protein). Receptor affinity (k_d) for ANG II was similar (0.75 ± 0.12, 0.78 ± 0.13, 0.81 ± 0.15, and 0.80 ± 0.13 nM) for SHR rats and WKY rats in either age group, and it did not change during maturation (Fig. 2).

Receptor occupancy by endogenous ANG II was assessed in intact glomeruli from young WKY and SHR rats (Fig. 3). Incubation of glomeruli from each group with [^{125}I]ANG II in the presence of 3 M $MgCl_2$ resulted in complete dissociation of

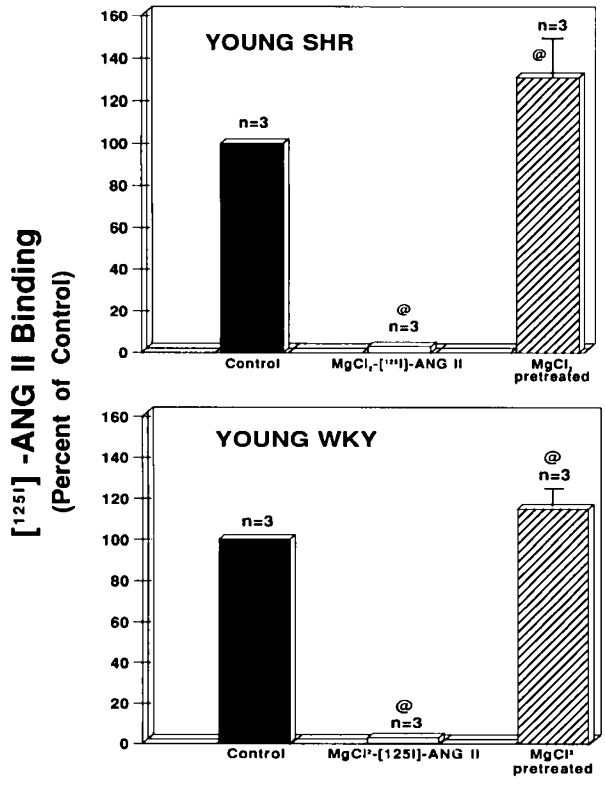

Fig. 3. Assessment of receptor occupancy in intact glomeruli from 4- to 5-wk-old SHR (*upper panel*) and 4- to 5-wk-old WKY rats (*lower panel*). Changes in receptor density are expressed as a percentage of control, defined as 100%. Complete dissociation of [^{125}I]ANG II from its receptor occurred in glomeruli incubated with radioligand in the presence of $MgCl_2$ (@, $p < 0.05$). Radioligand studies performed after glomeruli were preincubated with $MgCl_2$ for 3 min and then rinsed with PBS before radioligand study yielded increased receptor density in both groups. Values shown are the mean values from three separate experiments in which each determination was performed in triplicate.

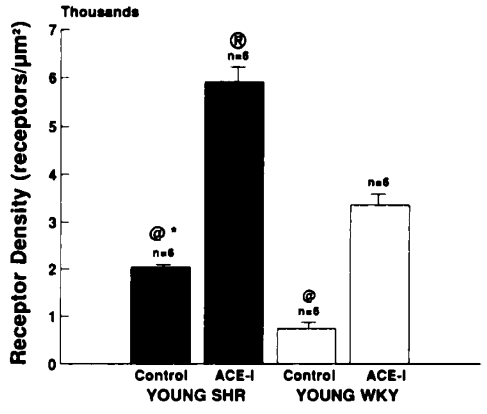

Fig. 4. Comparison of glomerular ANG II receptor density (receptors/μm^2 planar glomerular surface area) in young SHR and WKY rats after (*ACE-I*) and without (*Control*) treatment with converting enzyme inhibitor (enalaprilat). Glomerular ANG II receptor density was higher in control young SHR and WKY rats (*, $p < 0.05$). Glomerular ANG II receptor density increased in response to ACE-I in both young SHR and WKY rats (@, $p < 0.05$). Receptor density remained nearly 2-fold higher in young SHR compared with young WKY rats (®, $p < 0.05$) after treatment with ACE-I. Values are mean ± SEM.

