Importance of angiogenic action of angiotensin II in the glomerular growth of maturing kidneys

AGNES FOGO, YOSHIYUKI YOSHIDA, AIDA YARED, and IEKUNI ICHIKAWA

Departments of Pathology and Pediatrics, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

Importance of angiogenic action of angiotensin II in the glomerular growth of maturing kidneys. We studied the effect of three antihypertensive drugs on the growth of glomeruli in four- to five-week-old Munich-Wistar rats (N = 24), which were undergoing rapid maturation processes. Young rats were given an angiotensin converting enzyme inhibitor (ACEI, enalapril, 50 mg/liter drinking water), verapamil (50 mg/liter) or hydralazine (80 mg/liter) or no treatment for six weeks. Body weight increased comparably in the treatment groups and age-matched controls, reaching on average 197 ± 11, 214 ± 12 and 198 ± 3 g in ACEI-, verapamil- and hydralazine-treated rats, respectively, versus 218 ± 6 g in control rats. Glomerular hemodynamic patterns, including glomerular capillary pressure, measured in maturing rats after one and six weeks of ACEI treatment were unaffected by ACEI. Mean planar area of glomeruli (PAmean) achieved was smaller than control in ACEI rats (6.60 ± 0.20 × 10⁻³ mm² vs. 6.98 ± 0.22, respectively, P < 0.005), but not in rats treated with other antihypertensive drugs. Furthermore, the maturational PAmean increase in rats given ACEI for six weeks was, on average, only half that achieved by age-matched controls not given ACEI, in contrast to normal maturational growth with hydralazine or verapamil (29% increase in PAmean from normal baseline in ACEI vs. 52%, 53% and 59% increases in verapamil, hydralazine and control, respectively). In contrast, comparable PAmean values were found in adults with (7.08 ± 0.22 × 10⁻³ mm², N = 6) and without (6.98 ± 0.33 × 10⁻³ mm², N = 6) ACEI treatment given for six weeks. Therefore, ACEI, but not verapamil and hydralazine, causes growth retardation in maturing glomeruli. The studies suggest that the rapid growth of glomeruli in maturing kidneys is dependent upon the angiogenic effect of angiotensin II.

Angiotensin I converting enzyme inhibitors (ACEI) have been shown to ameliorate the progressive deterioration of glomerular structure and/or function secondary to experimental diabetes or subtotal renal ablation [1—4]. Common additional observations made in these studies are the antihypertensives' effect to suppress the marked glomerular hypertrophy preceding sclerosis [5, 6]. To determine if these drugs have a similar anti-hypertrophic effect on glomerular morphology in other conditions characterized by rapid glomerular growth, we studied the effect of ACEI and two other structurally-unrelated antihypertensive drugs on the size of glomeruli of young animals, which are undergoing a rapid maturational growth.

Of note, angiogenic actions of angiotensin II have been repeatedly demonstrated in other organ smooth muscle cells [7], and most recent preliminary in vitro studies using glomerular cells have also shown angiogenic potential of angiotensin II in the glomeruli [8]. It has therefore been postulated that endogenous angiotensin II may play a role in the development of glomerular hypertrophy in some in situ settings, at least in part, through mechanism(s) independent of its well known effect on glomerular hemodynamics. In designing our studies assessing the effect of ACEI on the structure and hemodynamics of the maturing glomeruli, in which a rapid angiogenic process is taking place, we speculated that the angiogenic effect of angiotensin II may be distinguished from its hemodynamic effect in these otherwise normal healthy animals [9, 10].

Methods

Studies were performed in twenty-four, four- to five-week-old and twelve more than three-month-old male Munich-Wistar rats. Animals were allowed free access to regular rat chow and tap water. Systolic arterial pressure was measured at six weeks by the tail cuff method [11] in awake animals.

Experimental groups

Experimental protocols employed in the present studies are illustrated in Figure 1.

Group 1: Six week treatment of maturing rats with or without antihypertensives. Four- to five-week-old rats were placed on one of the following antihypertensive drugs for six weeks, right up to the time of micropuncture, as described below.

Group 1a: Enalapril (ACEI, enalapril maleate, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey, USA), 50 mg/liter in drinking water (DW) (N = 7).

