Progression of hypertension and kidney disease in aging fawn-hooded rats is mediated by enhanced influence of renin–angiotensin system and suppression of nitric oxide system and epoxyeicosanoids

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ABSTRACT

The fawn-hooded hypertensive (FHH) rat serves as a genetic model of spontaneous hypertension associated with glomerular hyperfiltration and proteinuria. However, the knowledge of the natural course of hypertension and kidney disease in FHH rats remains fragmentary and the underlying pathophysiological mechanisms are unclear. In this study, over the animals’ lifetime, we followed the survival rate, blood pressure (telemetry), indices of kidney damage, the activity of renin–angiotensin (RAS) and nitric oxide (NO) systems, and CYP450-epoxygenase products (EETs). Compared to normotensive controls, no elevation of plasma and renal RAS was observed in prehypertensive and hypertensive FHH rats; however, RAS inhibition significantly reduced systolic blood pressure (137 ± 9 to 116 ± 8, and 159 ± 8 to 126 ± 4 mmHg, respectively) and proteinuria (62 ± 2 to 37 ± 3, and 132 ± 8 to 87 ± 5 mg/day, respectively). Moreover, pharmacological RAS inhibition reduced angiotensin (ANG) II and increased ANG 1–7 in the kidney and thereby may have delayed the progression of kidney disease. Furthermore, renal NO and EETs declined in the aging FHH rats but not in the control strain. The present results, especially the demonstration of exaggerated vascular responsiveness to ANG II, indicate that RAS may contribute to the development of hypertension and kidney disease in FHH rats. The activity of factors opposing the development of hypertension and protecting the kidney declined with age in this model. Therefore, therapeutic enhancement of this activity besides RAS inhibition could be attempted in the therapy of human hypertension associated with kidney disease.

Introduction

The fawn-hooded hypertensive (FHH) rat serves as a genetic model of spontaneous hypertension in which early development of systemic and renal glomerular hypertension results in glomerular hyperfiltration and proteinuria (1–3). The progression of hypertension and associated organ damage, especially of kidney failure, leads to premature death of the animals. The dilemma whether renal glomerulosclerosis observed in FHH rats is the consequence or the cause of hypertension has been extensively explored (3–5). The principal reasoning was that systemic hypertension may increase glomerular capillary pressure under the condition of reduced preglomerular resistance and thus accelerate the development of glomerular damage (6,7). Indeed, micropuncture measurements revealed that glomerular capillary pressure is higher in FHH rats as compared to their normotensive counterparts described as fawn-hooded low-pressure (FHL) rats, the strain that displays relative resistance to renal damage (5,8). Similarly, the resistance vessels of FHH rats displayed impaired control of the myogenic response, both in the renal and cerebral microcirculations (9,10). On the other hand, regional differences in the regulation of vascular reactivity were reported (11). The reduced myogenic reactivity of preglomerular arterioles to blood pressure (BP) elevations in FHH rats was paralleled by reduced reactivity to angiotensin II (ANG II) (6), suggesting that renal vascular dysfunction precedes glomerular damage (9).

The renin–angiotensin system (RAS) plays a major role in regulation of preglomerular resistance and glomerular pressure (6). Therefore, the RAS activity might significantly influence the progression of hypertension and chronic kidney disease (CKD) in FHH rats. Indeed, inhibition of the RAS was reported to reduce renal injury and proteinuria in many forms of renal disease and to slow down progression toward the end-stage renal failure (12,13). However, CKD is usually characterized by low-to-normal plasma renin activity (PRA), indicating rather local enhancement of RAS activity, if any at all. While the data on the role of PRA remain inconclusive in FHH strain, one study described enhanced expression of renin in the afferent arteriole (14). Other studies showed...

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correlation of elevated renin and BP levels with the extent of renal damage (15). Finally, inhibition of the RAS in young FHH rats effectively protected the animals from both hypertension and renal damage (12,13).