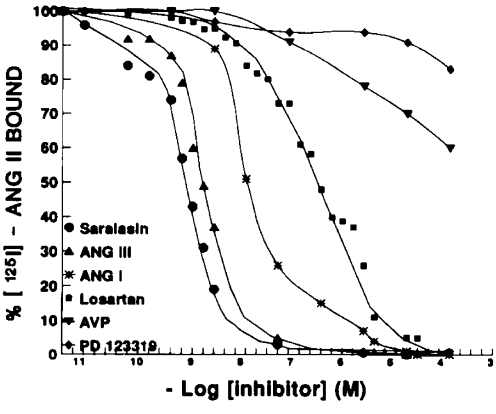

Fig. 5. Competitive binding curves illustrating displacement of specifically bound [^{125}I]ANG II by agonists and antagonists, using glomeruli from adult WKY rats. The percent binding of [^{125}I]ANG II is plotted as a function of increasing concentrations of agonist or antagonist. Values shown are the mean values from four separate experiments in which each determination was performed in triplicate. *AVP*, arginine vasopressin.

the radioligand from its receptor ($p < 0.05$). Freeing endogenous ANG II from its receptor by prior treatment with 3 M $MgCl_2$, along with inhibition of *de novo* synthesis of ANG II by 25 μM chymostatin (21), increased receptor density in glomeruli from both young SHR and WKY rats in an equivalent manner.

Pharmacologic inhibition of ANG II generation resulted in a 3-fold increase in glomerular ANG receptor density in young SHR rats (2033 ± 154 *versus* 5915 ± 318 receptors/μm^2, $p < 0.001$) and a 4.5-fold increase in receptor density in young WKY rats (742 ± 151 *versus* 3358 ± 234 receptors/μm^2, $p < 0.01$). Receptor density remained nearly 2-fold higher (Fig. 4) in young SHR compared with young WKY rats ($p < 0.05$). Competitive binding curves for the displacement of [^{125}I]ANG II binding to glomeruli from adult WKY rats by various ligands are depicted in Figure 5. Sarlasin, ANG II (not shown in the figure), ANG III, and ANG I, as well as the nonpeptide ANG II receptor antagonist specific for the AT_1 receptor subtype, losartan, displaced [^{125}I]ANG II binding in a concentration-dependent, monophasic manner. The order of potency was ANG II (not shown) > saralasin > ANG III > ANG I > losartan (IC_{50} 0.9 ± 0.3, 1.6 ± 0.2, 4.5 ± 1.2, 47 ± 16, and 210 ± 25 nM, respectively). In contrast, there was minimal displacement of [^{125}I]ANG II by either PD 123319 (specific antagonist for AT_2) or the unrelated peptide arginine vasopressin. Identical displacement curves were obtained with glomeruli from 4-wk-old and 10- to 12-wk-old SHR rats (Fig. 6). IC_{50} values for young and adult SHR rats were 0.9 ± 0.2 and 0.9 ± 0.1 nM, respectively, for ANG II (not shown in the figure), 1.6 ± 0.1 and 1.4 ± 0.2 nM for saralasin, and 210 ± 7 and 200 ± 42 nM for losartan. PD 123319 did not displace [^{125}I]ANG II in young or adult SHR rats.

