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Blood pressure and the susceptibility to renal damage after unilateral nephrectomy and L-NAME-induced hypertension in rats

Richard P. E. van Dokkum[1], Howard J. Jacob[2] and Abraham P. Provoost[1]

[1]Department of Pediatric Surgery, Erasmus University Medical School, Rotterdam, The Netherlands and [2]Department of Physiology, Medical College of Wisconsin, Milwaukee, WI, USA

Abstract

Background. Fawn-hooded hypertensive (FHH) rats carry several genes which determine the susceptibility to develop renal damage, while renal damage resistant August × Copenhagen Irish (ACI) rats do not. Kidneys from heterozygous (FHH × ACI) F_1 rats, appear to be largely, but not completely, protected after blood pressure elevation with N^{ω}-nitro-L-arginine methyl ester (L-NAME). We examined the role of an increased haemodynamic burden on the development of renal damage combining unilateral nephrectomy (UNx)- and L-NAME-induced hypertension in F_1 and ACI rats. Additionally, we investigated whether a general toxic effect of L-NAME, independent from a blood pressure elevation, caused renal damage in F_1 rats in animals simultaneously treated with L-NAME and the ACE inhibitor lisinopril.

Methods. Surgery was performed and L-NAME treatment (50 or 150 mg/l) was started at the age of 15 weeks. Systolic blood pressure (SBP) and urinary albumin excretion (UaV) were measured at 6 and 12 weeks post-UNx, followed by autopsy to determine the incidence of focal glomerulosclerosis (FGS). Using lisinopril (LIS) and L-NAME, another group of rats was evaluated at 12, 18, and 24 weeks after start of treatment.

Results. At similar L-NAME intake, F, rats developed more severe hypertension and more UaV than ACI rats. The increase in UaV per mmHg increase in SBP was fivefold higher in F_1 compared with ACI rats. In F_1 rats, the increase in UaV per percentage incidence increase in FGS was three times higher. In LIS treated F_1 rats, no significant UaV or FGS was measured at low blood pressure levels, indicating that renal damage in hypertensive F_1 rats is not a direct effect of L-NAME, but the result of the high blood pressure or another action of the renin–angiotensin system.

Conclusion. We conclude that heterozygosity for the genes influencing the development of renal damage in the FHH strain increases the susceptibility of the kidney to develop damage after UNx combined with systemic hypertension.

Keywords: fawn-hooded rats; genetic susceptibility; glomerulosclerosis; hypertension; renal damage

Introduction

The fawn-hooded hypertensive (FHH) rat is a unique model of hypertension-associated renal damage [1]. Males develop mild systolic hypertension, progressive albuminuria (UaV), and focal glomerulosclerosis (FGS) at a relatively young age. Previous studies in FHH rats showed the presence of hyperfiltration and glomerular hypertension preceding the development of functional and structural renal damage [2]. In the FHH rat, renal damage is greatly enhanced by unilateral nephrectomy (UNx) [3], a procedure that will further increase the haemodynamic burden to the glomeruli. A linear relationship was found between systolic blood pressure and intraglomerular pressure in three groups of UNx rats with different SBP levels [4]. A relatively high efferent arteriolar resistance in combination with a decreased ability to increase afferent tone was also found in the FHH rat, suggesting an impairment in the control of renal vascular resistance.

Genetic factors influencing the development and progression of renal damage are apparent in FHH rats. Using a back-cross of (FHH × August × Copenhagen Irish (ACI)) F_1 × FHH rats, we mapped two quantitative trait loci (QTLs) on chromosome 1, influencing proteinuria and structural renal damage [5]. These genes were denoted *Rf-1* and *Rf-2*. *Rf-1* appeared at least partly independent from blood pressure. In a subsequent study, the development of renal damage after UNx was assessed in a (FHH × ACI) F_2 progeny [6]. Linkage analysis in this F_2 cross not only confirmed the importance of *Rf-1* and *Rf-2*, but also revealed the presence of three additional QTLs (*Rf-3, Rf-4*, and *Rf-5*) influencing the development of proteinuria. Thus, susceptibility to

Correspondence and offprint requests to: Richard P. E. van Dokkum PhD, Erasmus University Medical School, Department of Pediatric Surgery, Laboratory for Surgery, Room Ce 040, Dr Molewaterplein 50, Post Office Box 1738, 3000 DR Rotterdam, The Netherlands.

renal damage after UNx is influenced by at least five susceptibility genes.