In the context of the above knowledge, we hypothesized that the development of hypertension and the progression of CKD in FHH rats could be influenced both by age-dependent enhancement of pro-hypertensive mechanisms, such as exaggerated systemic and intrarenal RAS activity, and by attenuated action of protective systems, such as nitric oxide (NO) or cytochrome P-450 (CYP450)-epoxyeicosanoids. No conclusive data are available to indicate the exact mechanisms that may contribute to the development of hypertension and progression of CKD in FHH rats. The main goal of this study was to follow BP, plasma and renal RAS activity, the levels of cytochrome CYP450-derived metabolites, the activity of NO synthases (NOS) in the kidney, and the degree of renal injury in FHH and in normotensive FHL strains during the course of aging process of the animals. In addition, we evaluated the effects of RAS blockade on BP and RAS activity. Finally, we tested acute BP and renal blood flow (RBF) responses to vasoactive substances with an aim to assess possible differences in the vascular responsiveness between pre-hypertensive and hypertensive FHH rats.

Methods

The study performed in male FHH and FHL rats (total n = 158) was approved by the Animal Care and Use Committee of the Institute for Clinical and Experimental Medicine and was in accordance with the regulations in the Czech Republic and in the European Union. The animals originated from the colony at the UMC Groningen, The Netherlands, and were housed in the institutional facility accredited by the Czech Association for Accreditation of Laboratory Animal Care.

Blood pressure monitoring, urine collection, and tissue harvesting

The experimental protocol was conducted in FHH and FHL rats aged 3, 6, 9, and 12 months to identify age-related differences in BP and the degree of renal damage, and to assess the progression of hypertension and CKD in the FHH model. Cardiovascular parameters were monitored by radiotelemetry system (Data Sciences International, St. Paul, MN, USA) using TA11PA-C40 radiotransmitters, as described previously (16,17). The animals were anesthetized with a combination of tiletamine, zolazepam (Zoletil, Virbac SA, Carros Cedex, France; 8 mg/kg), and xylasine (Rometar, Spofa, Czech Republic; 4 mg/kg), given intramuscularly. An abdominal midline incision was performed to expose the abdominal aorta that was briefly occluded to allow insertion of the transmitter catheter. The catheter was secured in place with tissue glue. The transmitter body was sutured to the abdominal wall along the incision line as the incision was closed. Due to the technical limitations of radiotelemetry, the BP recording could not be performed in the same animals from the 3rd to the 12th month of age. Therefore, the transmitters were implanted in separate age groups of animals. This was done two weeks before actual measurements to allow for appropriate recovery. After 10–12 days of recovery, data acquisition was initiated to record BP and collect the data daily as described previously (16,17). The animal body weight (BW) was monitored in the same time periods.

Urine collections were performed to determine proteinuria by commercial kits (Lachema, Brno, Czech Republic), as described previously (18,19). At the end of experiments, animals were decapitated to harvest the kidneys for biochemical and histological analysis. Plasma and renal ANG II and ANG 1–7 levels were measured by radioimmunoassay using a commercially available kit (Euro-Diagnostica Co., Malmö, Sweden and Immunotech s.r.o, Prague, Czech Republic, respectively) as described previously (16,18,19). CYP450 metabolites epoxyeicosatrienoic acids (EETs) and hydroxy-icosatetraenoic acids (HETEs) were determined in kidney tissue by high-performance liquid chromatography (HPLC) mass spectrometry. Samples were extracted, separated by reverse-phase high-performance liquid chromatography, and analyzed by negative-mode electrospray ionization and tandem mass spectrometry, as described previously (16,19). Activity of NOS was measured as conversion of radioactive labeled arginine to citrulline in the renal cortex and medulla (20) using an assay kit (Cayman Chemical, Ann Arbor, MI, USA). The progression of glomerular and tubular damage was assessed by high-power-field technique in the Periodic acid–Schiff (PAS) stained kidney slices; the assessment refers to the area visible under 400-fold magnification power of the objective used in the present study. A total number of 100 glomeruli in each kidney slice were examined on a semi-quantitative scale for the glomerulosclerosis index (GSI) and tubulointerstitial injury (TII) was assessed for at least 30 random and non-overlapping fields, as described previously (18–20).

