Tubuloglomerular feedback and prolonged ACE-inhibitor treatment in the hypertensive fawn-hooded rat

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Abstract

**Background.** The spontaneously hypertensive fawn-hooded (FHH) rat develops severe glomerulosclerosis with ageing. The afferent arteriolar resistance is low, resulting in a strongly elevated glomerular capillary pressure (P_GC).

**Methods.** Afferent arteriolar resistance is under the control of the tubuloglomerular feedback (TGF) system, and we studied whether young FHH rats, i.e. at a stage when only mild glomerulosclerosis was present, have diminished TGF responsiveness.

**Results.** Maximum TGF-mediated decreases in stop-flow pressure in response to late proximal perfusion with artificial tubular fluid were 9.0 ± 1.0 mmHg, a value not different or even slightly lower than observed in normal rats. P_GC was 59.9 ± 1.2 mmHg and the estimated P_GC at half-maximal activation of the TGF system was 54.5 ± 0.8 mmHg at 11 weeks of age (n = 11), a value higher than observed in normal rats. The second question of the present study concerns the effect of chronic angiotensin-I-converting enzyme inhibitor (ACE-i) administration on P_GC. ACE-i, by reducing angiotensin II (Ang II), diminishes TGF responsiveness, which would offset the beneficial effect on P_GC under normal flow conditions to the macula densa. Maximum TGF responses were 8.9 ± 1.0 and 17.5 ± 1.5 mmHg in 11- and 26-week-old rats that had been treated with the ACE-i lisinopril in the drinking water started when the animals were 7 weeks of age. P_GC was 44.3 ± 1.2 (n = 9) and operating P_GC was 40.1 ± 1.6 mmHg (n = 9) at 11, values significantly lower than in untreated rats. Values remained lower in the 26-week-old treated animals and were 40.9 ± 0.8 and 32.6 ± 1.1 mmHg.

**Conclusions.** The TGF system in this model of spontaneous hypertension and glomerulosclerosis is intact, despite the fact that the FHH rat has a characteristically low afferent arteriolar resistance as compared to other hypertensive rats; (2) the rat displays a normal or even enhanced function of the TGF system following prolonged administration of the ACE-i lisinopril. The latter finding indicates that the reduction of P_GC achieved by the ACE-i is not offset by a concomitant attenuation of TGF function.

Introduction

The spontaneously hypertensive fawn-hooded (FHH) rat develops severe glomerulosclerosis [1–5]. Systemic arterial pressure in this animal is increased, and the remarkably low afferent arteriolar resistance results in elevated glomerular capillary pressure (P_GC) [3,5]. This suggests that glomerular hypertension forms a major risk factor for the development of glomerulosclerosis in this species [2–5], as in several other models of glomerulosclerosis [6–9]. However, P_GC in these studies was calculated from proximal tubular stop-flow pressure, which eliminates any potential correction by the macula densa [10]. Besides, if the regulation of afferent arteriolar tone is diminished in this animal, one may suspect that TGF control of the afferent arteriolar tone is also reduced, which would further add to the glomerular hypertension in vivo. This would further differentiate this animal from other hypertensive rat strains, such as the spontaneously hypertensive rat (SHR) or the Milan hypertensive rat, which are characterized by an increased afferent arteriolar resistance [11–13], slightly enhanced TGF responsiveness [11–14], normal P_GC [11–13,15] and absence of major glomerulosclerosis [16]. Thus the first aim of our study was to assess TGF responsiveness in the FHH rat at a stage prior to the development of severe glomerulosclerosis.