Group 1b: Verapamil (VPL, isoptin, Knoll, Whipppany, New Jersey, USA), 50 mg/liter DW (N = 5).

Group 1c: Hydralazine (HLZ, Sigma Chemical Company, St. Louis, Missouri, USA), 80 mg/liter DW (N = 5).

Group 1d: No antihypertensives (CONT, N = 7).

At six weeks Group 1a and 1d animals were subjected to the micropuncture assessment of glomerular hemodynamics, followed by the morphometric analysis of glomeruli on fixed kidney tissues as described below. In a separate group of six young rats (Group 1a') given enalapril as above, micropuncture assessment was done after one week of treatment to assess the early hemodynamic pattern with this treatment. Kidneys from four- to five-week old age-matched rats, not shown in Figure 1

Received for publication April 16, 1990
and in revised form July 13, 1990
Accepted for publication July 24, 1990
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were examined morphometrically to determine normal glomerular size at the onset of the study.

**Group 2:** Six week treatment of adult rats with an angiotensin I converting enzyme inhibitor. Adult rats (>3 months old) were placed on one of the following experimental regimens for six weeks, right up to the time of micropuncture, as described below.

*Group 2a:* Enalapril, 50 mg/liter in drinking water (DW) (*N* = 6).
*Group 2b:* No antihypertensives (*N* = 6).

A separate group of adult rats (*N* = 4, >3 months old) was treated with enalapril, 50 mg/liter drinking water as Group 2a, for one week, and plasma renin and angiotensin converting enzyme activities determined to verify that this dose indeed has biologic activity in adult rats. Control rats (*N* = 4) received no drug. Plasma renin activity was markedly elevated in enalapril treated rats versus control, on average 96 ± 39 versus 25 ± 7 ng/ml, while angiotensin converting enzyme was substantially reduced below normal, on average 7.4 ± 0.1 versus 22.2 ± 0.7 U/liter, respectively (*P* < 0.0005).

At six weeks animals were sacrificed for the morphometric analysis of glomeruli specified below.

**Renal clearance and micropuncture measurements**

Animals were anesthetized with an intraperitoneal injection of Inactin (Byk, Gulden Konstanz, FRG; 100 mg/kg body wt, i.p.), placed on a temperature-regulated micropuncture table, and prepared as previously described [12]. Briefly, following tracheostomy, indwelling polyethylene catheters (PE-50, Clay Adams, Parsippany, New Jersey, USA) were placed into the left and right jugular veins for infusion of plasma, inulin and para-aminohippurate (PAH). The left femoral artery was catheterized to monitor mean systemic arterial pressure (MAP), measured by electronic transducer (Model p23XL, Gould Inc., Cleveland, Ohio, USA) connected to a recorder (Model 2200S, Gould Inc.). The left ureter was cannulated with PE-10 catheter for urine collections. The left kidney was suspended on a Lucite holder, its surface illuminated with a fiberoptic light source, and bathed with 0.9% NaCl. Volume losses consequent to surgical preparation were replaced with plasma, infused in a volume equal to 1% of body weight over 30 minutes, followed by a maintenance infusion at the rate of 1.2 ml/hr. A solution of 10% inulin and 1.0% PAH in 0.9% NaCl was infused throughout the duration of the experiment at the rate of 1.2 ml/hr.

After a 60-minute equilibration period, clearance and micropuncture measurements were made as detailed below. Exactly timed (1 to 2 mm) samples of tubule fluid were collected from surface proximal convolutions for the determinations of flow rate and inulin concentration for calculation of single nephron glomerular filtration rate (SNGFR). Coincident with the tubule fluid collections, femoral arterial blood was obtained for the determination of hematocrit and plasma concentrations of protein, inulin and PAH. Whenever appropriate, two or three samples of urine from the kidney were collected for determinations of flow rate, inulin and PAH concentrations, which were used in the calculation of whole kidney GFR and effective renal plasma flow rate (ERPF).