Dual RAS blockade

The effect of dual RAS blockade was tested in the second series of experiments. At the age of 3 and 9 months, FHH (n = 13) and FHL (n = 12) rats chronically implanted with radiotransmitters were treated with an angiotensin-converting enzyme inhibitor (ACEI, perindopril, Servier Laboratories, France, 5 mg/kg/day) together with an ANG II receptor blocker (ARB, losartan, Zentiva, Zentiva, Slovakia, 50 mg/kg/day) for 28 days (18). At the end of experiments, these animals were decapitated to harvest the kidneys for biochemical and histological analysis.

Acute vascular responses to vasoactive drugs

In the third series of experiments, the rats aged 3 or 9 months were anesthetized with Thiopental (VUAB Pharma, Czech Republic), 50 mg/kg intraperitoneally, and placed on a thermoregulated table to maintain body temperature at about 37°C. A tracheal tube was inserted to supply atmospheric air enriched with humidified gas mixture containing 95% oxygen and 5% carbon dioxide. The right jugular vein was cannulated with PE-50 tubing for infusion of solutions, additional anesthetic or drugs. The femoral artery was cannulated for continuous monitoring of mean arterial pressure (MAP) and blood sampling. The left kidney was exposed via a flank
incision, isolated, and placed in a lucite cup. An ultrasonic cuff probe (Transonic Systems Inc., Ithaca, NY, USA) was placed on the left renal artery to record RBF. The renal vascular resistance (RVR) was calculated as MAP-to-RBF ratio and expressed in mmHg/ml-min-g⁻¹. During surgery, an isotonic saline solution containing bovine serum albumin (5%) was infused at a rate of 25 μl/min. After surgery, isotonic saline solution containing albumin (2%) was infused at the same volume infusion rate. In both FHH and FHL strains, vasoactive drug boluses (200 μl) were administered in sequence via the jugular vein; 45 min stabilization/recovery periods were made between injections. ANG II and norepinephrine (NE) were given at doses of 65 and 300 ng/kg; acetylcholine (ACh) was given at doses of 150 and 650 ng/kg (21).

**Statistical analysis**

All values are expressed as means ± SEM. One-way analysis of variance and two-way repeated measures analysis of variance followed by the post hoc test were made, as appropriate, using GraphPad Prism Software (San Diego, California, USA). Values exceeding 95% probability limits (P < 0.05) were considered statistically significant.

**Results**

In order to define more clearly the characteristics of the selected animal model, we first assessed the mortality of FHH rats (n = 20) in comparison with FHL controls (n = 20). Beginning from the age of 11 months, FHH rats exhibited decreased survival rate, which fell to 80% and 40% at months 12 and 18, respectively. Prior to death, we recorded a considerable loss of BW, polydipsia, polyuria, hematuria, and uremia. On necropsy, obvious macroscopic morphological changes of the kidney tissue were detected. Therefore, we performed subsequent studies in the animals not older than 12 months. In FHL rats, the survival rate equaled 90% at 18 months.

Radiotelemetry monitoring of BP performed at 3, 6, 9, and 12 months of age confirmed that systolic BP (SBP) remained within the low normotensive range (113 ± 6 to 126 ± 4 mmHg) in FHL rats, whereas in FHH rats it progressed significantly from 138 ± 4 to 162 ± 5 mmHg at 3 and 12 months, respectively (Table 1). This increase in SBP was accompanied by progression of proteinuria. In FHL rats, however, proteinuria remained low and stable. The BW did not significantly differ between the two animal strains and increased with age at a usual rate.

Histological evaluation of renal damage expressed as a GSI (Figure 1A) and TII (Figure 1B) further confirmed the age-dependent progression of CKD. In contrast, normotensive FHL strain remained resistant to renal injury throughout the course of the animals’ aging process. Representative views of kidney sections are shown in the supplemental figure 1.

Table 1 shows the comparison of plasma and kidney tissue ANG II levels and kidney EETs and HETEs levels in FHL and FHH strains during aging. There was a significant suppression of plasma ANG II in adult FHH rats but no substantial difference in renal ANG II level compared to FHL. On the other hand, renal EETs’ concentrations significantly decreased with age in FHH rats while these remained unchanged in FHL rats. By contrast, no significant differences in renal HETEs concentration were found between both strains, although the levels appeared to decrease with age.

Figure 2 shows that kidney NOS activity did not differ between young FHH and FHL rats. However, both cortical and medullary NOS activity was significantly lower in the former group when measured at the age of 9 months.

