BLOOD PRESSURE AND RENAL FAILURE IN THE FAWN-HOODED RAT: COMBINING PHYSIOLOGY AND GENETICS

THESIS

R.P.E. van Dokkum

1999

FYSIOLOGIE EN GENETICA

De omslag van dit proefschrift laat de verbondenheid zien van de fysiologie met de genetica en het spectrum aan biologische niveaus dat zich daartussen bevindt. Voor het gebruik van de afbeelding werd goedkeuring verkregen van de American Physiological Society.

Lay-out: R.P.E. van Dokkum, G.H. Verseput
Omslag: American Physiological Society
Druk: Drukkerij Elinkwijk, Utrecht

De studies beschreven in dit proefschrift werden uitgevoerd bij het Instituut Kinderheelkunde op het Laboratorium voor Chirurgie van de Erasmus Universiteit Rotterdam en op het Department of Physiology van het Medical College of Wisconsin in Milwaukee, USA. Subsidies hiervoor werden verleend door de Koninklijke Nederlandse Academie van Wetenschappen (KNAW), de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), de National Institutes of Health (NIH) in de Verenigde Staten, het Trustfonds van de Erasmus Universiteit Rotterdam en Stichting De Drie Lichten.

Het drukken van dit proefschrift werd mede mogelijk gemaakt door een subsidie van de Nier Stichting Nederland.

ISBN 90-9012541-8

BLOOD PRESSURE AND RENAL FAILURE IN THE FAWN-HOODED RAT: COMBINING PHYSIOLOGY AND GENETICS

BLOEDDRUK EN NIERFALEN IN DE FAWN-HOODED RAT:
EEN COMBINATIE VAN FYSIOLOGIE EN GENETICA

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof.dr. P.W.C. Akkermans M.A.
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 21 april 1999 om 13.45 uur.

door

RICHARD PIERRE EDWIN VAN DOKKUM

geboren te Hoek

PROMOTIECOMMISSIE

Promotor: Prof.dr. J.C. Molenaar

Overige leden: Prof.dr. M.A.D.H. Schalekamp

Prof.dr. B.A. Oostra

Prof.dr. P.R. Saxena

Dr. A.P. Provoost (tevens copromotor)

We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.

T.S. Eliot (Little Gidding)

CONTENTS

ACEi	angiotensin-converting enzyme inhibit(ion)(or)
ACh	acetylcholine
ACI	August x Copenhagen Irish
AI	autoregulatory index
BSA	bovine serum albumin
BW	body weight
CON	control
EDRF	endothelium-derived relaxing factor
ESRF	end-stage renal failure
FGS	focal glomerulosclerosis
FHH	Fawn-Hooded Hypertensive
FHL	Fawn-Hooded Low blood pressure
F_1	F_1 (FHH x ACI) rat
FHR	Fawn-Hooded Rat
GFR	glomerular filtration rate
HD	high dose
ID	inner diameter
LD	low dose
LIS	lisinopril
L-NAME	N^{ω}-L-arginine methyl ester
MAP	mean arterial pressure
MHS	Milan Hypertensive Strain
MNS	Milan Normotensive Strain
NOi	nitric oxide synthase inhibit(ion)(or)
UNX	unilateral nephrectomy
PE	phenylephrine
P_E	efferent arteriolar pressure
P_{GC}	glomerular capillary pressure
P_{SF}	stop-flow pressure
P_T	proximal tubular pressure
QTL	quantitative trait locus
RBF	renal blood flow
Rf	renal failure
RPP	renal perfusion pressure
SBP	systolic blood pressure
SHR	Spontaneously Hypertensive Rat
T_a	active tension
TGF	tubuloglomerular feedback
UaV	urinary albumin excretion
URA	unilateral renal agenesis
UpV	urinary protein excretion

CHAPTER 1

INTRODUCTION

1.1 HYPERTENSION-ASSOCIATED RENAL FAILURE

1.1.1 Clinical settings

The question why not all patients with hypertension develop end-stage renal failure (ESRF) has become a major issue in nephrology and hypertension research over the past decade. There are indications for a relationship between hypertension and impaired renal function in individuals with no underlying renal disease.[31] It is widely believed that genetic factors play an important role in the susceptibility to hypertension-induced renal failure.[18,100] Epidemiological studies indicate that the risk for hypertension-associated renal failure varies with the ethnic background.[14,28] For instance, the presence of ESRF in an African-American individual results in a nine-fold increased risk of ESRF in a first-degree relative, even after controlling hypertension in the relative.[32]

Most information on familial clustering of renal failure and hypertension is derived from studies in patients with diabetic nephropathy, for which Seaquist *et al.* recently showed the involvement of genetic factors in its pathogenesis.[108] Other studies have reported a greater prevalence of hypertension and/or cardiovascular disease in the parents of children who developed diabetic nephropathy later in life.[27,133] Furthermore, Schmidt *et al.* found that a familial history of hypertension is not only more frequent in patients who develop chronic renal failure caused by diabetes but also in patients with different histologic types of primary glomerulonephritis.[107]

The factors responsible for an association between blood pressure and renal failure are not known, but an increased blood pressure is: (a) necessary and sufficient to cause ESRF, or (b) necessary but not sufficient to cause ESRF, or (c) neither necessary nor sufficient to cause ESRF; it accelerates the risk in individuals who are otherwise predisposed. Renal failure is hypertension-*induced* in the first two suppositions and hypertension-*associated* in the latter.[12]

In this context, we have to consider the possibility that hypertension and the predisposition to develop glomerular damage due to hypertension are influenced by different genes. Gene-gene and gene-environment interactions determine the final phenotype. It could be that hypertension alone and renal failure due to hypertension are two different phenotypes.

The genetic basis of complications in human diseases deserves more attention, and it would be useful to ascertain a large number of hypertensive affected sib-pairs to study whether risk of renal failure correlates between these sib-pairs and, if so, to map human susceptibility factors. Using the candidate gene approach, an insertion/deletion polymorphism of the angiotensin converting enzyme (ACE) gene was recently discovered, significantly influencing circulating ACE levels.

These levels might play a role in the development of target organ damage, such as left ventricular hypertrophy in essential hypertension and microalbuminuria in diabetes mellitus.[11,29,83] However, simple comparisons do not provide answers to these complex problems. Combining physiology and genetics, we might be able to dissect the susceptibility to hypertension and renal damage.

1.1.2 Physiology and genetics.

An important discovery of genetics research is that gene products, rather than acting alone, form complex webs of molecular interactions. Although this principle was already known, the ability to identify the genes involved in a given trait and the way in which simple molecules collaborate to create complex traits remain major research challenges. Accomplishing this task requires knowledge of gene identities, protein functions and interactions, and developmental and physiological pathways.

The original goal of the Human Genome Project was to provide the reagents, technologies and information, which would be the foundation for discovering disease genes and, ultimately, for exploring the pathways that would link genes and traits. Using this concept, gene discovery is now routine, especially for genetically simple Mendelian traits, like some forms of monogenic hypertension. With the sequence of an entire genome (the complete set of chromosomes and their genes) available and with the identity of many disease genes established, attention shifts to studies of gene function and to genotype and phenotype dissection of complex traits. Recent technological developments are now creating new opportunities for defining gene functions in developmental and physiological pathways.

Genetic analysis of developmental and physiological pathways will benefit both from the ability to analyze whole genomes and from the integration with traditional genetic, biochemical, computational and engineering paradigms. The approach to find the products generated by different genes and the way in which these products contribute to functional processes on the cellular or whole organism level can be indicated as physiological genomics.[79] Whole-genome methods enable integrated rather than individual approaches to complex problems. Integration of these approaches will close the phenotype gap between the information encoded by individual genes and the complex biological functions that emerge from interactions of gene products.