Hydraulic pressures were monitored in accessible surface structures with a continuous-recording servo-null micropipette transducer system (Model 3, Instrumentation for Physiology and Medicine, San Diego, California, USA). Micropipettes with outer diameter of 1 to 2 μm containing 2.0 M NaCl were used. Hydraulic output from the servo-null system was converted electronically to a recorder (Model 2200S, Gould) by means of a pressure transducer. Direct measurements of time-averaged

**Fig. 1. Schematic presentation of the protocols employed in the present study.**
hydraulic pressures were recorded in single capillaries of surface glomeruli (P_{GC}), Bowman’s space, and surface efferent arteriole. For estimation of oncotic pressures of plasma entering and leaving glomerular capillaries, protein concentrations of afferent (C_A) and efferent (C_E) arterioles were determined by analyzing femoral arterial and surface efferent arteriolar resistances were comparable between Group Ia ACE! and Group Ib control rats (31.6 ± 0.17 vs. 30.9 ± 0.12). Values for other glomerular microcirculatory parameters were also comparable between Group Ia ACE! and Group Ic control rats, demonstrating the typical patterns seen in rats of Munich-Wistar strain at their age [10, 19]. Thus, in Group Ic control rats, protein concentrations of surface efferent arteriolar plasma, respectively, using the fluorometric method previously described [13, 14].

### Analytic

The volume of fluid collected from individual proximal tubules by micropuncture was estimated from the length of the fluid column in a constant-bore capillary tube of known internal diameter. Plasma and urine inulin concentrations were assayed by the macroanthrone method of Führ, Kaczmarczyk and Krütgen [15]. Inulin concentrations in tubule fluid were determined by the method of Vurek and Pegram [16]. PAH concentration in plasma and urine was measured by the method of Bratton and Marshall, as modified by Smith et al [17].

### Statistical analysis

Results are expressed as mean ± 1 SEM. Comparisons between two groups were made using the unpaired t-test. Data from multiple groups were compared using one-way ANOVA followed by multiple comparisons by the Bonferroni method [18]. The results were deemed statistically significant when the P value was <0.05.

### Results

#### Systemic and whole kidney parameters

Data for body weight and dry and wet kidney weight measured at the completion of study are shown in Table 1. Thus, Group Ia, Ib, Ic and Id maturing animals were statistically indistinguishable in body weight regardless of the treatment given during the preceding six week period. Wet kidney weight was significantly less than control only for Group Ia, whereas dry kidney weight was significantly less in all the treated rats versus control. Group 2a adult animals given ACEI and Group 2b adult control animals were comparable with regards to body and kidney weight. Systemic systolic blood pressure at six weeks was similar between Groups Ia, Ib and Id (110 ± 6 mm Hg, 113 ± 5, and 109 ± 6, respectively), whereas Group Ic had higher level versus control Group Ia and ACEI Group Ia (123 ± 1 mm Hg, P < 0.0005 vs. Group Ia, P < 0.005 vs. Group Ia). Systemic blood pressure in Group 2A adult ACEI was normal (119 ± 5 mm Hg).

#### Whole kidney and glomerular hemodynamic parameters

Average values for several pertinent whole kidney and glomerular hemodynamic parameters measured in maturing rats of Groups Ia and Id are summarized in Table 2. Thus, values for mean systemic arterial pressure, MAP, were modestly but significantly lower in Group Ia animals given ACEI than Group Id control rats of the same age, on average by 12 mm Hg. Like those for whole kidney GFR and ERPF, mean values for SNGFR and SNFF were comparable in Group Ia ACEI rats (29.7 ± 2.1 nI/min and 0.23 ± 0.02, respectively) and Group Id control rats (31.6 ± 1.7 nI/min and 0.24 ± 0.01, respectively) so that Q_A values were calculated to be also comparable between the two groups. Values for other glomerular microcirculatory parameters were also comparable between Group Ia ACEI and Group Id control rats, demonstrating the typical patterns seen in rats of Munich-Wistar strain at their age [10, 19]. Thus, in Groups Ia and Id values for P_{GC} averaged 51.1 ± 1.3 and 48.7 ± 0.5 mm Hg, respectively. Values of arterial plasma protein concentration, C_A, were similar (Table 2). Likewise, values for afferent and efferent arteriolar resistances were comparable between the two groups. As shown in Table 3, values for body weight, mean systemic arterial pressure and P_{GC} in Group Ia' rats after one week of enalapril showed patterns indistinguishable from those measured in age-matched rats previously reported by us [10]. Exception to these resemblances in glomerular microcirculatory dynamics between the ACEI-treated and untreated maturing rats was the value of glomerular capillary ultrafiltration coefficient, K_f. Thus, values for K_f in Group Ia