Previously we have studied the effects of hypertension induced by chronic nitric oxide (NO) inhibition with N^{ω}-nitro-L-arginine methyl ester (L-NAME) on the development of renal damage in F_1 (FHH × ACI) rats and in both parental strains with two kidneys. It was shown that despite similar levels of systolic blood pressure (SBP), severe UaV, and FGS were present in FHH rats, while ACI rats developed hardly any renal damage [7]. The most important finding in the F_1 rats was that, although these hybrids developed less renal damage compared with FHH rats, being heterozygous for the susceptibility genes did not completely protect the kidney from developing UaV and FGS. Compared with ACI rats, the severity of UaV and FGS increased, especially at SBP levels above 180 mmHg.

The purpose of the present study was to assess the genetic susceptibility to develop renal damage after UNx in combination with L-NAME-induced hypertension. The changes in UaV and SBP at 6 and 12 weeks after UNx and L-NAME-induced hypertension were assessed in F_1(FHH × ACI) and ACI rats. At 12 weeks after surgery, rats were sacrificed, creatinine clearance was measured, and morphological studies were performed in kidney tissue to determine structural renal damage. FHH rats were not included in these studies because of their high susceptibility and mortality in response to chronic L-NAME treatment [7], especially in combination with UNx [4].

A second study was carried out under a similar protocol to exclude the possibility of renal damage being a direct toxic effect of L-NAME. In this study, F_1 rats were treated with L-NAME and the angiotensin-converting enzyme inhibitor (ACEI) lisinopril, to keep SBP at a low concentration. The SBP and UaV levels were measured 12, 18, and 24 weeks after UNx in control and treated groups.

Subjects and methods

Animals

Fifty-five animals were used in study 1, and 25 animals were used in study 2. All animals were 15 weeks of age at the time the study was started. The FHH and ACI rats were all derived from our own breeding nuclei, and the F_1(FHH × ACI) rats were specially bred for the study. The rats received acidified tap-water (pH 3.0) *ad libitum* and standard rat chow (Hope Farms, Woerden, The Netherlands) containing 1% NaCl.

UNx

The right kidney was removed using ethyl ether (Sigma Chemical Co., St Louis, MO) anaesthesia, administered through an evaporation system connected to an airtight box. Rats were allowed to recover from surgery in a warmed cage for 1–2 h.

Design study 1

Chronic nitric-oxide inhibition was started after the rats had recovered from surgery for 3–4 days using L-NAME, (Sigma Chemical Co., St Louis, MO) dissolved in the drinking water at a concentration of 50 or 150 mg/l to obtain various blood pressure elevations, and was based on previous experiments [7]. This dose was slightly lower compared with previous studies in two-kidney rats, because some F_1 animals died in that study as a result of L-NAME-induced complications. Control animals were provided with normal drinking fluid.

Animals from each strain were divided into three groups, and were age- and body weight-matched. The F_1 groups (n = 29) consisted of nine control rats, 10 rats treated with 50 mg L-NAME, and 10 rats treated with 150 mg L-NAME. The ACI groups (n = 26) consisted of 10 control rats, six rats treated with 50 mg L-NAME, and 10 rats treated with 150 mg L-NAME. Actual L-NAME intake was calculated in mg/kg from the metabolic data at 6 and 12 weeks after UNx.

Design study 2

Blood pressure was maintained at low levels by chronic angiotensin-converting enzyme inhibition (ACE-i), using lisinopril (LIS) (Novatec®, Merck Sharp & Dome, Haarlem, The Netherlands), that was administered through the drinking water at a concentration of 50 mg/l. This dose was used because it had previously proven to provide adequate blood pressure reduction in FHH rats [8]. As in study 1, hypertension was induced using L-NAME. The F_1 rats were divided into three age- and body weight-matched groups, and were measured 12, 18, and 24 weeks after UNx. The end-point of study 1 was taken as a starting-point for this study to obtain a longer follow-up period in F_1 rats with different blood pressure levels.

The control and L-NAME groups consisted of eight rats. The third group (NAME + LIS) consisted of nine rats that were simultaneously treated with L-NAME and LIS. Treatment was started after the rats had recovered from surgery for 3–4 days and continued until the final measurement at 24 weeks after UNx. Also in this study, actual L-NAME intake was calculated in mg/kg from the metabolic data at 12, 18, and 24 weeks after UNx.

SBP and metabolic measurements

SBP was measured as previously described [7]. The week before the measurement, animals were allowed to adapt to the equipment and the procedure to minimize stress-induced artefacts. Although SBP was measured every 3 weeks after UNx, only the values obtained at the time of the metabolic measurements will be presented. Urine excretion, food and water intakes were determined. gravimetrically in two 24-h collections at 6 and 12 weeks after UNx in study 1 and 12, 18, and 24 weeks after UNx in study 2, as described previously [7]. Rats were allowed to adapt to the metabolic cages over the weekend.