Physiological genomics represents a new phase of genome analysis and specifically refers to the development and application of genome-wide experimental approaches to study genes and their function. This way the

likelihood for a certain trait to be linked to a gene or gene product can thus be established. Physiology and genetics is going to bind sequence and function together and to provide new insights into the behavior of biological systems.[37,41,84] In general, the functional information will provide a basis for further analysis, much like a primary genetic screen identifies candidate genes for a certain disease that requires extensive subsequent validation.[30,79]

The definition of physiological genomics is not "official", but rather a concept or strategy that combines the strengths of genomics and physiology. For example, defining gene function via the use of positional cloning and physiology in congenic rats or expression profiling after giving a drug and comparing this to physiological changes. Finally, it is attaching biological processes to the genome via mapping, sequencing of genes or interference using transgenic technologies.

In this thesis, the concept of physiological genomics is simplified. What is known about the physiology and the genetics of an animal model is combined to come up with putative candidates for the genes of interest, rather than using a large-scale experimental methodology combined with statistical and computational analysis of the results.

1.1.3 Animal models for hypertension and renal failure

As humans, inbred rat strains also differ in the development of renal failure and genetic factors are suspected to affect the susceptibility to hypertension-induced or to hypertension or diabetic accelerated renal failure.[9,12,18,39,119,134] For instance, rats of the Milan Normotensive Strain (MNS) develop renal failure, but no hypertension, whereas rats of the Milan Hypertensive Strain (MHS) and the Spontaneously Hypertensive Rat (SHR) develop hypertension and no renal failure.[9,43] Other strains develop neither hypertension nor renal failure, not even after high protein intake, increases in blood pressure or reduction of renal mass, such as the Wistar Kyoto, the ACI/Nmh, and the PVG/c rat.[75,111,134,138] In fact, the ACI rat we use in our genetic and physiological studies as normotensive background is also resistant to the development of renal damage (chapters 2 through 5 of this thesis).

The mode of inheritance of renal failure has only been documented in a few strains of rats. Lozzio *et al.* found that a mutant strain of Gunn rats developed hydronephrosis, which was inherited as an autosomal dominant trait.[68] Development of severe nephrosis in Koletsky obese hypertensive rats and in nephrose Shionogi (NPS) rats has been assumed to be regulated by a single autosomal recessive gene.[1] Studies on the genetic regulation of glomerular sclerotic lesions in a cross of BUF/Mna and ACI/NMs rats and high proteinuria

and glomerular defects in the FGS/Nga mouse suggest the presence of two autosomal recessive genes.[44,75]

An example for the dissociation between hypertension and renal failure might be the MHS rat in whose selection process not only the genetic mechanisms that increase blood pressure and glomerular filtration rate, but also a specific susceptibility of the afferent arteriole to develop hypertrophy may have been selected by chance.[9] This would protect the glomerulus from exposure to increased pressure levels and hyperfiltration and thus prevent the development of glomerular damage.[10,14,43] From this viewpoint, studies of renal damage in primary hypertension should investigate the genetics of primary hypertension with renal hyperfiltration as well as the genetic background that, in the presence of hypertension and hyperfiltration, cause renal failure. Regarding the first type of studies, Cusi *et al.* have identified a point mutation in a gene encoding the α and β subunit of adducin, which has been linked to the development of hypertension in MHS rats and in humans.[8,18]

To dissect the genetics of both hypertension and renal failure, it was decided to study renal impairment in a hypertensive animal model, the Fawn-Hooded rat, which is the focus of this thesis. Combining physiology and genetics in this animal model may help to better understand the role of genetic factors in hypertension-associated renal failure. So far, physiological as well as genetic studies have been carried out in this rat model, which are described below.

1.1.4 Scope

Studies in the rat model of hypertension-associated renal failure used in our laboratory, the Fawn-Hooded Hypertensive (FHH) rat, showed that hypertension and renal failure have a genetic basis.[12] Because the genes and their products responsible for these phenotypes in the FHH rat are not known yet, we designed experiments to investigate the interaction between blood pressure and the susceptibility to renal damage in the FHH strain and related strains, and in a normotensive control strain.

To be able to come up with candidate genes for the loci identified in the genetic studies, we performed physiological experiments on the mechanism of renal failure in the FHH rat. The outcome of these studies, which are mainly in the area of renal autoregulation, will bring us closer to the identification of the genes for renal failure that are present in the FHH rat. Identifying the homologous genes of the FHH rat genes in man will enable us to better understand and treat the development of end-stage renal failure in humans.

1.2 PHYSIOLOGY OF THE FAWN-HOODED RAT

1.2.1 History of the Fawn-Hooded rat

The Fawn-Hooded Rat (FHR) originates from the Department of Physiology, University of Michigan, Ann Harbor, as a mutant resulting from unplanned cross-breeding of various populations of laboratory rats.[72,127] Researchers from the Museum of Natural History in New York have made the FHR available for biomedical research into various diseases. This thesis concentrates on the FHR as a model for hypertension-associated renal failure.

Introduced into Europe by Dr. Tschopp,[124-126] the breeding nucleus was provided for the FHR colony kept at Unilever Research Laboratories in Vlaardingen, where the spontaneous presence of systemic hypertension was observed.[59-64] From this strain, two inbred strains were developed on the basis of differences in awake systolic blood pressure (SBP) and urinary protein excretion (UpV). These strains were transferred to the animal facilities of the Erasmus University Rotterdam (EUR), where they were fully inbred. The strain with the highest SBP and UpV was named FHH/EUR and the strain with the lowest values FHL/EUR.[91,92,95] From this point forward, these strains will simply be indicated as FHH and FHL. For a complete review of the FHR and its history, the reader is referred to the theses of Kuijpers[60] and Simons.[110]

1.2.2 The Fawn-Hooded rat in biomedical research

Congenital hemorrhagic diathesis was the first-described abnormality in the FHR.[97] Later, this was found to be associated with a defective platelet release pool of adenosine di-phosphate (ADP), tri-phosphate (ATP) and serotonin.[87,120,124,125] This bleeding disorder resembles storage pool deficiency found in humans.[33,38,114] Both the uptake and release of serotonin in platelets is reduced and associated with a defect in membrane glycoproteins.[3,55] Because of an abnormal brain serotonin and tryptophan metabolism,[19,51] the FHR is thought to be a genetic model for psychiatric disorders of serotonergic dysfunction, including depression,[87,88] anxiety,[2] and eating disorders.[5,123] Moreover, the FHR is being used as a model for alcohol preference.[6,17,19,77,86,99,106]

The outbred FHR has been tried as model for experimental atherosclerosis.[121] However, Verseput *et al.* found that hyperlipidemia was secondary to UpV in the FHH rat.[131]

Although the coat color pattern is the same, FHR strains differ genetically as well as physiologically.[95] Currently, at least four substrains of Fawn-Hooded rats

are used in biomedical research.[95] In this thesis, if known, the appropriate strain abbreviation will be used. The abbreviation FH will be used for the original random bred Fawn-Hooded strain, which came to Europe from the Museum of Natural History, being the ancestors of the inbred FHH and FHL strains. The same FH rat was used by W. Jean Dodds for a limited inbreeding program at the New York State Department of Health, Albany (NY) to generate the FH/Wjd strain.[127] Another Fawn-Hooded strain, the Fawn-Hooded Iowa Reactive (FH/IR) strain, seems only partly related to the other strains and is used mainly in research on pulmonary hypertension and in some behavioral studies. Overstreet and Rezvani observed significant behavioral differences between the FH/Wjd and FH/IR strains.[87] Recently, we also noted marked strain differences with regard to blood pressure and renal damage.[95] Thus, characteristics of one strain are not necessarily present in the other.