### Table 1. Whole body parameters in animals with or without antihypertensive treatment

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Body wt g</th>
<th>Kidney wt dry g</th>
<th>Kidney wt wet g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group Ia (Maturing)</td>
<td>197</td>
<td>0.72</td>
<td>0.16</td>
</tr>
<tr>
<td>ACEI, N = 7</td>
<td>±11</td>
<td>±0.05</td>
<td>±0.01</td>
</tr>
<tr>
<td>Group Ib (Maturing)</td>
<td>214</td>
<td>0.81</td>
<td>0.17</td>
</tr>
<tr>
<td>VPL, N = 5</td>
<td>±12</td>
<td>±0.06</td>
<td>±0.00</td>
</tr>
<tr>
<td>Group Ic (Maturing)</td>
<td>198</td>
<td>0.79</td>
<td>0.15</td>
</tr>
<tr>
<td>HLZ, N = 5</td>
<td>±3</td>
<td>±0.04</td>
<td>±0.01</td>
</tr>
<tr>
<td>Group Id (Maturing)</td>
<td>218</td>
<td>0.86</td>
<td>0.19</td>
</tr>
<tr>
<td>No-Tx, N = 7</td>
<td>±6</td>
<td>±0.03</td>
<td>±0.01</td>
</tr>
<tr>
<td>Group 2a (Adult)</td>
<td>226</td>
<td>1.00</td>
<td>—</td>
</tr>
<tr>
<td>ACEI, N = 6</td>
<td>±9</td>
<td>±0.03</td>
<td>—</td>
</tr>
<tr>
<td>Group 2b (Adult)</td>
<td>242</td>
<td>1.04</td>
<td>—</td>
</tr>
<tr>
<td>No-Tx, N = 6</td>
<td>±4</td>
<td>±0.00</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. Abbreviations are: Body wt, weight; Kidney wt, kidney weight; Maturing, 4—5 week old at the onset of experiments; Adult, >3 month old at the onset of experiments; ACEI, Angiotensin converting enzyme inhibitor, enalapril; VPL, verapamil; HLZ, hydralazine; No-Tx, control rats not given ACEI.

* P < 0.05 vs. Group Id. Statistical comparison is not made between Groups 1 and 2.
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Table 2. Whole kidney and glomerular hemodynamic parameters at 6 weeks in maturing animals with or without angiotensin I converting enzyme inhibitor treatment

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>MAP (mm Hg)</th>
<th>GFR (ml/min)</th>
<th>ERPF (nl/min)</th>
<th>SNGFR (mm Hg)</th>
<th>Qg</th>
<th>POC (×10^9 dynes · sec · cm⁻²)</th>
<th>RA (g/dl)</th>
<th>RE (nl/sec · mm Hg)</th>
<th>Kₗ⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1a</td>
<td>103</td>
<td>±2</td>
<td>0.92</td>
<td>3.37</td>
<td>29.7</td>
<td>135</td>
<td>0.23</td>
<td>51.1</td>
<td>1.84</td>
</tr>
<tr>
<td>ACEI (N = 6 rats)</td>
<td>±2</td>
<td>±0.13</td>
<td>±0.38</td>
<td>±2.1</td>
<td>±13</td>
<td>±0.02</td>
<td>±1.3</td>
<td>±0.22</td>
<td>±0.16</td>
</tr>
<tr>
<td>Group 1b</td>
<td>115</td>
<td>±5</td>
<td>0.95</td>
<td>3.87</td>
<td>31.6</td>
<td>132</td>
<td>0.24</td>
<td>48.7</td>
<td>2.22</td>
</tr>
<tr>
<td>No-Tx (N = 6 rats)</td>
<td>±5</td>
<td>±0.04</td>
<td>±0.20</td>
<td>±1.7</td>
<td>±7</td>
<td>±0.01</td>
<td>±0.5</td>
<td>±0.21</td>
<td>±0.05</td>
</tr>
</tbody>
</table>