Combined RAS blockade with ACEI and ARB induced a distinct decrease in SBP in FHH rats aged 3 months (137 ± 9 to 116 ± 8 mmHg, P < 0.05) and even greater decrease in 9-month-old FHH (159 ± 8 to 126 ± 4 mmHg, P < 0.001) (Figure 3). However, dual RAS blockade in adult FHH animals did not result in decreases of SBP to the level observed in the age-matched FHL controls (Figure 3).

### Table 1. Age-dependent progression of hypertension and proteinuria in hypertensive fawn-hooded (FHH) rats compared to normotensive controls (FHL), determined at 3, 6, 9, and 12 months of age.

| Group          | SBP (mmHg) | Diastolic Blood Pressure (DBP) (mmHg) | Proteinuria (mg/24 h) |
|---------------|-----------|-------------------------------------|-----------------------|
| FHL 3 months  | 113 ± 6   | 81 ± 3                              | 17.8 ± 1.8            |
| FHL 6 months  | 115 ± 5   | 84 ± 2                              | 22.7 ± 2.9            |
| FHL 9 months  | 117 ± 4   | 88 ± 2                              | 26.3 ± 2.5            |
| FHL 12 months | 126 ± 4   | 91 ± 2                              | 34.5 ± 2.7            |
| FHH 3 months  | 138 ± 4*  | 99 ± 2*                             | 61.1 ± 4.5*           |
| FHH 6 months  | 148 ± 6** | 108 ± 3**                           | 97.3 ± 6.9**          |
| FHH 9 months  | 157 ± 4** | 112 ± 2**                           | 134.7 ± 7.8**         |
| FHH 12 months | 162 ± 5** | 116 ± 3**                           | 150.6 ± 9.5**         |

Means±SEM.

*P < 0.05 vs. corresponding FHL group, #P < 0.05 vs. FHH 3 months.

**Figure 1.** Age-related progression of renal injury in FHH rats in comparison with FHL controls assessed as the glomerulosclerosis index (GSI; A) and tubulointerstitial injury (TII; B). *P < 0.05 vs. FHL groups.
Table 2. Age-dependent changes in plasma and renal levels of angiotensin II (ANG II), epoxyeicosatrienoic acids (EETs), and hydroxy-eicosatetraenoic acids (HETEs) in hypertensive fawn-hooded (FHH) rats compared to normotensive controls (FHL), determined at 3, 6, 9, and 12 months of age.

| Group       | Plasma ANG II (fmol/ml) | Renal ANG II (fmol/g) | Renal EETs (ng/g) | Renal HETEs (ng/g) |
|-------------|-------------------------|-----------------------|-------------------|-------------------|
| FHL 3 months (n=6) | 32.9 ± 2.4 | 44.2 ± 3.8 | 935 ± 70 | 3147 ± 187 |
| FHL 6 months (n=8)  | 26.3 ± 1.5 | 56.8 ± 4.6 | 913 ± 64 | 3021 ± 117 |
| FHL 9 months (n=8)  | 23.2 ± 1.7 | 55.2 ± 6.4 | 812 ± 52 | 2675 ± 164 |
| FHL 12 months (n=8) | 24.6 ± 2.5 | 53.8 ± 6.1 | 877 ± 61 | 2566 ± 143 |
| FHH 3 months (n=6)  | 27.5 ± 3.1 | 49.8 ± 6.0 | 830 ± 43 | 3315 ± 214 |
| FHH 6 months (n=8)  | 22.3 ± 3.4 | 63.7 ± 5.7 | 454 ± 12 | 3021 ± 117 |
| FHH 9 months (n=8)  | 14.2 ± 2.6 | 63.7 ± 5.7 | 528 ± 23 | 2760 ± 139 |
| FHH 12 months (n=7) | 12.5 ± 2.1 | 62.4 ± 6.4 | 467 ± 35 | 2349 ± 114 |

Means ± SEM.
*P < 0.05 vs. corresponding FHL group, #P < 0.05 vs. FHH 3 months.