1.2.3 Systemic blood pressure

Elevated systemic blood pressure has been reported in 5.5-mo-old male outbred FH rats with a level of about 150 mmHg up to levels around 170 mmHg at one year of age.[59-64] The FHH rat we use in our studies develops moderate systolic hypertension as measured by tail-cuff with levels around 160 mmHg at one year of age, compared with 125 mmHg in FHL rats.[92,112,130] Under anesthesia, mean arterial pressure in FHH rats is between 130 and 140 mmHg, compared with 120 mmHg in FHL rats.[111-113,128,129]

Outbred FH rats with the highest level of systemic hypertension had a higher mean daily water intake and urine output between 7 and 11 weeks of age, the period during which systemic hypertension develops.[93] Moreover, enhanced sodium and water excretion in the FH/Wjd rat was observed by Gilboa *et al.*, compared with Wistar rats.[34,35] The same authors reported reduced kallikrein activity in the FH/Wjd rat preceding the onset of hypertension. This suggested that the renal kallikrein system is involved in the pathogenesis of hypertension in the FH/Wjd rat. Moreover, plasma renin activity and plasma angiotensin II levels were found to correlate with the active form of kallikrein, as well as with conversion of inactive to active kallikrein, in contrast to Wistar rats.[34,35] These observations suggest a close relationship between the renin-angiotensin and kallikrein systems in the FH/Wjd rat.

Early studies showed hypertension in the FH/Wjd strain was of a hyporeninemic type.[34,35] Jung *et al.* found that plasma and renal renin concentration were elevated in young FHH rats.[53] Also, an immature pattern of renin expression was found in the afferent arteriole in these rats with moderate

systemic hypertension. Aging of FHH rats is accompanied by a decline in renal and plasma renin, whereas renal immunostaining for angiotensin converting enzyme and angiotensinogen increases. Moreover, upregulation of angiotensin II (AT_1) receptors was found in the FHH rat.[52] These changes are associated by increases in UpV and focal glomerulosclerosis (FGS).

Furthermore, increased urinary catecholamine, as indicated by increased dopamine and noradrenaline levels, were measured in the outbred FH strain, indicating increased activity of the sympathetic nervous system.[71,136]

1.2.4 Proteinuria and glomerulosclerosis

Renal damage in the FHR was described by Kreisberg and Karnovsky for the first time: they found that upon aging male FH/Wjd rats developed progressive UpV and FGS.[56] Subsequently, UpV was also found in the random bred FH rat.[59-64] The severity of UpV was quite variable in the randomly bred strain. The variance coincided with variances in blood pressure, actually providing the basis for the inbreeding and generation of the FHH and FHL strains. Additional studies showed that the UpV almost exclusively consists of albuminuria (UaV). Studying four different FHR strains showed that FHH and FH/Wjd rats develop severe progressive UpV, whereas FHL and FH/IR rats do not.[95]

Detailed histopathological studies in the FHH rat by Kriz *et al.* showed that the development of FGS is consistently associated with the vascular pole of the glomerulus due to an elevated glomerular capillary pressure (P_{GC}).[57] The initial injury involves the expansion of primary branches of the afferent arteriole. Podocytes serving these largest glomerular vessels (in which wall tension would be greatest) are exposed to increased strain. Podocyte insufficiency in counteracting the expansile forces will lead to further capillary dilation, finally causing podocyte detachments. This triggers parietal cells to attach to bare areas of the glomerular basement membrane, establishing a starting point of parietal endothelium on the tuft. These events represent the initial lesion inevitably progressing to segmental sclerosis and eventually to ESRF.[57] In this process, fluid leakage into the periglomerular interstitium from perfused capillaries contained in a tuft adhesion plays a prominent role. A second study by Kriz *et al.* suggests that severely injured glomeruli continue to filter along interstitial routes, accounting for interstitial progression of the disease. Similar histopathologic phenomena in human kidneys showing FGS suggest that the pathogenetic pathways defined in rat models such as the FHH rat and the MNS rat operate in human renal disease as well.[58]

Male FH/Wjd rats develop significantly more hypertension and renal damage compared with female FH/Wjd rats, similar to FHH rats.[94] This suggests that there may be a protective effect of estrogens or, alternatively, a negative influence of androgens, as in other animal models and humans.[127] However, recent studies in male and female FHH rats did not show an influence of gonadectomy on the development of hypertension or renal damage in the FHH rat, suggesting some other form of sex-specific protective mechanism.[94]

1.2.5 Interaction between blood pressure and renal damage

As in several other experimental models of kidney disease, challenging the kidney by reducing the number of nephrons by unilateral nephrectomy (UNX) or an increase in dietary protein augments the progression of UpV and FGS in the FHH rat.[21,112]

Provoost *et al.* showed that two-kidney FHH and FH/Wjd rats had similar SBP and UpV levels, whereas both FHL and FH/IR rats did not show elevated SBP or UpV. After UNX, FH/Wjd rats developed more UpV and FGS compared with FHH rats despite similar SBP levels. FHL rats also developed some UpV after UNX, but showed no increases in SBP, whereas FH/IR rats did not exhibit any increases in SBP or UpV.[95]

The effects of UNX on renal damage and SBP at about five weeks of age were also assessed in hypertensive SHR rats, normotensive FHL rats, and F_1 crosses of (SHR x FHH) and (SHR x FHL) rats, all differing in basal blood pressure level. UNX had no significant effect on blood pressure in any of the strains. In this study, genetic differences appeared to determine the severity of renal damage after UNX. FHH and FHL rats developed renal damage, although FHL rats developed less renal damage than FHH rats, the differences being related to the different blood pressure levels. Renal damage in the F_1 rats in this study was markedly less than in both Fawn-Hooded strains. Despite high blood pressure, the SHR rat was quite resistant to renal damage.[96]

The relation between UpV and SBP was also investigated by Simons *et al.*, who showed a significant correlation between blood pressure and UpV in 12-wk-old FHH rat rats. Moreover, the incidence of FGS was also correlated with blood pressure and UpV.[111] Recent renal transplantation studies using kidneys of the FHH rat showed that renal damage, and not hypertension, travels with the FHH kidney, indicating that the renal abnormalities observed in this rat strain have their origin in the kidney and not in the systemic circulation.[90] The results suggest that an independent genetic mechanism is present for the development of renal

failure independent of the development of hypertension, which may be related to the regulation of P_{GC}.