Terminal analysis

Autopsy of the rats, processing of kidneys were performed as described previously [7]. The criteria for the assessment of the incidence of FGS were adhesion of the glomerulus to Bowman's capsule, periodic acid-Schiff-positive material in the mesangial region, and folding of the glomerular basement

membrane with entrapment of amorphous material. Final creatinine clearance as a measure of glomerular filtration rate (GFR) was calculated from the plasma creatinine concentrations from blood obtained at autopsy and from the urinary creatinine concentration obtained at the last metabolic measurement shortly before autopsy.

Analytic procedures

Plasma and urinary albumin concentration was measured with bromocresol green (Merck, Darmstadt, Germany), and creatinine with the Jaffé method without deproteinization using the semi-automatic ELAN system (Merck-Eppendorf, Hamburg, Germany).

Statistics

Data are presented as mean $\pm$ SEM in the figures, text, and tables. Differences in mean values between groups were compared by ANOVA and a subsequent Student–Newman–Keuls test to identify the groups that were different. In case of a non-normal data distribution, groups were compared using the Mann–Whitney rank sum test. In all tests, statistical significance was defined as $P < 0.05$.

The relationships between SBP and UaV, and between the level of UaV at week 12 and the incidence of FGS was assessed by linear regression analysis. All tests were performed using the Primer of Biostatistics (S. E. Glantz) software package.

Results

Study 1

Blood pressure and albuminuria The actual L-NAME intake was calculated from the fluid intake at the metabolic measurements and is given in Table 1. No statistical significant differences between strains at the measured time-points were detected.

The values for SBP in both F_1 and ACI control (ACI-CON) rats and after L-NAME treatments are summarized in Table 2. SBP in control F_1 (F_1-CON) rats was significantly higher compared with ACI-CON rats and averaged around 130 and 110 mmHg respectively. Chronic L-NAME treatment induced dose-dependent differences in SBP that were already statistically significant at the first time-point. Treated F_1 rats developed significantly higher SBP levels than treated ACI rats. These significant differences between F_1 and ACI remained present during the entire follow-up. From the first time-point onwards, SBP concentrations increased, although not dramatically, in both the ACI-50 and in ACI-150 groups respectively. The increase in SBP was also modest in the F_1-50 group. In contrast, SBP increased more steeply in the F_1-150 group. The groups that developed similar levels of SBP were the F_1-CON and the ACI-50, and the F_1-50 and the ACI-150 groups.

The level of functional renal damage, as indicated by the level of UaV, rose in a dose-dependent fashion in all strains, and is also shown in Table 2. The rise in UaV in control and treated F_1 rats was significantly higher than in the ACI rats, and was highest in the F_1-150 group. In contrast, ACI rats showed small increases in UaV during the follow-up, even after 12 weeks of 150 mg L-NAME treatment.

Structural renal damage and final GFR The level of structural damage, as indicated by FGS, is shown in Table 3. Both strains showed a dose-dependent increase in FGS. The incidence of FGS in the F_1-150 group was significantly higher compared with all other groups. The 50 mg-treated groups of both strains showed similar FGS incidence.

At autopsy, the calculated creatinine clearance, used as an estimate for final GFR, was significantly higher in all groups of F_1 rats compared with ACI-CON and 50 mg-treated rats, as shown in Table 3. GFR was significantly lower in the 150 mg-treated groups of both strains compared with the 50 mg-treated groups, but still significantly higher in the F_1 compared with the ACI rats.

Renal damage in groups with similar SBP levels The groups that develop similar levels of SBP appeared to be the FI-CON and the ACI-50, and the F_1-50 and the ACI-150 groups respectively. The SBP level in these groups averaged 132 ± 1 and 139 ± 1, and $165 \pm 1$and 160 ± 1 mmHg, averaged over all measured time-points. The UaV level in the F_1 rats over the same period was higher, although not statistical significantly different from ACI rats, due to the large variation. However, when we compared the groups that received the same amount of L-NAME (Table 1), the level of UaV in the F_1-150 group was significantly higher compared with every ACI group at 6 and 12 weeks post UNx.

Comparison of strains by linear regression analysis To determine whether a difference in the relationship between SBP and UaV was present between F_1 and ACI rats after UNx, linear regression analysis was performed on the data obtained at 6 and 12 weeks after start of the experiment. All control, 50-, and 150-mg

Table 1. Actual L-NAME intake adapted from metabolic measurements in F_1 and ACI rats in mg/kg at 6 and 12 weeks of treatment in studies 1 and 2

Group	*n*	Week 6	Week 12	Week 18	Week 24
F_1-50	10	3.3 ± 0.2	3.0 ± 0.2	NM	NM
F_1-150	10	9.4 ± 0.6	9.4 ± 0.7	NM	NM
ACI-50	6	3.1 ± 0.2	2.7 ± 0.2	NM	NM
ACI-150	10	8.6 ± 0.5	7.9 ± 1.0	NM	NM
P1/P2		NS/NS	NS/NS		
F_1-NAME	8	NM	8.3 ± 0.5	8.1 ± 1.3	8.7 ± 1.7
F_1-NAME + LIS	9	NM	8.6 ± 0.9	7.3 ± 1.1	6.3 ± 0.9
P3			NS	NS	NS