P value
<0.05 NS NS NS NS NS NS NS NS NS NS NS

Values are expressed as mean ± SEM. MAP, Mean arterial pressure; GFR, whole kidney glomerular filtration rate; ERPF, effective renal plasma flow rate; SNGFR, single nephron glomerular filtration rate; Qg, glomerular plasma flow rate; SPFF, single nephron filtration fraction; PGC, glomerular capillary hydraulic pressure; RA, afferent arteriolar resistance; RE, efferent arteriolar resistance; CA, systemic arterial plasma protein concentration; Kₗ⁰, glomerular capillary ultrafiltration coefficient; ACEI, angiotensin converting enzyme inhibitor, enalapril; No-Tx, control rats not given antihypertensives; NS, P > 0.05. Filtration pressure equilibrium was achieved in 2 ACEI rats and 3 No-Tx rats.

Table 3. Glomerular hemodynamic parameters at 1 week in maturing animals with or without angiotensin I converting enzyme inhibitor treatment

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Body wt (g)</th>
<th>MAP (mm Hg)</th>
<th>PGC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1a</td>
<td>106</td>
<td>87</td>
<td>38</td>
</tr>
<tr>
<td>ACEI 1 week (N = 6 rats)</td>
<td>±3</td>
<td>±2</td>
<td>±0.8</td>
</tr>
<tr>
<td>Control</td>
<td>96</td>
<td>78</td>
<td>37</td>
</tr>
<tr>
<td>No Tx (N = 6 rats)</td>
<td>±5</td>
<td>±5</td>
<td>±2</td>
</tr>
</tbody>
</table>

P value
NS NS NS NS

Values are expressed as mean ± SEM. Abbreviations are: Body wt, body weight; MAP, mean arterial pressure; PGC, glomerular capillary pressure. Control rat data are from our reference [10]. NS, P > 0.05.

rats given ACEI for six weeks averaged 0.057 ± 0.008 nl/(sec · mm Hg), a value 27% below the average value measured in age-matched Group 1d control rats (0.078 ± 0.008, P < 0.05).

Glomerular morphology

Figure 2 summarizes values for PAMEAN determined at the completion of study in all experimental groups. Baseline PAMEAN at 0 weeks in young rats was 4.16 ± 0.24 × 10⁻³mm² (Fig. 2A, horizontal line). Not surprisingly, all young rats showed continued growth after six weeks. Values for PAMEAN were smaller, but approached the adult size, in Group 1d control maturing animals. The PAMEAN was comparable to Group 1d control maturing rats, with increases of 52%, 53% and 59% over 0 week baseline values, respectively. In contrast, Group 1a rats given ACEI had significantly smaller PAMEAN (P < 0.005 ACEI vs. No-Tx; P < 0.025 ACEI vs. VPL or HLZ) and showed only half of the growth observed in the other groups (29% increase over baseline PAMEAN). In adults, by contrast, six weeks of ACEI treatment did not affect glomerular size: PAMEAN values were comparable between Group 2a and 2b, averaging 7.08 ± 0.22 × 10⁻³mm² versus 6.98 ± 0.33, respectively (P > 0.4).

Discussion

The renin-angiotensin system is active during intruterine life, and remains so in the neonate of several mammalian species as compared to the adult [20—22]. Physiological role for this active renin-angiotensin system during the early periods of life, however, has not been elucidated [23]. In fact, the finding of Jose et al [9] that the prevailing level of RBF in young animals did not increase after antagonism of angiotensin II was taken to indicate that this hormone does not participate in determining the high renal arteriolar vascular tone characterizing young Animals. Evidence that the same notion applies also to the five- to six-week-old maturing rats in the present study has recently been obtained in a series of experiments conducted by us and others. Both plasma renin activities [10] and angiotensin II concentrations [24] were shown to reach similarly low levels as those of adults by five to six weeks of age. Yet, in response to a given amount of exogenous angiotensin II, vasoconstrictive response of young animals is remarkably less than that of adults [10, 25]. In contrast to the lack of evidence for vasoconstrictive action of angiotensin II in maturing animals, recent studies suggest that angiotensin may have an important role in the growth and differentiation in the young animal. In this regard, angiotensin II receptors are highly expressed in the rat fetus [5]. Studies of the augmented expression of renin mRNA found maximal levels at the time of most marked nephrogenesis in the newborn rat [6]. Our observations further point to the importance of angiotensin II in glomerular maturational growth after completion of nephrogenesis in the rat.