Figure 4 shows how combined (“dual”) RAS inhibition over 28 days affected renal ANG II levels and proteinuria in young and adult FHH rats in comparison with adult FHL controls. The inhibition significantly decreased renal ANG II levels, similarly in both rat strains. In young and adult FHH rats, the treatment significantly decreased proteinuria. These effects varied between young and adult FHH rats. In young FHH, proteinuria was only moderately higher as compared to FHL controls. However, in adult FHH proteinuria remained substantially higher. Figure 5 shows that before RAS blockade plasma and kidney ANG 1–7 levels were comparable in the two rat strains. ACEi/ARB treatment significantly increased these levels, quite similarly in FHL and FHH strains.

In acute experiments designed to study renal hemodynamics, we first established that baseline RBF was significantly higher in prehypertensive young FHH rats (MAP = 124 ± 3 mmHg) than in age-matched FHL (8.2 ± 0.6 vs. 4.8 ± 0.5 ml.min⁻¹.g⁻¹, P < 0.05). Furthermore, these animals had significantly lower RVR (15 ± 1 vs. 21 ± 1 mmHg.ml⁻¹.min⁻¹.g⁻¹, P < 0.05). On the other hand, baseline RBF in hypertensive adult FHH rats (MAP = 141 ± 4 mmHg) was not significantly different compared to age-matched normotensive FHL controls (4.4 ± 0.5 vs. 5.7 ± 0.4 ml. min⁻¹.g⁻¹, difference NS). However, they exhibited significantly higher RVR than normotensive control rats (34 ± 3 vs. 20 ± 2 mmHg.ml⁻¹.min⁻¹.g⁻¹, P < 0.05).

Figure 6 shows acute MAP and RBF responses to intravenous ANG II in anesthetized young and adult FHH and FHL rats. Young FHH rats displayed significantly enhanced MAP and RBF responses to ANG II compared to age-matched FHL controls. These effects were associated with significantly greater increases in RVR (Table 3). In adult hypertensive FHH rats, MAP responses to ANG II were also enhanced, whereas the decreases in RBF were quite similar as in FHL controls. These responses were also associated with significantly greater RVR increases in FHH as compared with FHL rats (Table 3).

MAP and RBF responses to NE did not differ between young and adult FHH and FHL strains. On the other hand, adult FHH rats exhibited significantly smaller RBF responses to ACh (Figure 7) in parallel with significantly lower RVR responses (Table 3), which suggested progression of endothelial dysfunction in the kidney.

Discussion

The present study is the first to evaluate in detail the age-related dynamics of the main features of FHH rats, a unique genetic model of hypertension. It showed clearly an association between increasing arterial hypertension and development of CKD, reflected by progressing deterioration of indices of glomerulosclerosis and tubular interstitial damage and of proteinuria. Admittedly, a limitation of this study was that documentation of kidney injury did not include determination of specific markers. Based on the necessary framework of basic information, in
Figure 4. Effect of combined treatment with ACE inhibitor (ACEi) and AT1 receptor blocker (ARB) on renal ANG II levels (A) and proteinuria (B) in young and adult FHH rats in comparison with adult FHL controls. *P < 0.05 vs. untreated corresponding groups, #P < 0.05 vs. FHL groups.

Figure 5. Effect of combined treatment with ACE inhibitor (ACEi) and AT1 receptor blocker (ARB) on ANG 1–7 levels in plasma (A) and kidney (B) in young and adult FHH rats in comparison with adult FHL controls. *P < 0.05 vs. untreated corresponding groups.

Figure 6. Dose-dependent systemic and renal vascular responses to angiotensin II in young (A) and adult FHH rats (B) in comparison with age-matched FHL controls. *P < 0.05 vs. corresponding FHL group.
selected periods of the natural course of the disease we obtained data providing insights into the pathogenetic and protective mechanisms operating in the model of CKD. In addition, at such appropriate time points we carried out pharmacological interventions and functional tests aimed at further elucidation of the complex pathogenetic mechanisms involved.

The major novel findings of this study were that

1. FHH rats do not exhibit exaggerated plasma and renal RAS activity at any age in comparison with normotensive control FHL strain but they show enhanced vascular responsiveness to ANG II.
2. Compared to the young animals, adult FHH rats display diminished kidney level of important vasodilator agents, a fact that may contribute to endothelial dysfunction and progression of renal damage.
3. Antihypertensive effects of ACEI/ARB treatment might be partly mediated by ANG 1–7 whose plasma and kidney levels increased significantly after dual RAS blockade.