Furthermore, studies by Oliver *et al.* showed that increased UaV in UNX-FHH rats results from a specific defect in glomerular charge selectivity, rather than size selectivity, induced by chronic glomerular hypertension.[85]

1.2.6 Intrarenal hemodynamics

De Keijzer *et al.* found that the glomerular filtration rate (GFR) was significantly higher in 12-wk-old FH rats compared with age and weight matched control Wistar rats, whereas effective renal plasma flow was similar among groups.[23] Because the number of glomeruli in FH rats was similar to that in Wistar rats, GFR per glomerulus was one third higher in FH rats compared with Wistar rats. This suggests that in FH rats, hyperfiltration is present at a single-nephron level which coincided with enhanced urinary eicosanoid excretion.[20] The elevated levels of UpV together with the glomerular hyperfiltration were thought to be the result of glomerular capillary hypertension.[20,136]

Micropuncture studies by Simons *et al.* revealed the presence of glomerular capillary hypertension in the FHH rat. They also observed hyperfiltration at the single nephron level in 12- and 22-wk-old FHH rats and to a lesser extent in FHL rats compared with normotensive Wistar rats.[113]

In another set of experiments, Simons *et al.* found a close correlation between P_{GC} and systemic blood pressure in 12-wk-old FHH rats. Moreover, an increased efferent arteriolar resistance was found in combination with a low afferent resistance. They concluded that the elevated efferent vascular resistance and the inability to modulate afferent vascular tone contribute to the transmission of arterial pressure elevations to the kidneys of FHH rats.[110,111] These findings were confirmed by Verseput *et al.* in 11- and 26-wk-old FHH rats.[129,132]

Unilateral nephrectomy, which causes further increases in P_{GC} without changes in systemic pressure, further reduces afferent arteriolar resistance in FHH rats, leading to even more pronounced renal damage compared with two-kidney FHH rats.[112]

1.2.7 Vascular abnormalities

While the FH/Wjd and FHH/EUR strains develop systemic hypertension and renal damage, the FH/IR strain develops pulmonary hypertension but no renal failure.[4,54,102] Abnormal responses of the aortic and pulmonary vascular wall to ADP and serotonin in the FH/IR rat could be involved in the development of pulmonary hypertension in this strain, indicating altered endothelial function and vessel wall reactivity.[104,105,115-117] Ashmore *et al.* studied vascular reactivity in isolated pulmonary arteries of the FH/IR rat. They found that the responses to the platelet-derived endothelium dependent vasodilator ADP were markedly impaired in the pulmonary artery, but mildly impaired in the aorta of these rats. Furthermore, they found that vasodilatory responses to acetylcholine were also impaired.[4]

The production of endothelin-1 was increased by epithelial cells in the airway of the FH/IR rat.[118] In addition, the conversion of arachidonic acid (AA) to 6-keto-prostaglandin-$F_{1\alpha}$ was reduced in the aorta of FH/IR rats. This could be partially normalized by cholesterol feeding. Accordingly, the AA-induced aggregation of platelet-rich plasma from FH/IR rats was also stimulated by cholesterol feeding, whereas collagen-induced aggregation remained absent despite high cholesterol intake.[139] Furthermore, abnormal urinary eicosanoid excretion was noted, consisting of increased levels of tromboxane B_2, 6-keto-prostaglandin $F_{1\alpha}$, and prostaglandin-E_2. A shift in the pattern of excretion from vasodilatory to vasoconstrictor eicosanoids was observed over time, which was accompanied by progressive UpV.[20]

Results of a study by Resch *et al.*[98] assessing the nitric oxide system in the macula densa of FHH and FHL rats suggested an upregulated activity. Such dysregulation could explain low inflow resistance to the glomerulus. The nitric oxide system abnormality may be linked or associated to other vasoregulatory abnormalities.

1.3 IMPACT OF CHANGES IN BLOOD PRESSURE

1.3.1 Effects of antihypertensive treatment in the Fawn-Hooded rat

Magro *et al.* first described antihypertensive effects of various classes of drugs, including angiotensin converting enzyme inhibition (ACEi) in FH/Wjd rats.[72] The response to captopril in FH/Wjd rats was stringer than that in normotensive Wistar control rats. The responses to nifedipine and furosemide suggested that hypertension in FH/Wjd rats is due to vasoconstriction rather than volume expansion.

Weening *et al.* tested the antihypertensive effects of the serotonin receptor blocking agent, ketanserin, and found no beneficial effects of this drug on blood pressure or UpV in the FH rat. This led to the conclusion that hypertension and renal disease in the FH rat are not due to abnormal serotonin metabolism in this strain.[135]

Westenend *et al.* studied the effects of ACEi using captopril in the FH rat and showed protection from structural renal damage even when treatment was started after the development of glomerular injury. This effect was attributed to the antihypertensive effects of ACEi.[137]

Verseput *et al.* studied the efficacy of ACEi in the prevention and treatment of chronic renal damage in FHH rats treated with lisinopril at different stages of renal failure. They concluded that early-onset ACEi treatment very effectively protects against renal damage in the FHH rat and that this protection is associated with normalization of P_{GC}. ACEi could not completely prevent progression of FGS in rats with established glomerular disease despite lowering P_{GC}. Short-term ACEi had no long-term effect on arterial pressure or P_{GC}, and could not prevent the development of FGS.[128,130,132]

Simons *et al.* suggested that the beneficial effect of enalapril after UNX in FHH rats primarily results from decreasing transcapillary pressure and ameliorating UaV by preserving glomerular charge-selectivity, rather than size-selectivity.[85,112] A lag of approximately one week was observed between the new level of blood pressure and UpV. This latter finding indicates that in addition to the direct hemodynamic effects of ACEi, alterations in the filtration membrane affect renal protein excretion.

Verseput *et al.* studied the effects of four different classes of antihypertensive drugs and showed that lowering blood pressure is not the exclusive renoprotective mechanism of ACEi in the FHH rat. In the FHH rat, early-onset clonidine offers the same degree of renoprotection compared with ACEi despite higher blood pressure levels, and late-onset triple therapy offers no renoprotection despite equally effective blood pressure control by ACEi.

The ability of ACEi to reduce P_{GC} and intrarenal angiotensin II levels in the FHH rat may be the most important renoprotective effect.[130]

Simons *et al.* studied the effects of enalapril in UNX-FHH rats and found that with high levels of glomerular hyperfiltration and hypertension in addition to glomerular enlargement, treatment with ACEi prevents the rise in P_{GC} but not in glomerular size and abolishes the development of renal damage.[111]

Ziai *et al.* and Mackenzie *et al.* tested the efficacy of the angiotensin receptor antagonist (AT_1) irbesartan, and enalapril in UNX-FHH rats and confirmed the results by Simons *et al.*[111] Chronic treatment with both drugs in the FHH rat normalized systemic blood pressure and P_{GC}, suggesting that the antihypertensive and renoprotective effects of enalapril were largely mediated by inhibiting the effects of angiotensin II on the AT_1 receptor.[69,140] These findings support a critical role for angiotensin-dependent mechanisms in the development of hypertension and FGS in the FHH rat.[113,131]

1.3.2 L-NAME treatment and renal hemodynamics

In general, systemic inhibition of nitric oxide synthase leads to dose-dependent increases in blood pressure and renal vascular resistance, a large fall in renal plasma flow and a slight reduction in GFR. The kidney is particularly sensitive to nitric oxide inhibition (NOi), which leads to increases in efferent arteriolar resistance, decreases in the ultrafiltration coefficient and a variable effect on the efferent arteriolar resistance. When systemic NOi leads to increases in blood pressure, efferent arteriolar resistance rises and causes marked elevations in P_{GC}.[7]

The degree of renal damage after chronic NOi also differs among rat strains. The effects may be caused by increases in blood pressure or by the effects of the specific inhibitor used. Simons *et al.* studied the effects of the NOi L-NAME in UNX-FHH rats. They found that rats treated with L-NAME exhibited high systemic blood pressure, heavy UpV and FGS, and further elevation of the ultrafiltration pressure with a decreased ultrafiltration coefficient after only eight weeks of treatment.[111] Moreover, the elevated resistance of both the afferent and efferent arteriole caused an increase in P_{GC}. In this study, L-NAME treatment which lowered values for single nephron GFR and glomerular volume but caused a further elevation in P_{GC} did not reduce glomerular damage. On the contrary, FGS eight weeks after UNX in L-NAME rats was as severe as 12 weeks after UNX in control animals. Studies described in chapters 2 through 4 of this thesis deal with the subject of chronic NOi in rats with different genetic backgrounds.