Administration of angiotensin I converting enzyme inhibitor in normal adult rats regularly fails to induce alteration in glomerular circulatory dynamics [26, 27]. Therefore we verified that the dose given indeed has biologic effect in the adult rats, by showing inhibition of angiotensin converting enzyme and elevated plasma renin levels in adult rats given ACEI. Given the observations made in our laboratories [10] that the renin-
**Fig. 2. Values for mean planar area (PAmean) measured in various experimental groups at the completion of study.** The horizontal line in A represents normal PAmean at 0 weeks (that is, 4 to 5 week old). Average increases over this baseline (100%) PAmean were calculated to be 29%, 52%, 53% and 59%, respectively, for ACEI, VPL, HLZ and no treatment (No-Tx) control rats.

In contrast to these effects found with ACEI, both VPL and HLZ failed to affect the maturational growth of glomeruli, suggesting that ACEI's suppressive effect on the glomerular growth is not shared with other antihypertensive drugs which are structurally unrelated to ACEI. In fact, glomerular planar area of HLZ rats, relative to their slightly smaller dry kidney weight, was on average even higher than that of control treated rats. In view of the well-known effect of HLZ to activate the renin-angiotensin system [30], our findings with HLZ are further supportive of the notion that angiotensin II is involved in maturational growth. Nevertheless, one may argue that if higher dosages of VPL and HLZ were given to the maturing animals, they, too, may have caused similar retardation in glomerular growth.

We are, however, intrigued by another possibility which is also consistent with the contention that the physiological significance of angiotensin II in maturing kidneys resides primarily in its angiogenic rather than vasoconstrictive action. The readers may recall that the two final functional expressions of angiotensin II's actions, that is, vasoconstriction and angiogenesis, share common receptor and immediate post-receptor signal transduction processes, yet the intermediary metabolites directly linked to vasoconstriction and angiogenesis are believed to be different beyond the hydrolysis of phosphatidylinositol bisphosphate (PIP$_2$). Thus, the immediate products of PIP$_2$, that is, inositol 1,4,5-trisphosphate (IP$_3$) and diacylglycerol (DAG), are believed to be linked to contraction and angiogenesis, respectively [7]. It is therefore conceivable that unlike immature animals, local level of angiotensin II in baseline condition of adult animals is extremely low, or that normal mature animals lack the post-DAG processes which lead to glomerular growth.

IP$_3$, the other product of PIP$_2$ leads to mobilization of calcium from both intracellular and extracellular sources. Hydralazine and verapamil's [31] vasodilatory actions are channeled through their capacity to interfere with these processes, which are crucial steps for the initial and sustained contractility of the smooth muscle cells, respectively. Therefore it is conceivable that angiogenic actions of angiotensin II, and potentially other growth promoting substances, which are in part independent of this intracellular calcium transfer, may remain intact during the administration of VPL or HLZ. In support of this notion prenatal and postnatal hydralazine treatment was found not to prevent renal vessel wall thickening in genetically spontaneously hypertensive rats despite its effectiveness to ameliorate the characteristic hypertension [32]. Verapamil administration, in a certain dosage, in subtotally nephrectomized rats was actually found to augment glomerular deterioration characteristic of remnant nephrons [33]. In contrast, converting enzyme inhibitor (captopril) in one-kidney one-clip hypertensive rats resulted in decreased arteriolar cross-sectional wall area and also a decrease in the number of small arterioles, although ACEI was without effect on the systemic hypertension. Similar