DISCUSSION

There has been renewed interest recently in the role of the intrarenal RAS in the development of hypertension (23–25). This study evaluated glomerular ANG II receptor density and affinity during the development of hypertension in the SHR rats. We found glomerular ANG II density to be 3-fold greater in young SHR than in young WKY rats. Our observations regarding ANG II receptors in the kidney are in agreement with the findings of other investigators who have described an enhanced intrarenal RAS during the development of hypertension in this animal model (23–25). As such, our findings provide further evidence that changes in the intrarenal RAS may be partly responsible for the initiation of hypertension in the SHR rats. Moreover, the increase in glomerular ANG II receptors in the young SHR rats is consistent with the micropuncture data of Dilley *et al.* (5), who demonstrated a 30% reduction in GFR and ultrafiltration coefficient in young SHR compared with age-matched WKY rats. Furthermore, we have shown that glomerular ANG II receptors are similar in both adult SHR and WKY rats. These data are in agreement with "resetting" of kidney function in SHR rats after

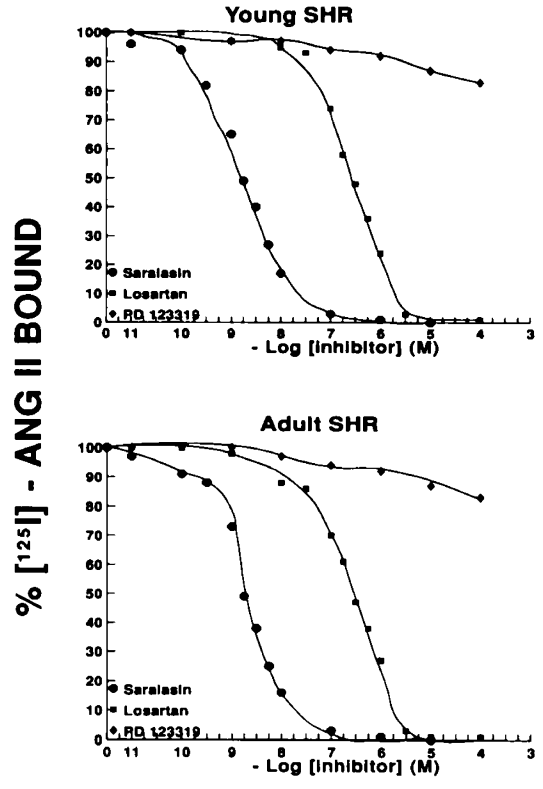

Fig. 6. Competitive binding curves illustrating displacement of specifically bound [^{125}I]ANG II by the ANG II antagonists saralasin, losartan, and PD 123319. The percent binding of [^{125}I]ANG II is plotted as a function of increasing concentrations of antagonist. Glomeruli from 4- to 5-wk-old SHR (*upper panel*) and 10- to 12-wk-old SHR (*lower panel*) rats were studied. Values shown are the mean values from four separate experiments in which each determination was performed in triplicate.

the onset of hypertension. The "reset" hypothesis refers to the observation that once hypertension is established, extracellular fluid volume and cardiac output are reset to normal levels (5, 26, 27). The reset phenomenon and our data are in agreement with the findings of Dilley *et al.* (5), who reported that GFR and ultrafiltration coefficients are comparable in adult SHR and WKY rats.

We further examined whether the difference in glomerular ANG II receptor-ligand binding between young and adult SHR rats is primary or whether it is secondary to altered receptor occupancy by endogenously produced ANG II. Pretreatment with $MgCl_2$, which displaces ANG II from its receptor binding sites (20), and incubation with chymostatin, which inhibits *de novo* synthesis of glomerular ANG II (21), did not increase sites available for radiolabeled ANG II binding in either young SHR or WKY rats (Fig. 3). In addition, we evaluated the role of endogenously produced ANG II in the disparity between glomerular receptors observed in young SHR and WKY rats. We have previously shown that intraperitoneal administration of enalaprilat decreases glomerular ANG II production by 43% (21). Enalaprilat treatment increased glomerular ANG II receptor density similarly in both young SHR and young WKY rats (Fig. 4). It is worth mentioning that the difference in glomerular ANG II receptor density observed in young SHR compared with young WKY rats is maintained after enalaprilat treatment. Moreover, differences in receptor density between young SHR and WKY rats are unrelated to alterations in plasma ANG II concentration (Table 1). These findings are in agreement with earlier studies that demonstrated plasma ANG, plasma renin activity, and plasma renin concentration are similar in the young SHR and WKY rats (8, 23, 24).