1.3.3 Renal autoregulation

The development of intraglomerular hypertension is thought to be one of the key features in the development of renal damage. Regulation of filtration and pressure in the kidney is ensured by the autoregulation mechanism. This means the ability to maintain relative constancy of blood flow, GFR and P_{GC}, despite variations in arterial pressure. The afferent arteriole is the primary source for the control of resistance changes.

Renal autoregulation is thought to involve two processes: (a) the myogenic response and (b) tubuloglomerular feedback (TGF). The myogenic response involves an intrinsic property of arterial smooth muscle enabling it to contract or relax in response to increases or decreases in vascular wall tension, respectively. TGF is referred to as the negative feedback loop that is elicited by changes in volume and composition of the fluid entering the thick ascending limb of the loop of Henle and leads to vascular responses in the afferent arteriole.[101] This mechanism was recently found to be intact in 11-wk-old FHH rats.[129] Therefore, we surmised that the abnormalities might well be caused by an impairment in the myogenic response.

The autoregulation mechanism controls the transmission of systemic pressure to the glomeruli. Without this mechanism, increases in systemic blood pressure would parallel increases in P_{GC} causing damage to the glomeruli, which is the case in several animal and human disease states.

The SHR rat presents with early hypertrophy of the afferent arteriole, increasing its resistance which protects the glomerulus from increases in P_{GC}. After UNX, P_{GC} increases in the SHR kidney accelerating the development of UpV and FGS.[26] In contrast, MHS rats have a reduced total number of nephrons and elevated afferent arteriolar resistance and single nephron filtration rate before the development of hypertension. The development of renal damage does not further increase after hypertension is fully developed. This strain appears to be relatively resistant to the development of progressive glomerular damage, which seems to be prevented by hypertrophy of the afferent arteriole.[9] The FHH rat, which has a normal number of nephrons,[92] develops intraglomerular hypertension due to the increased efferent arteriolar resistance without the ability to modulate afferent arteriolar tone.[111] The MNS rat, which is also thought to have an impaired modulation of afferent vascular tone,[9] develops glomerular sclerotic lesions similar to those in the FHH rat. Lesions in these animal models resemble some forms of FGS observed in humans.[58]

In general, hypertension produces hemodynamic and mechanical stress, which may lead to glomerular endothelial dysfunction. Both of these mechanisms may lead to increased glomerular leakiness, which may further damage the kidney.

Hypertension may also stimulate the production of many vasoactive substances that influence the tone of the afferent and efferent arterioles. They include a variety of vasoconstrictor and vasodilator hormones, which are potent stimulators for growth factors and cytokines, leading to the production of extracellular matrix proteins.[50]

1.4 GENETICS OF THE FAWN-HOODED RAT

The first study on the genetics of the FHR strain was performed by LaVail *et. al.*, who described genes determining the coat and eye color.[65] Regarding these genes, the FHR was found to be homozygous for the non-agouti (aa), black (BB), non-albino (CC), and hooded (hh) genes. The fawn color was found to be due to the presence of the autosomal recessive red-eyed (rr) dilution gene.[89] The bleeding diathesis and lack of ATP secretion were associated with homozygosity for this gene, which was found to be close to the albino gene (cc) on chromosome one.[122] The homozygous presence of the recessive aa, hh, and rr genes forms the basis for the unique coat color pattern of the FHR.

1.4.1 Inheritance of hypertension and renal damage in the FHH/EUR rat

The recessive coat color genes were used to start a search for the inheritance of hypertension and renal damage in the FHH rat. The outcome of this study has been reported by Brown *et. al.*[12] The August x Copenhagen Irish (ACI) rat was chosen because it is normotensive and resistant to renal damage and carries the dominant forms of the genes at the r, a, and h loci. The mode of inheritance was determined by comparing the F_1 (FHH x ACI) with the two parental strains. F_1 rats showed normal UpV levels, indicating that renal impairment was inherited as a recessive trait. Accordingly, it was decided to study the inheritance of the hypertension and renal failure in a (FHH x ACI) FHH backcross. Progression of renal impairment was mainly followed by UpV and by visible lesions at macroscopic examination of the kidneys. Systolic blood pressure was measured at six months of age.

For genotyping, we used randomly chosen markers as well as markers representing genes that are known to be involved in blood pressure regulation (including angiotensin converting enzyme, atrial natriuretic factor, 11-β-hydroxylase, and renin) and in certain regions reported to show linkage to hypertension in other crosses (such as carboxypeptidase 8 and tumor necrosis factor). Moreover, markers flanking the S_A locus and guanyl cyclase associated

with the atrial natriuretic factor receptor) were included.[15,40,42,46,103] Linkage analysis (analysis determining the degree of association between a marker and a phenotype) was used to detect quantitative trait loci (QTL) (genetic loci responsible for quantitative traits such as blood pressure) for renal impairment and blood pressure.

By comparing the genetic variance for SBP and UpV in F_0, F_1 rats and in the backcross progeny, it was possible to determine the proportion of genetic variance in the backcross. Moreover, it was possible to determine the proportion of genetic variance in this cross and to estimate the effective number of genetic factors controlling the traits.[24] Most of the backcross progeny showed some degree of hypertension and showed a weak, but statistically significant correlation between SBP and UpV.

A gene involved in renal impairment was found on rat chromosome 1. Linkage was clear for six of the seven phenotypes related to renal impairment, and this locus was referred to as *Rf-1* (for *r*enal-*f*ailure-1). The strength of the evidence for linkage emerged from the correlation of genotypes with UpV: *Rf-1* homozygotes had twice as much UpV as *Rf-1* heterozygotes. Using the macroscopic index score of renal impairment, the *Rf-1* locus accounted for almost half of the total variance. Although the *Rf-1* locus was positively correlated with the measures of renal impairment, it had no significant effect on SBP. There was also no significant difference in mean blood pressure between *Rf-1* homozygotes and heterozygotes, indicating that *Rf-1* caused renal impairment through a mechanism other than increasing blood pressure. When the UpV phenotype was adjusted by eliminating the effect of blood pressure, it showed similar correlation to *Rf-1*. This confirms that *Rf-1* causes renal impairment through an independent mechanism and not simply by increasing blood pressure.

Since *Rf-1* explained only about half of the genetic component of renal impairment and did not explain a significant proportion of blood pressure, the genome was searched (a) for additional loci responsible for renal impairment and (b) for loci responsible for systolic hypertension. Evidence was found for two additional loci, which were also on chromosome 1, but were at a considerable distance from *Rf-1*. The first had a significant effect on SBP, but less on renal impairment. This locus was referred to as *Bpfh-1* (for *b*lood *p*ressure in *f*awn-*h*ooded 1). The second locus, *Rf-2* (for *r*enal *f*ailure-2) had a strong effect on one measure of renal impairment (plasma albumin level), as well as a significant but weaker effect on plasma creatinin levels and the macroscopic injury score. This region containing *Rf-2* also showed some linkage to blood pressure.

The *Rf-1* locus could not be identical with the *Bpfh-1* or *Rf-2* loci because the physical distance on chromosome 1 was sufficiently large to exclude a significant correlation between genotypes. The relationship between *Bpfh-1* and *Rf-2* was

less clear. The most likely locations for both genetic loci did not overlap. Moreover, *Bpfh-1* proved to be close to an important candidate locus, the S_A gene, a gene of unknown function that showed higher expression in the kidney of SHR compared with WKY rats[45,66,81,103] and cosegregates with blood pressure in a number of crosses.[25,40,49] This provided additional confidence in the localization of *Bpfh-1* and it was concluded that *Bpfh-1* and *Rf-2* represent distinct loci.