The second question of the present study concerns the effect of chronic angiotensin-I-converting enzyme inhibition on P_GC. We have shown that angiotensin-I-converting enzyme inhibitor (ACE-i) administration effectively prevents or reduces renal injury in the FHH rat [2,4,5], as it does in other rat models of glomerulosclerosis [6–9] and in humans [17]. This protection...
was accompanied by a fall in systemic blood pressure and \( P_{GC} \). Again, it should be noted that \( P_{GC} \) was assessed during interrupted proximal tubular flow, which eliminates TGFn activity. This is particularly relevant since ACE-i, by reducing angiotensin II (Ang II) availability, attenuates TGFn responsiveness [18], which would offset the beneficial effect on \( P_{GC} \) in vivo. Attenuation of TGFn responsiveness during ACE-i treatment has been demonstrated in acute experiments [18]; however, data on the function of the TGFn system during chronic ACE-i administration are not available.

Conceivably, given the pivotal role that the TGFn mechanism plays in the control of sodium balance, TGFn activity becomes restored during prolonged ACE-i treatment. Thus our second aim was to measure glomerular haemodynamics and TGFn function in the FHH rat during chronic ACE-i treatment, to allow more insight in the effects of chronic ACE-i administration on \( P_{GC} \) in vivo.

With these goals, glomerular haemodynamics and TGFn function were assessed in FHH rats at the age of 11 weeks, i.e. prior to development of severe glomerulosclerosis under control conditions and following 4 weeks of ACE-i treatment, and at the age of 26 weeks after prolonged ACE-i administration. Effectiveness of ACE inhibition in these chronic experiments was verified by assessment of intrarenal Ang II levels and by testing the blood pressure response to intravenous bolus injections of Ang I.

Subjects and methods

Animals

The male FHH rats were obtained from our colony maintained at the animal facilities of the Utrecht University. The rats had free access to standard rat chow containing 0.40% sodium and 24% protein by weight (Hope Farms, Woerden, The Netherlands) and tap water ad libitum. Sentinel animals were monitored regularly for infection by nematodes and pathogenic bacteria as well as antibodies for a large number of rodent viral pathogens [19] and were consistently negative throughout the course of the experiments. The protocol was approved by the board for studies in experimental animals of the Utrecht University.

General study design

Micropuncture studies were carried out at 11 and 26 weeks of age. Rats studied at 11 weeks had been treated with the ACE-i, lisinopril (LIS), 50 mg/l drinking water. from week 7 (11LIS, \( n = 9 \)), or had been left untreated (11CON, \( n = 8 \)). Rats studied at 26 weeks of age had been similarly treated with lisinopril from week 7 (26LIS, \( n = 9 \)). In the week preceding the micropuncture experiments two consecutive 24-h urine samples were collected to determine albumin excretion (\( U_{\text{ALB}} \)) and systolic blood pressure (SBP) was measured in the conscious state with the tail-cuff method (ITC, Woodland Hills, CA, USA) [5].

Micropuncture preparations

Animals were prepared as described previously [5]. Briefly, rats were anaesthetized with 60 mg/kg sodium pentobarbitone i.p. and placed on a servo-controlled heated operating table. The trachea was intubated and the left femoral artery and vein were cannulated for continuous monitoring of mean arterial pressure (MAP), blood sampling, and intravenous infusion. Immediately following cannulation, approximately 75 \( \mu l \) of arterial blood was collected to determine a baseline value for the \( \beta \)-aminohippurate (PAH) assay. The left kidney was approached by a flank incision, freed from connective tissue and placed in a Lucite cup; the left ureter was cannulated. Warm agar was dripped around the kidney and on the Lucite cup to form a well, which was filled with saline or mineral oil. Following surgery, a 60-min equilibration period was observed before initiating the micropuncture procedures. Because of the plasma volume depletion reported during micropuncture experiments [20], the rats were infused 6% bovine serum albumin in 0.9% NaCl as described previously [5]. Thereafter, infusion was continued throughout the experiment with 0.9% NaCl containing 15% inulin, 0.5% PAH and 1% BSA at 30 \( \mu l/min \). To maintain ACE inhibition during the experiment, rats that had received lisinopril treatment were administered 0.2 mg/kg/h lisinopril i.v.