Contrary to our findings, Chatziantoniou and Arendshorst (12) found that the glomerular ANG II receptor density is similar between 6-wk-old SHR and WKY rats, and Messenger *et al.* (11) reported that glomerular ANG II receptor density is greater in glomeruli from adult SHR rats than from 20-wk-old normotensive Wistar rats. The reason for the disagreement is unclear. A difference in methodology may account for part of this disparity (28, 29). In the present study, radiolabeled ANG II binding to glomerular receptors achieved equilibrium at approximately 105 min of incubation, whereas in the other reports the duration of incubation was 45–50 min. We have recently reported the presence of glomerular peptidase activity degrading ANG II in the same experimental model (21). Moreover, in our hands, [^{125}I] ANG II degraded when incubated with isolated glomeruli, unless ACTH was added to the incubation medium. This suggests that ACTH acted as a peptidase inhibitor. Several other investigators have also reported that ACTH prevents degradation of ANG II (16), and recent studies in rat brain have shown that ANG II receptor (AT_1) number increased 2.3-fold when peptidase inhibitors were used during incubation (30). In addition, an age difference between rats used in our experiment and those used by Chatziantoniou and Arendshorst (12) (4 *versus* 6 wk) and Messenger *et al.* (11) (10 *versus* 20 wk) could also be responsible for different results. Moreover, it is often assumed that SHR and WKY rats are fully inbred, but genetic and biologic variability among different breeding colonies has been reported (31, 32). We used a rat strain for control experiments different from that used by Messenger *et al.* (WKY *versus* Wistar rats), and our WKY and SHR rats were obtained from a different breeding source compared with that used in the previous studies (Harlan Farms, Indianapolis, IN *versus* In bred, NC and Olac Ltd., Stoke-on-Trent, UK).

Glomerular ANG II receptor subtypes were also evaluated in the present study. Several biochemical and pharmacologic studies indicate that at least two subtypes exist within various tissues, and recent studies have suggested that receptor subtype may change during development (13). In the present study, agonists and antagonists competed for the binding of [^{125}I]ANG II (Fig. 5), with an order of potency characteristic of binding to ANG II receptors (33). The AT_1-specific antagonist losartan completely inhibited the binding of [^{125}I]ANG II to glomerular ANG II receptors. IC_{50} for losartan is 210 nM, which is comparable to data reported for intact human glomeruli (an IC_{50} for losartan of 300 nM) (34). Furthermore, Fontoura *et al.* (35) have shown in isolated perfused rats kidneys that the IC_{50} for losartan is approximately 200 nM. In contrast, membrane preparations from isolated glomeruli (36), cultured mesangial cells (37), and cloned AT_1 (38) have exhibited lower IC_{50} values (20, 7.9, and 6.3 nM, respectively). We postulate that this lower receptor affinity may be caused by a change in the physical properties of the receptor protein during membrane preparation. Alternatively, the physical properties of the losartan molecule may affect its propensity to bind ANG II receptor sites embedded deep in a three-dimensional structure. The discrepancy in affinity between membrane preparations and whole structures may be of clinical relevance. For losartan to have a protective glomerular effect, it must bind receptor sites *in vivo*. Hence, modification of the molecular structure of losartan may improve its glomerular protective effect. AT_2-specific antagonist PD 123319 does not displace the labeled ligand. Our data confirm previous reports that PD 123319 does not displace ANG II from renal receptors (39). This suggests that glomerular ANG II receptors are mainly of the AT_1 subtype (34–39). LIGAND analysis indicated a single population of receptors in both young and mature SHR as well as WKY rats.