Together, *Rf-1* and *Rf-2* explained about 40% of the total variance in both UpV and renal injury score. Animals homozygous at both *Rf-1* and *Rf-2* showed substantially higher indexes of renal impairment than animals heterozygous at either or both QTLs, indicating that both QTLs cumulatively contribute to the development of renal impairment. *Rf-1* and *Rf-2* contrasted in that the former showed the strongest effect on UpV, but weak effects on plasma albumin concentration, whereas *Rf-2* exhibited the opposite pattern. These differences suggest that *Rf-1* and *Rf-2* may act through different mechanisms.

Brown *et al.* recently revealed the presence of sexual dimorphism in the development of hypertension and UpV in FHH rats.[13] They found that in females, SBP cosegregates close to the *Rf-1* area and not to the locus previously mapped in males,[12] being close to *Rf-2*. Moreover, no significant logarithm-of-the-odds score (lod; statistical measure for the correlation between a locus and a trait) for the *Rf-2* area in the female cohort was found.

1.4.2 Additional F_2 cross

Subsequently, a new and larger F_2 cross of FHH and ACI rats was studied to extend the genetic analysis of loci involved in the regulation of blood pressure and those defining the susceptibility to develop renal damage in the FHH rat after UNX.[109] Compared with the backcross study described above, the density of the genetic map published by Jacob *et al.* in 1995 was almost tripled.[47]

At the *Rf-1* locus, significant linkage was found with UpV and FGS. The inheritance patterns at *Rf-1* were consistent with the FHH alleles acting in a recessive or additive manner to increase urinary protein levels. A lod score scan for UpV showed that *Rf-1* may have two peaks, which could not be definitively ruled out from being independent. At the maximum-point-likelihood position for *Rf-1*, the lod score for SBP was below the threshold level, indicating that *Rf-1* causes renal impairment through a mechanism independent from blood pressure, confirming the backcross data. *Rf-2* also showed high lod scores for UpV. Suggestive linkage in the same area was found with SBP (*Bpfh-1*). Therefore, independence of these two loci could not be established.

In addition to *Rf-1* and *Rf-2*, significant evidence was obtained for additional QTLs affecting UpV on chromosome 3 (*Rf-3*) and on chromosome 14 (*Rf-4*). Two more QTLs on chromosome 17 (*Rf-5* and *Bpfh-2*) showed suggestive linkage for UpV and SBP, respectively, and were independent. Similarly, for FGS, the lod score was significant for *Rf-3* and suggestive for *Rf-4* and *Rf-5*.

Rf-1 proved to have the strongest effect on UpV among all other *Rf*-loci. However, animals that were homozygous for FHH on *Rf-1* and heterozygous or homozygous for ACI on other *Rf*-loci did not develop marked UpV. Although *Rf-1* shows the strongest linkage, the effect was less pronounced when other *Rf*-loci did not carry homozygous *Rf*-alleles. This suggests that *Rf-1* has a strong additive effect on other *Rf*-loci. The combination of *Rf-1* and one of the other *Rf*-loci severely impaired renal function. The combinations of *Rf-1* and *Rf-4* or *Rf-5* showed the highest UpV levels.

The use of molecular genetics in the (FHH x ACI) F_2 intercross progeny not only confirmed the presence of two loci for renal damage (*Rf-1* and *Rf-2*), but also identified three new gene loci, named *Rf-3*, *Rf-4*, and *Rf-5*. The susceptibility to develop renal failure in the FHH rat seems to be largely a recessive trait in that the F_1 rats do not develop high UpV. *Rf-1* appears to act through a mechanism independent of blood pressure and additive effects on other *Rf*-loci. The data underscore the complexity of the development of progressive renal damage in the FHH rat model and the interaction between hypertension and renal failure. To understand the phenotype in light of the genotype, especially with regard to polygenic control,[80] the strength of physiology and genetics have to be combined, which is the concept of physiological genomics and the scope of this thesis.

1.5 OUTLINE

The studies described in this thesis were undertaken to combine physiology and genetics in the FHR model of hypertension-associated renal damage. This approach eventually requires the determination of the impact of each *Rf*-region separately and of the various combinations of the different regions on renal damage. This requires the generation of congenic strains. Congenic strains carry a part of the FHH genome containing an *Rf*-locus on the ACI background. The generation of such strains requires at least three years of selective breeding. During this breeding process, we further investigated the impact of hypertension on renal damage in different genotypes. Moreover, we studied the mechanism of renal autoregulation in the FHH rat because previous studies pointed towards impairments in this mechanism.[23,111,113] The outcome of these studies might effect the interpretation of the genetic studies. While most studies so far were focused on the role of *lowering* blood pressure, the studies described in this thesis describe the impact of *elevating* blood pressure on the development of renal damage in rats with different genetic background. First, we tested the role of L-NAME-induced increases in blood pressure on the development of renal failure in the FHL and the ACI rat (Chapter 2). The FHL rat was used because it shares most of the markers in the *Rf*-regions with the FHH rat, but does not develop hypertension.[95] The ACI rat was used because it is little susceptible to renal damage. Secondly, we compared the impact of L-NAME-induced increases in blood pressure on the development of renal failure in ACI and FHH rats, and in the F_1 progeny of these two strains, which are heterozygous for the *Rf*-genes (Chapter 3). Thirdly, we studied the combination of UNX and increased blood pressure on the development of renal damage in ACI and F_1 (FHH x ACI) rats. In this study, we also investigated whether differences in susceptibility to developing renal damage were caused by increases in blood pressure alone or also by actions of L-NAME itself (Chapter 4).

Another set of experiments served to better characterize the mechanisms underlying the high susceptibility to develop renal failure in FHH rats. First, the autoregulation of renal blood flow and glomerular filtration rate in FHH and FHL rats were studied (Chapter 5). Secondly, similar studies were done regarding the autoregulation of intraglomerular pressure and *in vitro* studies of renal microvascular reactivity (Chapter 6) to evaluate the myogenic response in FHH rats.

REFERENCES

1. Abramowsky CR, Aikawa M, Swinehart GL. Spontaneous nephrotic syndrome in a genetic rat model. *Am J Pathol.* 1984;117:400-408.
2. Altemus M, Smith MA, Diep V, Aulakh CS, Murphy DL. Increased mRNA for corticotrophin releasing hormone in the amygdala of fawn-hooded rats: a potential animal model of anxiety. *Anxiety* 1994;1:251-257.
3. Arora RC, Tong C, Jackman HL, Stoff D, Meltzer HY. Serotonin uptake and imipramine binding in blood platelets and brain of Fawn-Hooded and Sprague-Dawley rats. *Life Sci.* 1983;33:437-442.
4. Ashmore RC, Rodman DM, Sato K, Webb SA, O'Brien RF, McMurtry IF, Stelzner TJ. Paradoxical constriction to platelets by arteries from rats with pulmonary hypertension. *Am J Physiol.* 1991;260:H1929-H1934.
5. Aulakh CS, Hill JL, Murphy DL. A comparison of feeding and locomotion responses to serotonin agonists in three rats strains. *Pharmacol Biochem Behav.* 1988;31:567-571.
6. Badishtov BA, Overstreet DH, Kashevskaya OP, Viglinskaya IV, Kampov-Polevoy AB, Seredenin SB, Halikas JA. To drink or not to drink: open field behavior in alcohol-preferring and non-preferring rat strains. *Physiol Behav.* 1995;57:585-589.
7. Baylis C, Qiu C. Importance of nitric oxide in the control of renal hemodynamics. *Kidney Int.* 1996;49:1727-1731.
8. Bennett V: Spectrin based membrane skeleton: A multipotential adaptor between plasma membrane and cytoplasm. *Physiol Rev.* 1990;70:1029-1065
9. Brandis A, Bianchi G, Reale E, Helmchen U, Kuhn 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.
10. Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Eng J Med.* 1982;307:652-659.
11. Broeckel U, Shiozawa M, Kissebah AH, Provoost AP, Jacob HJ. Susceptibility genes for end-organ damage. New strategies to understand diabetic and hypertensive nephropathy. *Nephrol Dial Transplant.* 1998;13:840-842.
12. 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. *Nat Genet.* 1996;12:44-51.
13. Brown DM, Van Dokkum RPE, Korte MR, McLauglin MG, Shiozawa M, Jacob HJ, Provoost AP. Genetic control of suceptibility for renal damage in hypertensive fawn-hooded rats. *Ren Fail.* 1998;20:407-411.