Experimental protocol

To determine whole kidney glomerular filtration rate (GFR) and renal plasma flow (RPF), urine was collected over six 30-min periods and plasma samples were obtained at the start of the first and after the second, fourth, and sixth urine collection. Stop-flow pressure responses to step increases in late proximal perfusion were obtained as described previously [21]. Briefly, an early proximal tubular segment was localized with a 4–6 \( \mu m \) tip diameter localization pipette, containing artificial tubular fluid (ATF; composition see [21]) stained with 0.2% Fast Green (Sigma Chemicals, St Louis, MO, USA). A wax block was inserted into an early proximal tubular segment. SFP was measured with a 3–4 \( \mu m \) tip diameter localization pipette, containing a continuous recording servo-null pressure system (Model 5A, Instruments for Physiology and Medicine, San Diego, CA). A 5–7 \( \mu m \) tip-diameter perfusion pipette filled with stained (0.2% Fast Green) ATF was introduced into a late proximal tubular segment and connected to a micropuncture pump (Effenberger, Pfaffing/Attel, Germany). SFP was measured at late proximal perfusion rates of 0, 10, 15, 20, 25, 30, 40 and 50 \( nl/min \). Proximal tubular pressure (\( P_{\text{TP}} \)) was measured in at least four tubules. Efferent arteriolar pressure (\( P_{\text{E}A} \)) was assessed from at least four different star vessels. When pressure measurements were completed, the saline in the well was replaced with light mineral oil (Sigma Chemicals) and, three to five exactly timed fluid samples were collected from randomly selected proximal tubular segments to determine single-nephron glomerular filtration rate (SNGFR) as described previously [5,21]. At the end of the experiment, the MAP response to a bolus injection of 0.1 ml isotonic saline containing 25 ng of angiotensin I was evaluated. Ten minutes later, the left kidney was clamped, removed, weighed, and processed for the measurement of Ang II in renal tissue as described below. The right kidney was processed for light-microscopic evaluation of glomerular damage as described previously [3–5]. Glomerulosclerosis is expressed as the percentage of glomeru-
Biological analysis

Albumin concentration in 24-h urine collections, creatinine concentrations, inulin concentrations, PAH and haematocrit were determined using procedures reported previously from our laboratory [5,21]. Colloid osmotic pressure was measured using a strain gauge micro-oncometer. After determination of the volume of the proximal tubular fluid samples, inulin concentrations were measured by microfluorometry [22]. Kidney ANG II samples were extracted and determined according to the procedure of Fox et al. [23] and also reported by our group [21].

Calculations

Values for 24-h urinary albumin excretion in each individual rat are calculated as the mean of two 24-h urinary albumin excretion rates on consecutive days. Values for SBP in each individual rat are the mean calculated from measurements obtained at 2 consecutive days. GFR and RPF were assessed from inulin and PAH clearance respectively. Whole kidney filtration fraction (FF) is calculated from the equation: FF = (U_inulin / P_inulin) - (U_PAH / P_PAH), where U and P represent urinary and plasma concentrations, respectively. The glomerular ultrafiltration coefficient (Kf) could be estimated using the model described by Deen [24]. The differential equation was solved numerically on a single-nephron basis using the values of SNGFR, SNPF, πg, and ΔP.

Statistics

Each parameter was averaged per rat, and results are expressed as means ± SEM calculated from these averages. Data were compared by t test with Bonferroni protection using statistical software (SigmaStat™, Jandel Scientific, Erkrath, Germany). P < 0.05 was considered statistically significant. The V1/2max was calculated using the logistic equation and a non-linear curve-fitting routine (SigmaPlot® Jandel Scientific, Erkrath, Germany).

Results

Whole kidney, systemic parameters and histology

The 11-week-old FHH rats were hypertensive (Table 1). Treatment with lisinopril (LIS) prevented the rise in blood pressure, and this effect was maintained in animals studied at 26 weeks of age. ACE-i treatment had no significant effects on GFR, but filtration fraction was consistently lower and RBF higher during ACE-i. At 11 weeks of age a significant albuminuria was present, which was not observed in the rats administered ACE-i. The level of glomerulosclerosis in 11-week-old FHH rats was significantly prevented by ACE-i administration (Table 1). This protective effect was still observed at 26 weeks of age.