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1 Numerically, ΔP was slightly higher in group Ia ACEI vs. group Id control, 36.6 ± 1.0 vs. 34.5 ± 0.5 (P > 0.4), despite lower average value for SNGFR in the former. Since the relationship between SNGFR and its determinants is not a simple linear one, P values for each parameter can vary, as in this study, where P was less than 0.05 for Kf only.
results were seen in ACEI-treated normal rats, indicating that
ACEI resulted in inhibition of vascular growth independent of
effects on hypertension [34]. Experiments in a rat model of
hypertensive cardiac hypertrophy comparing dihydralazine, the
calcium antagonist nifedipine and the ACEI ramipril in equipo-
tent antihypertensive dosages, found that only the ACEI pre-
vented and also caused regression of the cardiac hypertrophy.
Even lower, non-antihypertensive doses of the ACEI were
effective in this regard, further indicating the role of angiotensin
II in vascular growth separate from its vasoconstrictive effects
[35]. In another hypertensive animal model, ACEI also re-
versed the hypertrophy of vascular smooth muscle cells while
absolute collagen content remained elevated [36]. This consid-
eration of angiotensin II-induced vascular constriction and angio-
genesis, which are regulated by different second messengers,
raises an interesting possibility that warrants future investiga-
tion.

Acknowledgments

These studies were supported by the National Institutes of Health
Grants DK 37868, 37869, 42131, and 39343.

Dr. Ikunji Ichikawa and Dr. Agnes Fogo are recipients of the
Established Investigatorship Award and Clinician Scientist Award,
respectively, from the American Heart Association.

The authors express their thanks to Ms. Teresa Bills for her technical
assistance, and to Ms. Mary Beehan for her secretarial assistance.

Portions of these studies were presented at the Annual Meeting of the
American Society of Nephrology, Washington, D.C., December 15,
1987, and published in abstract form in Kidney International 33:296,

Reprint requests to Agnes Fogo, M.D., Division of Pediatric
Nephrology, Vanderbilt University Medical Center, C-4202 Medical
Center North, 21st and Garland Avenue, Nashville, Tennessee 37232-
2584, USA.

References

1. ANDERSON S, MEYER TW, RENNKE HG, BRENNER BM: Control of
glomerular hypertension limits glomerular injury in rats with re-

2. ANDERSON S, RENNKE HG, BRENNER BM: Therapeutic advantage of
converting enzyme inhibitors in arresting progressive renal
failure associated with systemic hypertension in the rat. J Clin

3. ANDERSON S, RENNKE HG, GARCIA DL, BRENNER BM: Short and
long term effects of antihypertensive therapy in the diabetic rat.

4. YOSHIDA Y, KAWAMURA T, IKOMA M, FOGO A, ICHIKAWA I:
Effects of antihypertensive drugs on glomerular morphology. Kid-
ney Int 36:626–635, 1989

5. MILLAN MA, CARVALLO P, ITZUMI S-I, ZEMEL S, CATT KJ,
AGUILERA G: Novel sites of expression of functional angiotensin II
receptors in the late gestation fetus. Science 244:1340–1342, 1989

6. ROSENBERG ME, CORREA-ROTTER R, PEREZ-CASTILLO A,
HOSTETTER TH: Renin gene expression in the growing rat kidney.

7. SMITH JB: Angiotensin-receptor signaling in cultured vascular

8. HOMMA T, HOOVER RL, ICHIKAWA I, HARRIS RC: Angiotensin II
(AlI) induces hypertrophy and stimulates collagen production in

9. JOSE PA, SLOTKOFF LM, MONTGOMERY S, CALCAGNO PL, EISNER
229:983–988, 1975

10. YARED A, YOSHIOKA T: Uncoupling of the autoregulation of renal
blood flow and glomerular filtration rate in immature rats: Role of
the renin-angiotensin system. (abstract) Kidney Int 33:414, 1988

11. PFEFFER JM, PFEFFER MA, FROHLICH ED: Validity of an indirect
tail-cuff method for determining systolic arterial pressure in unan-
esthetized normotensive and spontaneously hypertensive rats. J
Lab Clin Med 78:957–962, 1971

12. ICHIKAWA I, MADDOX DA, COGAN MG, BRENNER BM: Dynamics of
glomerular ultrafiltration in euvoetric Munich-Wistar rats. Ren-
al Physiol (Basel) 1:121–131, 1978