A special attention was focused on the role of RAS as a potential determinant of hypertension and kidney disease, an issue that was also the subject of other studies (12,13,22). Until now, the information about association of RAS and plasma and renal ANG II concentrations over the lifetime of FHH animals and the course of the CKD was not conclusive in this model. Our finding that circulating and kidney tissue concentration of ANG II remained stable throughout the lifetime of FHH rats might indicate that the RAS plays only a marginal role in the pathogenesis of hypertension and the progression of renal damage in this model. However, we also provided evidence that the renal vasculature exhibits exaggerated responsiveness to exogenous ANG II, a finding that strongly suggests enhanced intracellular signaling mechanisms involving activation of AT1 receptors. The latter observation seems to contradict an earlier report indicating reduction of the responsiveness found in the hydronephrotic kidney of FHH rats (6). However, it is not very surprising that the responses of the whole-kidney vascular resistance, as determined in our experiments, may differ from those of the arteriole segments studied in the hydronephrotic kidney preparation. It needs to be noticed that in the same quoted study reduced reactivity of preglomerular arterioles to ANG II was observed in both normotensive and hypertensive fawn-hooded rats when compared with Wistar rats (6).

Despite our observation that plasma and kidney ANG II levels were not abnormally high in FHH rats, we found that “dual” RAS inhibition decreased kidney ANG II level and efficiently reduced hypertension. This was the case even though SBP of RAS-inhibited FHH rats remained significantly higher than in FHL controls. Such observation is in agreement with earlier evidence on alleviation of hypertension in young and somewhat older FHH rats by ACE inhibition (12,13). We

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Table 3. Relative (%) changes in RVR responses to angiotensin II (ANG II) and acetylcholine (ACh) in young and adult FHL and FHH rats.

| Group          | ANG II 65 ng/kg | ANG II 300 ng/kg | ANG II 150 ng/kg | ANG II 650 ng/kg | ACh 150 ng/kg | ACh 650 ng/kg |
|----------------|-----------------|-----------------|-----------------|-----------------|--------------|--------------|
| FHL 3 months (n = 9) | 79 ± 8          | 211 ± 23        | −31 ± 2         | −48 ± 4         |              |              |
| FHH 3 months (n = 8) | 119 ± 15*       | 336 ± 21*       | −26 ± 2         | −39 ± 3         |              |              |
| FHL 9 months (n = 9) | 92 ± 14         | 279 ± 26        | −29 ± 2         | −45 ± 2         |              |              |
| FHH 9 months (n = 8) | 197 ± 22*       | 524 ± 43*       | −18 ± 3*        | −27 ± 4*        |              |              |

Means ± SEM. *P < 0.05 vs. the corresponding FHL group, #P < 0.05 vs. FHH 3 months.

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Figure 7. Dose-dependent systemic and renal vascular responses to acetylcholine in young (A) and adult FHH rats (B) in comparison with age-matched FHL controls. *P < 0.05 vs. corresponding FHL group.
also observed that existing renal damage in adult FHH rats was not reverted by RAS inhibition initiated in the presence of established marked functional and structural renal damage. Thus, the kidneys seem to be partly protected just due to appreciable reduction of BP, decreased renal ANG II, and substantial increases in plasma and renal ANG 1–7 concentrations in response to RAS inhibition. However, it remains an open question whether this sign of renal function improvement was some direct effect of the decrease in intrarenal ANG II or an increase in ANG 1–7 content or simply a consequence of BP reduction.

Notwithstanding the primary role of genetic factors determining the development of hypertension and progression of renal damage in FHH rats (10,23,24), there is no doubt that nongenetic mechanisms are also involved. While in FHH rats BP did not reach the high level observed in spontaneously hypertensive rats SHR, the progression of renal damage from young prehypertensive age was prominent. Therefore, it can be assumed that BP elevation may not be the only cause of CKD development in this model. The loss of protective mechanisms in the kidney may also play a role in the progression of the age-related renal disease (25). However, diverse functional interactions complicate the assessment of the role of any specific prohypertensive or antihypertensive mechanisms, also in FHH rats. The present data show that at least some of the protective mechanisms known to be involved in the regulation of BP and renal function are maintained in prehypertensive FHH rats.