Single-nephron haemodynamics

Single-nephron GFR was similar in all groups (Table 2). Stop-flow pressure and PGC measured under conditions of zero-flow to the macula densa were significantly lower in the 11-week-old rats treated with ACE-i as compared to the untreated rats, and this effect persisted in the 26-week-old rats. ACE-i consistently decreased both afferent and efferent arteriolar resistances, and increased Kf.

TGF responses

In the 11-week-old untreated FHH rats the maximum TGF-mediated decrease in stop flow pressure was 9.0 ± 1.1 mmHg. Complete TGF response curves showed an average V1/2max of 32.0 ± 0.9 nl/min (Figure 1). Operating PGC, calculated from stop-flow pressure (PGC) minus half of the maximum TGF-mediated decrease in stop flow pressure, was averaged 54.5 ± 0.8 mmHg (Figure 2).

Maximum TGF responses were not different in 11-week-old rats treated with ACE-i, averaging 8.9 ± 1.0 mmHg. The V1/2max was also similar (Figure 1); however, operating PGC was significantly lower (40.1 ± 1.6 mmHg) than in the untreated rats (Figure 2). Remarkably, the 26-week-old ACE-i-treated rats showed exaggerated maximum TGF responses, averaging 17.5 ± 1.5 mmHg (Figure 1). Again, V1/2max was similar to that in the 11-week-old treated rats, and a further reduction in PGC to 32.6 ± 1.1 mmHg was observed (Figure 2).

Ang I challenge and kidney Ang II levels

To test the degree of ACE inhibition during the micro-puncture experiments, we documented the change in

| Table 1. Systemic and whole kidney parameters at 11 and 26 weeks of age |
|-----------------|-----------------|-----------------|
| Groups          | 11 CON          | 11 LIS          | 26 LIS          |
| Number          | 8               | 9               | 9               |
| Body wt (g)     | 297 ± 8         | 278 ± 7         | 350 ± 13*       |
| MAP (mmHg)      | 127 ± 2         | 78 ± 2          | 85 ± 2          |
| GFR (ml/min)    | 1.54 ± 0.07     | 1.44 ± 0.06     | 1.22 ± 0.08     |
| FF              | 0.37 ± 0.01     | 0.24 ± 0.01b    | 0.21 ± 0.01     |
| RBF (ml/min)    | 8.4 ± 0.4       | 11.3 ± 0.8*     | 10.0 ± 0.7      |
| U_ALB (mg/day)  | 50 ± 9          | 10 ± 2*         | 10 ± 1          |
| GS index (%)    | 5.0 ± 0.4       | 0.4 ± 0.2b      | 0.7 ± 0.2       |

MAP, mean arterial pressure; GFR, glomerular filtration rate; FF, filtration fraction; RBF, renal blood flow; U_ALB, 24-h urinary albumin excretion; GS index, glomerulosclerosis index. Values are means ± SEM. *P < 0.05 vs 11CON, †P < 0.01 vs 11CON, ‡P < 0.05 vs 11LIS.
mean arterial pressure in response to a single intravenous Ang I challenge, and measured the renal concentration of Ang II. ACE-i treatment virtually abolished the blood-pressure response to Ang I in both 11- and 26-week-old rats (Figure 3). Renal Ang II levels were 282 ± 66 pM in untreated rats (n = 6) and significantly lower in 11-week-old ACE-i-treated rats (53 ± 6 pM; n = 9, P < 0.01, t test with Bonferroni correction). There was no significant difference in renal Ang II levels between the 7-week and 26-week-old ACE-i-treated rats (48 ± 7 pM; n = 9). These findings indicate adequate ACE inhibition in both the 11- and 26-week-old treated rats.