Values are given as mean $\pm$ SD. L-NAME, N^{ω}-nitro-L-arginine methyl ester; LIS, lisinopril; F_1, F_1(FHH $\times$ ACI) rats; ACI, August $\times$ Copenhagen Irish; P1, ACI-50 *vs* F_1-50; P2, ACI-150 *vs* F_1-150; P3, F_1-NAME *vs* F_1-NAME + LIS; NM, no measurement; S, $P < 0.05$; NS, $P > 0.05$.

Table 2. Body weight (g), systolic blood pressure (mmHg), and albuminuria (mg/24 h) in ACI, and F_1 rats at 6 and 12 weeks of follow-up

		Week 6			Week 12		
Group	*n*	BW	SBP	UaV	BW	SBP	UaV
F_1-CON	9	349±14	129±1	9.6±1.3	387±13	134±2	16.5±2.9
F_1-50	10	345±12	160±3	12.3±2.7	382±14	170±4	27.1±4.7
F_1-150	10	351±12	182±3	18.1±3.7	366±12	212±4	109±15
ACI-CON	10	262±6	109±2	3.4±0.5	286±7	113±2	5.3±0.7
ACI-50	6	254±6	133±3	5.6±0.6	272±13	148±3	10.9±0.9
ACI-150	10	262±6	158±4	9.2±0.9	276±8	165±5	21.6±2.8
P1/P2/P3		S/S/S	S/S/S	NS/NS/S	S/S/S	S/S/S	NS/NS/S

Values are given as mean±SEM. BW, body weight; SBP, systolic blood pressure; UaV, albuminuria; CON, control. Other abbreviations as in Table 1. P1, ACI-CON *vs* F_1-CON; P2, ACI-50 *vs* F_1-50; P3, ACI-150 *vs* F_1-150; S, $P<0.05$; NS, $P>0.05$.

Table 3. Body, relative kidney, and heart weights, incidence of glomerulosclerosis and calculated creatinine clearance at autopsy after 12 weeks of follow-up in F_1 (FHH×ACI) and ACI rats

Group	*n*	BW (g)	LKW/g	GS (%)	HW/g	C_{Cr}/g
F_1-CON	9	388±16	495±6	6.2±1.0	254±5	0.39±0.03
F_1-50	10	398±19	481±9	9.6±1.2	248±5	0.42±0.02
F_1-150	10	358±15	533±13	23.4±4.2	314±5	0.33±0.02
ACI-CON	10	289±8	480±8	3.2±0.9	248±3	0.28±0.01
ACI-50	6	259±15	509±21	8.5±0.9	266±9	0.32±0.02
ACI-150	10	279±8	481±5	10.9±1.9	267±6	0.28±0.01
Pl/P2/P3		S/S/S	NS/NS/S	NS/NS/S	NS/NS/S	S/S/S

Values are given as mean±SEM. *n*, number of rats; BW, body weight; LKW/g, left kidney weight per 100 g BW; GS, glomerulosclerosis; HW/g, HW per 100 g BW; C_{Cr}/g, creatinine clearance per 100 g body weight. Other abbreviations as in Table 1. P1, ACI-CON *vs* F_1-CON; P2, ACI-50 *vs* F_1-50; P3, ACI-150 *vs* F_1-150; S, $P<0.05$; NS, $P>0.05$.

treated ACI and F_1 rats were included in the regression analysis, which is shown in Figure 1A,B respectively. Already at 6 weeks, the slope of the best-fit regression line was significantly higher in F_1 compared with ACI rats, and averaged 0.17±0.01 ($r=0.397$, $P=0.033$) and 0.10±0.01 ($r=0.709$, $P<0.01$) mg/24 h per mmHg. At week 12 after UNx, the increase in UaV per mmHg increase in SBP was five times more in F_1 than in ACI rats. Values averaged 1.24±0.04 ($r=0.807$, $P<0.001$) and 0.25±0.01 ($r=0.70$, $P<0.001$) mg/24 h per mmHg respectively, and indicate that the amount of functional damage between week 6 and 12 was sevenfold in F_1, and almost threefold in ACI.

Furthermore, a significant correlation between FGS and UaV was present in both strains. The increase in UaV per percentage increase in FGS incidence was three times higher ($P<0.001$) in F_1 compared with ACI rats and averaged 3.98±0.09 ($r=0.844$, $P<0.001$), and 1.31±0.05 ($r=0.695$, $P<0.001$) mg/24 h respectively. Furthermore, per percentage increase in FGS, F_1 rats showed an increase in LKW/100 g BW of 0.25±0.01 ($r=0.643$, $P=0.01$), whereas ACI rats did not show a significant correlation between those parameters.