13. VIETS JW, DEEN WM, TROY JL, BRENNER BM: Determination of
serum protein concentration in nanoliter blood samples using

14. DEEN WM, TROY JL, ROBERTSON CR, BRENNER BM: Dynamics of
glomerular ultrafiltration in the rat. IV. Determination of the

15. FÖHR J, KACZMARCZYK J, KRÜTTGEN CD: Eine einfache colori-
metrische Methode zur Inulinbestimmung für Nieren-Clearance-Un-
tersuchungen bei Stoßwechselgesunden und Diabetikern. Klin
Wochenschr 33:729–730, 1955

16. VUREK GG, PEGRAM SE: Fluorometric method for the determina-
tion of nanogram quantities of inulin. Anal Biochem 16:409–419,
1966

17. SMITH HW, FINKELSTEIN N, ALIMINOSA L, CRAWFORD B, GRAB-
BER M: The renal clearances of substituted hippuric acid deriva-
tives and other aromatic acids in dog and man. J Clin Invest
24:388–404, 1945

18. WALLENSTEIN S, ZUCKER CL, FLEISS J: Some statistical methods

19. ICHIKAWA I, MADDOX DA, BRENNER BM: Maturational develop-
ment of glomerular ultrafiltration in the rat. Am J Physiol 236:F465-
F471, 1979

20. KOTCHEN TA, STRICKLAND AL, RICE TW, WALTERS DR: A study of
the renin-angiotensin system in newborn infants. J Pediatr
80:938–946, 1972

21. FISLER TJW, LUJEN P, MONNIENS L, VAN MUNSTER P, JANSEN
M, PEER P: Levels of renin, angiotensin I and II, angiotensin-
converting enzyme and aldosterone in infancy and childhood. Eur J
Pediatr 141:3–7, 1983

22. WALLACE KB, HOOK JB, BAILEY MD: Postnatal development of
the renin-angiotensin system in rats. Am J Physiol 238:R432–R437,
1980

23. ROBILLARD JE, NAKAMURA KT, MATHERNE GP, JOSE PA: Renal
hemodynamics and functional adjustments to postnatal life. Sem-
nars in Perinatology 12:143–150, 1988

24. WALLACE KB, ROTH RA, HOOK JB, BAILEY MD: Age-related
differences in angiotensin I metabolism by isolated perfused rat

25. SIEGEL SR: Decreased vascular and increased adrenal and renal
sensitivity to angiotensin II in the newborn lamb. Circ Res 48:34-
38, 1981

26. ICHIKAWA I, FERRONE RA, DUCHIN KL, MANNING M, DZAU VJ,
BRENNER BM: Relative contribution of vasopressin and angioten-
sin II to the altered renal microcirculatory dynamics in two-kidney

27. ICHIKAWA I, PFEFFER JM, PFEFFER MA, HOSTETTER TH, BRENN-
NER BM: Role of angiotensin II in the altered renal function of

28. ICHIKAWA I, PURKERSON ML, KLAHR S, TROY JL, MARTINEZ-
Maldonado M, BRENNER BM: Mechanism of reduced glomerular

29. ROSENBERG ME, CHMIELEWSKI D, HOSTETTER TH: Effect of dietary
protein on rat renin and angiotensinogen gene expression. J

30. RUD P, BLASCHKE TF: Antihypertensive agents and the drug
therapy of hypertension, in Goodman and Gilman's The Pharma-
cologic Basis of Therapeutics, edited by AG GILMAN, LS GOOD-
MAN, TW RALL, F MURAD, (7th ed.) New York, Toronto, London,

31. FLECKENSTEIN A, GRÖN G, TRUTHARTH H, BYON K: Uterine
relaxation durch hochaktive Ca**-antagonistische Hemmstoffe der
elektromechanischen Koppelung wie Isotop (Verapamil, Iprover-
atriol), Substanz D600 und Segontin (Prenylamin). Klin Wochenschr
49:32–41, 1971

32. SMEDA JS, LEE RMKW, FORREST JB: Prenatal and postnatal
hydralazine treatment does not prevent renal vessel wall thickening

Fogo et al: ACE inhibitor effect on glomerular maturation

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