**Discussion**

The present study was performed to evaluate glomerular pressure (P GC ) in the presence of an operating TGF mechanism in the FHH rat. The first question was whether the low afferent arteriolar tone which is characteristic of this rat is associated with impaired TGF regulation. Young FHH rats displayed substantial TGF responses, not less than expected in normotensive Sprague–Dawley rats [18, 21], but insufficient to control operating P GC to normal levels. The second question was whether chronic ACE-i treatment would diminish TGF responsiveness, since that would abate the beneficial effect of ACE-i on P GC under normal flow conditions to the macula densa. Remarkably, this was not the case, since FHH rats at 11 weeks of age showed normal TGF responses and rats at 26 weeks of age even showed exaggerated responses during ACE-i.

The FHH rat is a model of spontaneous hypertension, glomerulosclerosis, and chronic renal failure. Observations in this rat made under different conditions indicate that glomerular hypertension may well contribute to the renal damage [2–5]. Characteristic for this rat is a low afferent-to-effenter resistance ratio of about one [5], a finding confirmed in the present study in young FHH rats. This confirms that FHH rats, compared to other hypertensive rat strains [11–13], have a low afferent arteriolar resistance, so that the elevated systemic pressure is transmitted to the glomerular capillaries [5]. However, so far the P GC in the FHH rat has been assessed under stop-flow conditions, thus without the influence of the TGF system on P GC as may occur in vivo.

That the effects of the TGF system cannot be neglected follows from the finding of large TGF-mediated diminutions of P GC in other hypertensive rat strains such as the SHR or the Milan hypertensive rat [11–15]. In SHR the proximal tubular stop-flow pressure and P GC in the absence of TGF activity are normal, i.e. around 40 mmHg and 54 mmHg respectively [13,15]. In view of the systemic hypertension, this implies that SHR have a relatively high afferent arteriolar resistance [13,15]. The maximum TGF-dependent decrease in stop-flow pressure is 10–12 mmHg [13,15], so that in SHR the operating P GC is approximately 48–50 mmHg, a value roughly similar as estimated in normotensive rats [18,21]. Comparable figures have been found in the Milan hypertensive rat [11,12]. This indicates that these rat strains probably have a normal P GC in vivo.

Quite different findings have been made currently in the FHH rat, in which P GC in the absence of TGF activity was 60 mmHg, and the maximum TGF-dependent decrease in stop-flow pressure was 9 mmHg, indicating that the operating P GC is 55 mmHg. This implies that the maximum TGF-mediated decrease in glomerular pressure, although normal in absolute terms, is relatively low, and not able to control glomerular pressure at normal levels. Notably, we studied young FHH rats at a stage prior