Study 2

Blood pressure and albuminuria The actual L-NAME intakes of the F_1 rats used in this study are also given in Table 1, and showed no significant differences between the F_1 groups.

Longitudinal values for the tail-cuff SBP and UaV at 12, 18, and 24 weeks after UNx are shown in Figure 2A,B respectively. Also in this study, SBP in L-NAME-treated rats was significantly higher than that of the control group. In contrast, SBP in the L-NAME+LIS rats was significantly lower compared with the control rats. The SBP in the L-NAME group increased to around 200 mmHg at week 24 after UNx. The SBP levels in the control and L-NAME+LIS group remained stable around 135 and 100 mmHg, respectively.

A similar picture emerged for the UaV levels, which increased steeply with time in the NAME treated group. UaV in the control group also progressively increased, although much less than in the L-NAME treated group. The rats treated simultaneously with L-NAME+LIS did not develop any significant UaV during the entire period, the average level being around 2 mg/24 h.

Final GFR and structural renal damage Final creatinine clearance as an indicator of final GFR were equal in the NAME and NAME+LIS groups and averaged 1.21±0.07 and 1.2±0.1 ml/min respectively. The clearance in the control group was slightly, but not significantly, higher compared with the other groups and averaged 1.42±0.07 ml/min. Final GFR in the CON

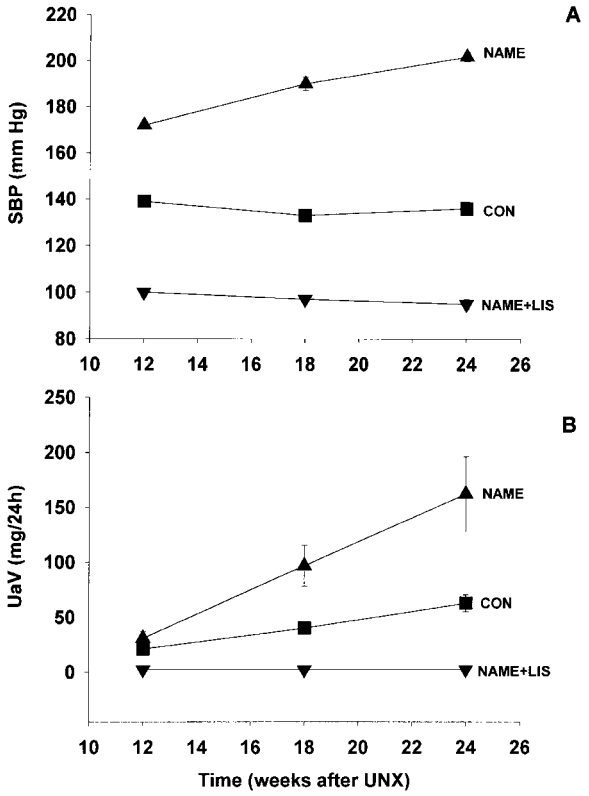

Fig. 1. Tail-cuff systolic blood pressure (SBP, mmHg; **A**, upper) and urinary albumin excretion (UaV, mg/24 h; **B**, lower) in F_1(FHH × ACI) rats at 12, 18, and 24 weeks after UNx. Groups are: untreated controls (CON, ■, $n=8$), rats treated with 150 mg/l L-NAME (NAME, ▲ $n=8$), and rats treated with 50 mg/l LIS and 150 mg/l L-NAME directly after UNx (NAME+LIS, ▼, $n=9$). Values are given as mean ± SE (error bars).

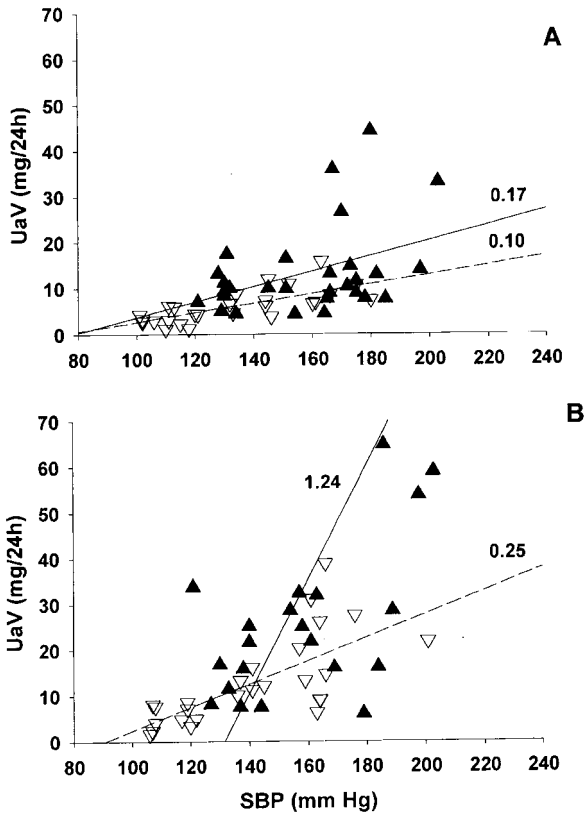

Fig. 2. Relationship between SBP and UaV at 6 weeks (**A**, upper) and 12 weeks (**B**, lower) after UNx in F_1 (FHH × ACI) (▲, $n=29$) and ACI (▽, $n=26$). Slopes are indicated next to the regression lines.