In summary, we have demonstrated that glomerular ANG II receptor density in young SHR is 3-fold higher than in age-matched control WKY rats and 1.5-fold higher than in adult SHR rats. The greater receptor density in young SHR rats cannot be explained by differences in receptor occupancy. Furthermore, we have shown that the predominant ANG II glomerular receptor in young and adult SHR and WKY rats is the AT_1, or

losartan-sensitive, subtype. We postulate that these differences in ANG II receptor density may underlie the altered glomerular function observed in young SHR rats, thereby providing further evidence for a role of the intrarenal RAS in the development of hypertension in this model.

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REFERENCES

1. Beierwaltes WH, Arendhorst WJ, Klemmer PJ 1982 Electrolyte and water balance in young spontaneous hypertensive rats. Hypertension 4:908–915
2. Bianchi G, Fox U, DiFrancesco GF, Giovannetti AM, Pagetti D 1974 Blood pressure changes produced by kidney cross-transplantation between spontaneously hypertensive rats and normotensive rats. Clin Sci Mol Med 47:435–448
3. Brenner BM, Garcia DL, Anderson S 1988 Glomeruli and blood pressure. Less of one, more of the other? Am J Hypertens 1:335–347
4. Coleman TG, Guyton AC 1969 Hypertension caused by salt loading in the dog: III. Onset transients of cardiac output and other circulatory variables. Circ Res 15:153–158
5. Dilley JR, Stier Jr CT, Arendshorst WJ 1984 Abnormalities in glomerular function in rats developing spontaneous hypertension. Am J Physiol 246:F12–F20
6. Blantz RC 1987 The glomerular and tubular actions of angiotensin II. Am J Kidney Dis 10(suppl):2–6
7. Hall JE 1986 Regulation of glomerular filtration rate and sodium excretion by angiotensin II. Fed Proc 45:1431–1437
8. Ausiello DA, Kreisberg JI, Roy C, Karnovsky MJ 1980 Contraction of cultured rat glomerular cells of apparent mesangial origin after stimulation with angiotensin II and arginine vasopressin. J Clin Invest 65:754–760
9. Camargo MJF, von Lutterotti N, Pecker MS, James GD, Timmermans PBMWM, Laragh JH 1991 DuP 753 increases survival in spontaneously hypertensive stroke-prone rats fed a high sodium diet. Am J Hypertens 4:341S–345S
10. Matsushima Y, Kawamura M, Akabane S, Imanishi M, Kuramochi M, Ito K, Omae T 1988 Increases in renal angiotensin II content and tubular angiotensin II receptors in prehypertensive spontaneously hypertensive rats. J Hypertens 6:791–796
11. Messenger EA, Stonier C, Aber GM 1988 Differences in glomerular binding and response to angiotensin II between normotensive and spontaneously hypertensive rats. Clin Sci 75:191–196
12. Chatziantoniou C, Arendshorst WJ 1991 Angiotensin and thromboxane in genetically hypertensive rats: renal blood flow and receptor studies. Am J Physiol 261:F235–F247
13. Grady EF, Sechi LA, Griffin CA, Schambelan M, Kalinyak JE 1991 Expression of the type 2 angiotensin receptor in the developing rat fetus. FASEB J 5:A869(abstr)
14. Fong JSC, Drummond KN 1968 Method for preparation of glomeruli for metabolic studies. J Lab Clin Med 71:1034–1039
15. Skorecki KL, Ballermann BJ, Rennke HG, Brenner BM 1983 Angiotensin II receptor regulation in isolated renal glomeruli. Fed Proc 42:3064–3070
16. Wilkes BM 1988 Increased angiotensin II receptor binding with age in isolated rat glomeruli. Kidney Int 34:241–247
17. Harms PG, Ojeda SR 1974 A rapid and simple procedure for chronic cannulation of the rat jugular vein. J Appl Physiol 36:391–392
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