<table>
<thead>
<tr>
<th>Groups</th>
<th>11CON</th>
<th>11LIS</th>
<th>26LIS</th>
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<tbody>
<tr>
<td>Number</td>
<td>8</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>SNFGR (nl/min)</td>
<td>55.1 ± 3.0</td>
<td>59.7 ± 3.8</td>
<td>49.0 ± 3.9</td>
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<tr>
<td>SNBF (nl/min)</td>
<td>303 ± 24</td>
<td>476 ± 49*</td>
<td>407 ± 41</td>
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<tr>
<td>P a (mmHg)</td>
<td>12.2 ± 0.2</td>
<td>11.5 ± 0.4</td>
<td>9.6 ± 0.2*</td>
</tr>
<tr>
<td>P e (mmHg)</td>
<td>11.1 ± 0.5</td>
<td>8.9 ± 0.6*</td>
<td>7.8 ± 0.4</td>
</tr>
<tr>
<td>C a (g/dl)</td>
<td>45.9 ± 0.9</td>
<td>47.8 ± 1.0</td>
<td>46.8 ± 1.7</td>
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<tr>
<td>C e (g/dl)</td>
<td>73.3 ± 2.8</td>
<td>61.3 ± 2.8</td>
<td>59.9 ± 2.7</td>
</tr>
<tr>
<td>p a (mmHg)</td>
<td>13.7 ± 0.4</td>
<td>14.5 ± 0.4</td>
<td>14.7 ± 0.5</td>
</tr>
<tr>
<td>p e (mmHg)</td>
<td>27.9 ± 1.7</td>
<td>21.2 ± 1.4*</td>
<td>20.5 ± 1.3</td>
</tr>
<tr>
<td>SFP (mmHg)</td>
<td>46.2 ± 1.1</td>
<td>30.4 ± 1.0*</td>
<td>26.2 ± 0.9*</td>
</tr>
<tr>
<td>P GC (mmHg)</td>
<td>59.9 ± 1.2</td>
<td>44.3 ± 1.2*</td>
<td>40.9 ± 0.8</td>
</tr>
<tr>
<td>AP (mmHg)</td>
<td>47.8 ± 1.1</td>
<td>32.8 ± 1.0*</td>
<td>31.3 ± 0.7</td>
</tr>
<tr>
<td>R a (10^10 dyne·s·cm^-1)</td>
<td>1.15 ± 0.15</td>
<td>0.79 ± 0.12*</td>
<td>0.80 ± 0.09</td>
</tr>
<tr>
<td>R e (10^10 dyne·s·cm^-1)</td>
<td>0.033 ± 0.001</td>
<td>0.066 ± 0.005*</td>
<td>0.059 ± 0.004</td>
</tr>
</tbody>
</table>

SNFGR, single-nephron glomerular filtration rate; SNBF, single-nephron blood flow; P a, proximal tubular pressure; P e, efferent arteriolar pressure; C a and C e, afferent and efferent arteriolar plasma protein concentrations; p a and p e, afferent and efferent arteriolar colloid osmotic pressures; SFP, stop-flow pressure; P GC , glomerular capillary pressure; AP, glomerular transcapillary pressure gradient; R a and R e, afferent and efferent arteriolar resistances; Kf, glomerular capillary ultrafiltration coefficient. Values are means ± SEM, *t test with Bonferroni correction: *P < 0.05 vs 11CON, 9P < 0.01 vs 11CON, 3P < 0.05 vs 11LIS, 6P < 0.01 vs 11LIS.
to the development of severe glomerulosclerosis [5], to avoid altered TGF responsiveness due to loss of functioning nephrons. The latter has been found in other rat models of impaired renal function, although the direction varied from enhancement to suppression [10].

The present data, in aggregate with previous findings, allow further characterization of the FHH rat in relation to other hypertensive rats. Apparently, in vivo operating \( P_{GC} \) in the young FHH rats is elevated, and insufficiently controlled by afferent arteriolar resistance and the TGF system, in contrast to normal operating \( P_{GC} \) in the SHR and the Milan hypertensive rat [11–15]. Since of these hypertensive strains only the FHH rat is predisposed to develop glomerulosclerosis, this gives further support to the pathogenetic role of glomerular hypertension in this process. Notably, the severity of hypertension in the FHH rat appears less than in the SHR and Milan hypertensive rat [2–5,11–15]. It has been emphasized that a relatively high TGF responsiveness may contribute to the development of hypertension, because of the associated depression of SNGFR [25]. Glomerular pressure and TGF activity in the FHH rat allow glomerular filtration to be higher at lower systemic pressure than other hypertensive strains. The glomerular haemodynamics in the FHH rat may thus limit the degree of systemic hypertension, however, at the cost of increased risk of glomerulosclerosis.