NO is known to buffer vasoconstrictor influences in the kidney and inhibition of its synthesis markedly accelerates the development of hypertension and renal damage in the FHH model (23,26). Moreover, it has been shown that the total NO synthesis falls with age, particularly in the male rat (25). In the present study, we showed that NOS activity in the kidney is significantly lower in adult FHH rats compared to the age-matched FHL controls but comparable in prehypertensive FHH animals and age-matched FHL controls. Further evidence supporting the notion that in FHH rats the functional integrity of the intrarenal NO system is age-dependent comes from our studies of renal vascular responses to ACh. We showed that the vasodilator response, measured as a change in RVR, was well preserved in young but greatly reduced in adult rats, in agreement with earlier evidence from in vitro studies in isolated renal vessels (9,26). We have to admit that the putative role of the endothelial vasodilator system would be much strengthened if the vascular response to ACh were compared with the response to a vasodilator not dependent on endothelial NO release.

Considering the known vasodilator and organoprotective actions of CYP450-epoxygenase metabolites (16,17,20), our novel data on EETs appear to be of special interest. We found that renal concentrations of EETs were similar in young FHH and FHL rats but the former showed a significant decrease in EETs progressing with age. This is important because we and others reported previously that the deficit in renal EETs significantly contributed to the pathophysiology of hypertension and renal damage in several models (16,17,19,20,27). Taken together, these findings indicate that a progressing deficit of intrarenal vasodilatory factors, such as NO or EETs, and, in general, local endothelial dysfunction could contribute to the progression of CKD in FHH.

There is increasing evidence on pathophysiological significance of the vasodilator axis of the RAS (ACE2, ANG 1–7, Mas receptor), which, at least in some conditions, could counteract the classical vasopressor axis (28–30). We found here that baseline plasma and kidney levels of ANG 1–7 did not change over the lifetime of FHH and were quite similar as in FHL rats. Therefore, a possible deficiency of this peptide was not a factor in the development of hypertension and renal damage in the FHH strain. On the other hand, we saw that the ability of the dual RAS blockade to increase plasma and kidney ANG 1–7 was well preserved in FHH rats aged 3 and 9 months, and this mechanism could contribute to alleviation of hypertension seen after RAS blockade.

An interesting observation was that, compared to the age-matched FHL controls, our young FHH rats exhibited significantly higher RBF and lower RVR. Thus, at moderately elevated arterial pressure, RBF increased considerably. Such response indicates inadequate or nonexistent constriction of renal resistance vessels, which supports previous evidence that in young FHH rats renal arteriolar myogenic response (especially in the afferent arteriole) and RBF autoregulation are impaired (7,9). Remarkably, in our adult FHH rats, at markedly higher BP, RBF was close to that in FHL rats and RVR was greatly elevated, which was compatible with substantial glomerular damage. It must be admitted that studies of RBF autoregulation in response to graded changes in renal perfusion pressure when performed in specific time points could substantially strengthen our conclusions. Nevertheless, the available data indicate that abnormal control of RVR may contribute to the development of hypertension and renal damage in FHH strain. This observation provides a better insight into the pathogenesis of experimental chronic kidney failure and a necessary basis for treatment of end-stage kidney disease in humans.

In summary, the present study indicates that changing status of the RAS may contribute to the development of hypertension and kidney disease in FHH rats. These animals were found to exhibit exaggerated vascular responsiveness to ANG II, which suggests an enhancement of intracellular signaling mechanisms involving AT1 receptor activation. Furthermore, RAS inhibition proved an efficient antihypertensive treatment that reduced ANG II and increased ANG 1–7 content in the kidney and thus may have delayed the progression of kidney disease. The activity of other factors opposing the development of hypertension and protecting the kidney, such as NO and EETs, declined with age. Therefore, therapeutic enhancement of this activity could, besides RAS inhibition, be attempted in the therapy of human hypertension associated with kidney disease.

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Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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