group was slightly, but not significantly, higher than in the NAME and NAME+LIS groups. The level of structural damage, indicated by the incidence of FGS was significantly higher in the NAME group compared with the other groups, and averaged 27.1 ± 2.7%. The incidence of sclerosis was significantly higher in the control compared with the NAME+LIS group and averaged 16.3 ± 2.9, and 4.8 ± 0.8% respectively. Long-term concomitant ACEI treatment completely prevented the development of FGS after UNx and L-NAME treatment. Furthermore, as evidenced by the significantly lower incidence in the NAME+LIS group, ACEI also reduced the incidence of FGS when compared with the control group. Thus the lower SBP levels in NAME+LIS-treated F_1 rats appears to prevent the development of both functional (UaV) and structural (FGS) renal damage.

Regression analysis study 2 Linear regression analysis using the SBP and UaV data obtained at 12, 18, and 24 weeks after UNx showed significant relationships between these parameters ($r=0.787$, $P<0.001$; $r=0.828$, $P<0.001$; $r=0.825$, $P<0.001$). Furthermore, a significant correlation between FGS and UaV was present at 24 weeks after UNx ($r=0.805$, $P<0.001$). This indicates that in this group an even stronger correlation was present between the SBP level and UaV compared with study 1.

Discussion

The primary finding of the first study was that in F_1 and ACI rats, L-NAME in combination with UNx caused a dose-dependent increase in blood pressure and renal damage. However, with the same L-NAME dose, F_1 rats developed more severe hypertension and more functional and structural renal damage per mmHg increase in SBP than did the treated ACI rats. Additional regression analysis showed significant linear relationships between functional and structural parameters 12 weeks post-UNx in both strains, being three times more severe in F_1 than in ACI rats. The primary finding of the second study was that the development of renal damage in F_1 rats appeared to depend on the L-NAME-induced hypertension and not on a direct toxic effect of L-NAME. With a similar L-NAME intake, prevention of the rise in blood pressure by LIS completely prevented the development of functional and structural renal damage.

By comparing the creatinine clearance in the present study with those obtained in a previous study performed in F1 and ACI rats with two kidneys (2K)

after similar L-NAME intake [7], we were able to calculate the compensatory increases in GFR after UNx. It appears that the compensatory increase in F_1 rats was significantly higher compared with ACI rats. In control F_1 rats, the final GFR after UNx on average increased by 59% compared with 2K-F_1 rats. In control ACI rats, the level after UNx was only 17% higher than in 2K-ACI rats. This data indicates that F_1 rats were less efficiently protected from an increase in GFR than ACI and might indicate differences in renal haemodynamic regulatory mechanisms between the strains. Our data support the view that, at low-dose L-NAME, the renal vasoconstriction caused by L-NAME leads to an increase in filtration and an increase in FGS due to a larger haemodynamic burden compared with the control groups in ACI and F_1 rats. At high dose L-NAME, increasing constriction causes a decrease in filtration and a further increase in FGS due to ischaemic factors. The presence of an increase in filtration solely due to the reduction of renal mass might also play an additional role in this process [9,10].

The male FHH rat, the parental strain of the F_1 rats used in the present study, develops mild systolic, but marked glomerular hypertension, and is extremely susceptible to develop renal damage [1–5]. Glomerular hypertension and the severity of renal damage in the FHH is further increased by UNx [3,4]. The glomerular capillary pressure (P_{GC}) level in UNx-FHH rats was found to be higher than those reported following UNx in MW or Wistar Kyoto (WKY) rats [11,12], strains that are less susceptible to the development of renal damage than the FHH rat. However, P_{GC} in UNx-FHH is comparable to that reported for MW rats with remnant kidneys [13]. In general, normotensive rat strains appear less susceptible than hypertensive strains. We have previously studied the normotensive WAG, in which survival time after UNx is only slightly reduced compared to the 2K WAG [9,14]. In ACI rats in the present study, hardly any renal damage develops even after a substantial elevation of systemic blood pressure. However, normal blood pressure is no guarantee for low susceptibility. The Milan normotensive rat, in contrast to the Milan hypertensive rat spontaneously develops renal damage without the development of hypertension [15].