It remains to be resolved why afferent arteriolar resistance is low and its control by the TGF mechanism relatively inefficient in the FHH rat. The present and previous studies have shown that the hypertension in this animal is very sensitive to blockers of the renin–angiotensin system [4,5], indicating that the (micro)vasculature is under profound influence of Ang II. Since
a high Ang II activity is associated with enhanced TGF activity in hypertensive models such as the two-kidney, one-clip model [18,26] and the transgenic mRen-2 rat [27], one could surmise that in the FHH rat the endogenous defence against the afferent arteriolar constriction by Ang II is pronounced by a paracrine disturbance. In this respect, it may be relevant that the FHH rat also has a bleeding diathesis and platelet dysfunction due to defective serotonin and ADP release [28], and that urinary excretion of eicosanoids is increased [29].

Our second aim was to explore whether maintenance ACE-i treatment would reduce TGF responsiveness, as may be deduced from acute studies. It has been shown that the TGF responsiveness is enhanced by acute Ang II administration [30,31] and attenuated by acute Ang II inhibition [18,26]. One study assessed TGF responses 4 days after initiation of ACE-i and meclofenamate, and observed a greatly diminished TGF function in hypertensive rats [39]. However, this is difficult to accept, in view of the great sensitivity of the circulation of this animal to ACE-i [4,5].

Summarizing, the present study evaluated the TGF system in the FHH rat, a model of spontaneous hypertension and chronic renal failure. The first observation is that the TGF system is intact, despite the fact that the FHH rat has a characteristically low afferent arteriolar resistance as compared to other hypertensive rats. The second observation is that the TGF responses have never been assessed in rats chronically treated with ACE-i (or AT1 receptor antagonists). In that regard, the present data fill an essential gap in our understanding of the actions of ACE-i on the kidney, in particular since they show that prolonged ACE-i treatment clearly fails to diminish the TGF function. In fact, the rats studied at 26 weeks showed enhanced TGF responses. As found previously in the FHH rat [5], the fall in renal vascular tone after ACE-i was equally distributed over afferent and efferent resistances, so that the fall in P_GC resulted from the fall in systemic pressure rather than from preferential efferent arteriolar relaxation.

The prime conclusion that we can draw from these findings is that the ACE-i-induced fall in stop-flow pressure measured during interrupted flow to the macula densa does not overestimate the fall in actual P_GC during normal flow to the macula densa. In other words, long-term treatment with ACE-i causes an even further reduction of P_GC than would have been achieved if the TGF were still attenuated such as found after acute ACE administration [18]. Obviously this further adds to the protective effect of ACE-i against glomerulosclerosis, since P_GC under normal flow conditions was estimated to be similar or even lower than found in normal rats [18,21]. Indeed, similar to the data we reported previously, ACE-i started at young age efficiently prevented the development of glomerulosclerosis [5].

Clearly, the finding that prolonged ACE-i does not attenuate TGF responsiveness as does acute ACE-i also raises multiple questions, which are beyond the direct focus of the present study. The main reason to assume a priori that TGF function might not be suppressed by prolonged ACE-i treatment was the notion that the TGF mechanism serves an important function. We presumed that this function, i.e. prompt regulation of distal solute delivery in dependence of extracellular fluid volume [10,33], is indispensable, and conserved by multiple regulating factors besides Ang II. For example, it is known that the TGF responsiveness increases by administration of vasoconstrictive eicosanoids [34] or nitric oxide synthase inhibitors [35,36]. Perhaps, during continued ACE-i a new balance between TGF modulators is established that restores TGF function, but the literature provides no direct clues. TGF modulation is a function of multiple paracrine factors [37], and the answer to such questions will need detailed studies of the renal microcirculation. Another option that would explain normal TGF function during maintenance ACE-i administration is escape from its pharmacological effect, by Ang II generation independent from ACE [38]. However, this option is unlikely, since effective inhibition of ACE was confirmed by absent blood pressure response to Ang II, and the intrarenal concentrations of Ang II were clearly suppressed. Finally, it is possible that angiotensin II does not play a role in modulating TGF in the FHH, as was recently found in euvaolemic normotensive rats [39]. However, this is difficult to accept, in view of the great sensitivity of the circulation of this animal to ACE-i [4,5].

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