14. Chung O, Rohmeiss P, Sponer G, Strauch M, Gretz N. Renal involvement in spontaneously hypertensive rats. In: *Rat Models of Chronic Renal Failure* (Gretz N, Strauch M, eds) Karger, Basel 1993;357-370.
15. Cicila GT, Rapp JP, Wang JM, St. Lezin E, Ng SC, Kurtz TW. Linkage of 11-β-hydroxylase mutations with altered steroid biosynthesis and blood pressure in the Dahl rat. *Nature Genet.* 1993;3:346-353.
16. Cilia GT, Rapp JP, Bloch KD, Kurtz TW, Pravenec M, Klen V, Hong CC, Quertermous T, Ng SC. Cosegation of the endothelin-3-locus with blood pressure and relative heart weight in inbred Dahl rats. *J Hypertens.* 1994;12:643-651.
17. Criswell HE, Overstreet DH, Rezvani AH, Johnson KB, Simson PE, Knapp DJ, Moy SS, Breese GR. Effects of ethanol, MK-801, and chlordiazepoxide on locomotor activity in different rat lines: dissociation of locomotor stimulation from ethanol preference. *Alcohol Clin Exp Res.* 1994;18:917-923.
18. Cusi D, Tripodi G, Casari G, Robba C, Bollini P, Merati G, Bianchi G. Genetics of renal damage in primary hypertension. *Am J Kidney Dis.* 1993;21:2-9.
19. Daoust M, Compagnon P, Legrand E, Boucly P. Ethanol intake and ^{3}H-serotonin uptake: a study in fawn-hooded rats. *Life Sci.* 1991;48:1969-1976.
20. De Keijzer MH, Provoost AP, Zijlstra FJ. Enhanced urinary excretion of eicosanoids in Fawn-Hooded rats. *Nephron* 1992;62:454-458.
21. De Keijzer MH, Provoost AP. Effects of dietary protein on the progression of renal failure in the Fawn-Hooded rat. *Nephron* 1990;55:203-209.
22. De Keijzer MH, Provoost AP, Molenaar JC. Proteinuria is an early marker in the development of progressive renal failure in hypertensive Fawn-Hooded rats. *J Hypertens.* 1989;7:525-528.
23. De Keijzer MH, Provoost AP, Molenaar JC. Glomerular hyperfiltration in hypertensive Fawn-Hooded rats. *Renal Physiol Biochem.* 1988;11:103-108.
24. Dietrich W et al. Genetic identification of Mom-1, a major locus affecting Min-induced intestinal neoplasia in the mouse. *Cell* 1993;75:631-639.
25. Dubay C, Vincent M, Samani NJ, Hilbert P, Kaiser MA, Beressi J-P, Kotelevtsev Y, Beckmann JS, Soubrier F, Sassard J, Lathrop GM. Genetic determinants of diastolic and pulse pressure map to different loci in Lyon hypertensive rats. *Nature Genet.* 1993;3:354-357.
26. Dworkin LD, Feiner HD. Glomerular injury in uninephrectomized spontaneously hypertensive rats. A consequence of glomerular capillary hypertension. *J Clin Invest.* 1986;77:797-809.
27. Earle K, Walker J, Hill C, Viberti G. Familial clustering of cardiovascular disease in patients with insulin dependent diabetes and nephropathy. *N Eng J Med.* 1992;326:673-677.
28. Ferguson R, Grim CE, Opgenorth TJ. A familial risk of chronic renal failure among blacks on dialysis ? *J Clin Epidemiol.* 1988;41:1189-1196.
29. Fernandez-Llama P, Poch E, Oriola J, Coll E, Darnell A, Rivera F, Revert L. Angiotensin converting enzyme gene I/D polymorphism in essential hypertension and nephroangiosclerosis. *Kidney Int.* 1998;53:1743-1747.

30. Fields S. The future is function. *Nature Genet.* 1997;5:325.
31. Fliser D, Ritz E. Does essential hypertension cause progressive renal disease ? *J Hypertens.* 1998;16:S13-S15.
32. Freedman BI, Spray BJ, Tuttle AB, Buckalew VM. The familial risk of end-stage renal disease in African Americans. *Am J Kidney Dis.* 1993;21:387-393.
33. Gawaz MP, Bogner C, Gurland HJ. Flow-cytometric analysis of mepacrine-labelled platelets in patients with end-stage renal failure. *Hemostasis* 1993;23:284-292.
34. Gilboa N, Rudofsky UH, Phillips MI, Magro AM. Modulation of urinary kallikrein and plasma renin activities does not affect established hypertension in the fawn-hooded rat. *Nephron* 1989;51:61-66.
35. Gilboa N, Rudofsky UH, Magro AM. Urinary and renal kallikrein in hypertensive fawn-hooded (FH/Wjd) rats. *Lab Invest.* 1984;50:72-78.
36. Gonzalez AM, Smith A, Emery C, Higenbottam T. Pulmonary hypertension, family and environment. *J Hum Hypertens.* 1997;11:559-561.
37. Goodfellow P. A celebration and a farewell. *Nature Genet.* 1997;16:209.
38. Gordge MP, Faint RW, Rylance PB, Neild GH. Platelet function and the bleeding time in progressive renal failure. *Thromb Haemostasis* 1989;60:83-87.
39. Hampton JA, Bernardo DA, Khan NA, Lacher DA, Rapp JP, Gohara AF, Goldblatt PJ. Morphometric evaluation of the renal arterial system of Dahl salt-sensitive and salt-resistant rats on a high salt diet. II. Interlobular arteries and intralobular arteries. *Lab Invest.* 1989;60:839-846.
40. Harris EL, Dene H, Rapp JP. S_A gene and blood pressure cosegregating using Dahl salt-sensitive rats. *Am J Hypertens.* 1993;6:330-334.
41. Hieter P, Boguski M. Functional genomics: it's all how you read it. *Science* 1997;278:601-602.
42. Hilbert P, Lindpaintner K, Beckmann JS, Serikawa T, Soubrier F, Dubay C, Cartwright P, De Gouyon B, Julier C, Takahashi S, Vincent M, Ganten D, Georges M, Lathrop GM. Chromosomal mapping of two genetic loci associated with blood-pressure regulation in hereditary hypertensive rats. *Nature* 1991;353:521-529.
43. 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-F93.
44. Hyun BH, Wakasugi N, Nose M, Saito T, Tomita T. A new mouse strain manifesting high proteinuria and kidney glomerular defect. Lab Anim Sci. 1991;41:442-446.
45. Iwai N, Inagami T. Isolation of preferentially expressed genes in the kidneys of hypertensive rats. *Hypertension* 1991;17:161-169.
46. Iwai N, Ohmichi N, Hanai K, Nakamura Y, Kinoshita M. Human SA gene locus as a candidate locus for essential hypertension. *Hypertension* 1994;23:375-380.
47. Jacob HJ, Brown DM, Bunker RK, Daly MJ, Dzau VJ, Goodman A, Koike G, Kren V, Kurtz T, Lernmark A, Levan G, Mao Y, Pettersson A, Pravenec M,

Simon JS, Szpirer J, Trolliet MR, Winer ES, Lander ES. A genetic linkage map of the laboratory rat, Rattus Norvegicus. *Nature Genet.* 1995;9:63-69.