Similar to male FHH rats, male Munich–Wistar-Fromter (MWF) rats spontaneously develop glomerular and systemic hypertension [16], which is also greatly worsened by UNx [17]. In spontaneously hypertensive rats (SHR), where systemic blood pressure is much higher, glomerulosclerosis and renal failure do not develop early in life [13]. When renal mass is reduced, the resistance of the afferent arteriole decreases in SHR and allows the transmission of systemic hypertension into the glomerular capillary network [13]. Subsequent proteinuria (UpV) develops, albeit much more slowly than in FHH and MWF rats following UNx [10,11]. Even a further increase in systemic pressure using L-NAME does not severely impair renal function in SHR because of adequate control of afferent arteriolar resistance [18]. Thus, it appears that *adequate* renal autoregulation is of major importance in protecting the glomerulus from the transmission of systemic pressure into the capillary bed. We recently observed an *impaired* renal autoregulation leading to glomerular hypertension in the FHH rat [19]. Moreover, isolated perfused interlobular arteries of the FHH rat showed an impaired myogenic response, which contributes to the development of renal damage in this animal model [20].

Regulation of renal vascular resistance, intraglomerular pressure, and glomerular filtration might be genetically determined and thereby predispose to renal failure if P_{GC} is excessively high. The recent findings that genes determining renal failure and blood pressure in the FHH rat are separate and distinct from each other could be a possible explanation for the strain differences in the susceptibility to develop glomerular damage. Experiments in which rats of an F_2 cross between ACI and FHH rats were studied after UNx, not only confirmed the results of an earlier study in a back-cross of both strains, but also revealed the presence of three additional loci for renal failure [5,6]. These findings underscore the complexity of the genetics to progressive renal failure and might point towards specific genetically regulated mechanisms related to changes induced by UNx.

Alterations in the glomerular permeability of proteins, allowing the development of UpV, can occur while GFR remains within normal limits in different renal diseases both in animals and humans [21]. The persistence of UpV in the presence of normal GFR can be considered an independent risk factor for the development of renal failure, since no correlations were found between UpV and GFR in the rats used in the first study after UNx and in the second study after L-NAME and ACEI treatment. The levels of final GFR in the present study showed that, compared with ACI rats, the compensatory increase in GFR after UNx in F_1 rats is larger than the decrease in GFR caused by NO synthase inhibition.

Although our data strongly suggest an important role for the elevated blood pressure in the development of glomerular damage, the effects of reducing synthesis may have other effects on the kidney as well. Likewise, chronic ACE-i mediates more than reverse the hypertension. When angiotensin II (Ang II) formation is chronically inhibited with ACEI, glomerular hypertension is controlled while glomerular hyperfiltration is usually unaffected. In contrast, when Ang II activity is increased by chronic infusion, intraglomerular pressure, proteinuria, and FGS are further elevated [22]. Although the role of the renin–angiotensin system (RAS) in chronic NO inhibition remains somewhat controversial, the development of hypertension in this model is thought to be renin-dependent and may result partly from increased vascular Ang II receptor expression [23]. Various reports suggest that the endothelial NO synthase system and the RAS interact as regulators of the glomerular microcirculation [24].

Another interesting finding was that, at similar doses of L-NAME, SBP increased significantly more in F_1

rats than ACI rats at both 6 and 12 weeks after UNx. In our previous studies [7], higher doses of L-NAME induced similar increases in SBP both in F_1 and ACI rats. Furthermore, the enhancement of blood pressure values in F_1-UNx rats seems to be higher in than in F_1-2K rats with a dose of L-NAME $\sim$9 mg/kg BW. These data suggest an alteration in the synthesis or in the metabolism of endothelial NO in F_1 rats.

In conclusion, after UNx and chronic NO synthase inhibition, the F_1 (FHH $\times$ ACI) rat, which is heterozygous for genes that determine the susceptibility to renal damage in the FHH rat, is not completely protected from developing hypertension-associated renal damage. Furthermore, we conclude that renal damage in hypertensive F_1 rats is not a direct effect of L-NAME, but the result of the high blood pressure or another action depending on the activation of the RAS.