19. Seikaly MG, Arant Jr BS, Seney Jr FD 1990 Endogenous angiotensin concentrations in specific intrarenal fluid compartments of the rat. J Clin Invest 86:1352–1357
20. Aguilera G, Catt K 1981 Regulation of vascular angiotensin II receptors in the rat during altered sodium intake. Circ Res 49:751–758
21. Atiyeh BA, Arant Jr BS, Seikaly MG 1991 *In vitro* production of angiotensin (ANG) II by isolated glomeruli. Pediatr Res 29:337A(abstr)
22. Munson PJ, Robard D 1980 Ligand: a versatile computerized approach for characterization of ligand-binding system. Anal Biochem 107:220–239
23. Kawashima K, Shiono K, Sokabe H 1980 Variation of plasma and kidney renin activities among substrains of spontaneously hypertensive rats. Clin Exp Hypertens 2:229–245
24. Shiono K, Sokabe H 1976 Renin-angiotensin system in spontaneously hypertensive rats. Am J Physiol 231:1295–1299
25. Henrich WL, Levi M 1991 Ontogeny of renal renin release in spontaneously hypertensive rat and Wistar-Kyoto rat. Am J Physiol 260:F530–F535
26. Roman RJ, Cowley AW 1985 Abnormal pressure-diuresis-natriuresis response in spontaneously hypertensive rats. Am J Physiol 248:F199–F205
27. Dilley JR, Arendshorst WJ 1984 Enhanced tubuloglomerular feedback activity in rats developing spontaneous hypertension. Am J Physiol 247:F672–F679
28. Brown GP, Douglas JG, Krontiris-Litowitz J 1980 Properties of angiotensin II receptors of isolated rat glomeruli: factors influencing binding affinity and comparative binding of angiotensin analogs. Endocrinology 106:1923–1929
29. Jacobs SK, Chang J, Cutrecasas P 1975 Estimation of hormone receptor affinity by competitive displacement of labeled ligand: effect of concentration of receptor and of labeled ligand. Biochem Biophys Res Commun 66:687–692
30. Saylor DL, Speth RC, Rowe BP 1993 Effects of peptidase inhibitors on binding at angiotensin receptor subtypes in the rat brain. Biochem Pharmacol 45:2109–2114
31. Kurtz TW, St. Lezin EM 1992 Gene mapping in experimental hypertension. J Am Soc Nephrol 3:28–34
32. Kurtz TW, Montano M, Chan L, Kabra P 1989 Molecular evidence of genetic heterogeneity in Wistar-Kyoto rats: implications for research with the spontaneously hypertensive rat. Hypertension 13:188–192
33. Chiu AT, Herblin WF, McCall DE, Ardecky RJ, Carini DJ, Duncia JV, Pease LJ, Wong PC, Wexler RR, Johnson AL, Timmermans PBMWM 1989 Identification of angiotensin II receptor subtypes. Biochem Biophys Res Commun 165:196–203
34. Chansel D, Czekalski S, Pham P, Ardaillou R 1992 Characterization of angiotensin II receptor subtypes in human glomeruli and mesangial cells. Am J Physiol 262:F432–F441
35. Fontoura BMA, Nussenzveig DR, Timmermans PBMWM, Maack T 1991 DuP 753 is a potent nonpeptide antagonist of angiotensin II receptors in isolated perfused rat kidney and cultured renal cells. Am J Hypertens 4:303S–308S
36. Edwards RM, Stack EJ, Weidley EF, Aiyar N, Keenan RM, Hill DT, Weinstock J 1992 Characterization of renal angiotensin II receptors using subtype selective antagonists. J Pharmacol Exp Ther 260:933–938
37. Ernsberger P, Zhou J, Damon TH, Douglas JG 1992 Angiotensin II receptor subtypes in cultured rat renal mesangial cells. Am J Physiol 263:F411–F416
38. Murphy TJ, Alexander RW, Griendling KK, Runge MS, Bernstein KE 1991 Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. Nature 351:233–236
39. de Gasparo M, Whitebread S, Mele M, Motani AS, Whitcombe PJ, Ramjoue HP, Kamber B 1990 Biochemical characterization of two angiotensin II receptor subtypes in the rat. J Cardiovasc Pharmacol 16(suppl 4):S31–S35