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References

1. Provoost AP, De Keijzer MH. The fawn-hooded rat: a model for chronic renal failure. In: Gretz N, Strauch M (eds). *Experimental and Genetic Rat Models of Chronic Renal Failure*. Karger, Basel, 1993; 100–114
2. Simons JL, Provoost AP, Anderson S *et al*. Pathogenesis of glomerular injury in the fawn-hooded rat: early glomerular capillary hypertension predicts glomerular sclerosis. *J Am Soc Nephrol* 1993; 3: 1775–1782
3. Simons JL, Provoost AP, De Keijzer MH, Anderson S, Rennke HG, Brenner BM. Pathogenesis of glomerular injury in the fawn-hooded rat: effect of unilateral nephrectomy. *J Am Soc Nephrol* 1994; 4: 1362–1370
4. Simons JL, Provoost AP, Anderson S, Rennke HG, Troy JL, Brenner BM. Modulation of glomerular hypertension defines susceptibility to progressive glomerular injury. *Kidney Int* 1994; 46: 396–404
5. Brown DM, Provoost AP, Daly MJ, Lander ES, Jacob HJ. Renal disease susceptibility and hypertension are under independent genetic control in the fawn-hooded rat. *Nature Genet* 1996; 12: 44–51
6. Provoost AP, Shiozawa M, Majewski RR, Van Dokkum RPE, Jacob HJ. Genetic analysis of the susceptibility to develop renal damage after unilateral nephrectomy in rats. *Am J Hypertens* 1998; 11: 5A (Abstract)
7. Van Dokkum, RPE, Jacob HJ, Provoost AP. Genetic differences define severity of renal damage after L-NAME-induced hypertension. *J Am Soc Nephrol* 1998; 9: 363–371
8. Verseput GH, Provoost AP, Braam BB, Weening JJ, Koomans HA. Angiotensin-converting enzyme inhibition in the prevention and treatment of chronic renal damage in the hypertensive fawn-hooded rat. *J Am Soc Nephrol* 1997; 8: 249–259
9. Provoost AP, Baudoin P, De Keijzer MH, Van Aken M, Molenaar JC. The role of nephron loss in the progression of renal failure: Experimental evidence. *Am J Kidney Dis* 1991; 5: 27–32
10. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *Am J Physiol* 1981; 241: F85–93
11. Anderson S, Meyer TW, Rennke HG, Brenner BM. Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J Clin Invest* 1985; 76: 612–619
12. Deen WM, Maddox DA, Robertson CR, Brenner BM. Dynamics of glomerular ultrafiltration in the rat. VII. Response to reduced renal mass. *Am J Physiol* 1974; 227: 556–562
13. Dworkin LD, Feiner HD. Glomerular injury in uninephrectomized spontaneously hypertensive rats: a consequence of glomerular hypertension. *J Clin Invest* 86; 77: 796–809
14. Provoost AP, De Keijzer MH, Molenaar JC. Effect of protein intake on life long changes in renal function in rats unilaterally nephrectomized at young age. *J Lab Clin Med* 1989; 114: 19–26
15. Brandis A, Bianchi G, Reale E, Helmchen U, Kühn K. Age-dependent glomerulosclerosis and proteinuria occurring in rats of the Milan normotensive strain and not in rats of the Milan hypertensive strain. *Lab Invest* 1986; 55: 234–243
16. Remuzzi A, Puntorieri S, Battaglia C, Bertani T, Remuzzi G. Angiotensin converting enzyme inhibition ameliorates glomerular filtration of macromolecules and water and lessens glomerular injury in the rat. *J Clin Invest* 1990; 85: 541–549
17. Remuzzi A, Benigm A, Malanchini B, Bruzzi I, Foglieni C, Remuzzi G. ACE inhibition prevents renal failure and death in uninephrectomized MWF/Ztm rats. *Kidney Int* 1995; 47: 1319–1326
18. Reverte M, Flores O, Gallego B, Leston A, Lopez-Novoa JM. Effect of chronic N^G-nitro-L-arginine methyl ester (L-NAME) on blood pressure and renal function in conscious uninephrectomized spontaneously hypertensive rats. *Can J Physiol Pharmacol* 1996; 27: 176–183
19. Van Dokkum RPE, Alonso-Galicia M, Provoost AP, Jacob HJ, Roman RJ. Impaired autoregulation of renal blood flow in the fawn-hooded rat. *Am J Physiol* 1999; 276: 8189–8196
20. Van Dokkum RPE, Sun C-W, Provoost AP, Roman RJ. Altered renal hemodynamics and impaired myogenic responses in the fawn-hooded rat. *Am J Physiol* 1999; 276: 8855–8863
21. Kayseri GA, Meyers BD, Couser WG. Mechanisms and consequences of proteinuria. *Lab Invest* 1986; 54: 479–484
22. Baylis C. Renal responses to acute angiotensin II inhibition and administered angiotensin II in the aging, conscious, chronically catheterized rat. *Am JKidney Dis* 1993; 22: 842–850
23. Yang Y, Macdonald GJ, Duggan KA. A study of angiotensin II receptors after chronic inhibition of nitric oxide synthase in the spontaneously hypertensive rat. *Clin Exp Pharmacol Physiol* 1996; 23: 441–443
24. Raij L. Nitric oxide and the kidney. *Circulation* 1993; 87: V26–29

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