48. Jacob HJ, Shiozawa M, Broeckel U, Brown DM, Naff B, Majewski RR, Gauthier S, Kissebah A, Provoost AP. Genetics of end stage renal disease (ESRD): Of Rat and Man ? *Am J Hypertens.* 1997;10:216A (Abstract).
49. Jacob HJ, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao Y-P, Ganten D, Dzau VJ, Lander ES. Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. *Cell* 1991;67:213-224.
50. Johnston CI, Risvanis J, Naitoh M, Tikkanen I. Mechanism of progression of renal disease: current hemodynamic concept. *J Hypertens.* 1998;16:S3-S7.
51. Joseph MH. Brain tryptophan metabolism on the 5-hydroxytryptamine and kynurenine pathways in a strain of rats with a deficiency in platelet 5-HT. *Br J Pharmacol.* 1978;63:529-533.
52. Jung FF, Provoost AP, Haveran L, Ingelfinger JR. Upregulation of angiotensin II (AT_1) receptors in the hypertensive fawn hooded rat. *J Am Soc Nephrol.* 1995;6:680 (Abstract).
53. Jung FF, Provoost AP, Bouyounes B, Ingelfinger JR. Persistence of immature renin pattern expression in young fawn-hooded rats. *J Am Soc Nephrol.* 1993;4:773 (Abstract).
54. Kentera D, Susic D, Veljkovic V, Tucakovic G, Koko V. Pulmonary artery pressure in rats with hereditary platelet function defect. *Respiration* 1988;54:110-114.
55. Kirchmaier CM, Meyer M, Spangenberg P, Heller R, Haroske D, Breddin HK, Till U. Platelet membrane defects in fawn-hooded bleeder rats. *Thromb Res.* 1990;57:353-360.
56. Kreisberg JI, Karnovsky MJ. Focal glomerular sclerosis in the fawn-hooded rat. *Am J Pathol.* 1978;92:637-652.
57. Kriz W, Hosser H, Hähnel B, Simons JL, Provoost AP. Development of vascular pole associated glomerulosclerosis in the fawn-hooded rat. *J Am Soc Nephrol.* 1998;9:381-396.
58. Kriz W, Hosser H, Hähnel H, Gretz N, Provoost AP. From segmental glomerulosclerosis to total nephron degeneration and interstitial fibrosis: a histopathological study in rat models and human glomerulopathies. *Nephrol Dial Transplant.* 1998;13:2781-2798.
59. Kuijpers, MHM, Jong W de. Relationship between blood pressure level, renal histopathological lesions and plasma renin activity in fawn-hooded rats. *Br J Exp Pathol.* 1987;68:179-189.
60. Kuijpers MHM. Hypertension in the Fawn-Hooded rat. PhD-thesis, 1986, Utrecht, The Netherlands.
61. Kuijpers MHM, Gruys E. Spontaneous hypertension and hypertensive renal disease in the fawn-hooded rat. *Br J Exp Pathol.* 1984;65:181-190.
62. Kuijpers MHM, Gruys E. Nephrosclerosis and hypertension in the fawn-hooded rat. *Berl Munch Tierartztl Wochenschr.* 1981;94:39.

63. Kuijpers MHM, De Jong W. Spontaneous hypertension in the Fawn-Hooded rat: a cardiovascular disease model. *J Hypertens Suppl.* 1986;4:S41-S44.
64. Kuijpers MHM, Provoost AP, De Jong W. Development of hypertension and proteinuria with age in Fawn-Hooded rats. *Clin Exp Pharmacol Physiol.* 1986;13:201-209.
65. LaVail MM. Fawn-hooded rats, the fawn mutation and interaction of pink-eyed and red-eyed dilution genes. *J Hered.* 1981;72:286-287.
66. Lindpaintner K, Hilbert P, Ganten D, Nadal-Ginard B, Inagami T, Iwai N. Molecular genetics of the S_A-gene: cosegregation with hypertension and mapping to rat chromosome 1. *J Hypertens.* 1993;11:19-23.
67. Lommel A van, Lauweryns JM. Neuroepithelial bodies in the Fawn Hooded rat lung: morphological and neuroanatomical evidence for a sensory innervation. *J Anat.* 1993;183:553-566.
68. Lozzio BB, Chernoff AI, Machado ER. Hereditary renal disease in a mutant strain of rats. *Science* 1967;156:1742-1744.
69. Mackenzie HS, Provoost AP, Troy JL, Sandstrom S, Rennke HG, Brenner BM. Irbesartan prevents renal injury in uninephrectomized (UNX) Fawn-Hooded hypertensive (FHH) rats. *J Am Soc Nephrol.* 1995;6:1022 (Abstract).
70. Magro A, Bizios R, Catalfamo J, Blumenstock F, Rudofsky U. Collagen-induced rat platelet reactivity is enhanced in whole blood in both the presence and absence of dense granule secretion. *Thromb Res.* 1992;68:345-356.
71. Magro AM, Rudofsky UH, Gilboa N, Seegal R. Increased catecholamine output in the hypertensive fawn-hooded rat. *Lab Anim Sci.* 1986;36:646-649.
72. Magro A, Gilboa N, Rudofsky UH. The Fawn-Hooded (FH/Wjd) rat as a genetic hypertensive animal model. In: *Central and peripheral mechanisms of cardiovascular regulation.* (Magro A, Osswald W, Reis D, Vanhoutte P, eds) Plenum Publishing Corporation 1986:219-267.
73. Magro AM, Brai M. Evidence for lipoxygenase activity in induction of histamine release from rat peritoneal mast cells by chelated iron. *Immunology* 1983;49:1-8.
74. Magro AM. Histamine release from fawn-hooded rat mast cells is not potentiated by phosphatidylserine. *Immunology* 1981;44:1-10.
75. Matsuyama M, Ogiu T, Kontani K, Yamada C, Kawai M, Hiai H, Nakamura T, Shimizu F, Toyokawa T, Kinoshita Y. Genetic regulation of the development of glomerular sclerotic lesions in the BUF/Mna rat. *Nephron* 1990;54:334-337.
76. McKusick VA. Genomics: structural and functional studies of chromosomes. *Genomics* 1997;45:244-249.
77. McMillen BA, Williams HL. Volitional consumption of ethanol by fawn-hooded rats: effects of alternative solutions and drug treatments. *Alcohol* 1995;12:345-350.
78. Michalak E, Walkowiak B, Paradowski M, Cierniewski CS. The decreased circulating platelet mass and its relation to bleeding time in chronic renal failure. *Thromb Haem.* 1991;65:11-14.

79. Miklos GL, Rubin GM. The role of the genome project in determining gene function: insights from model organisms. *Cell* 1996;86:521.
80. Morel L, Rudofski UH, Longmate JA, Schiffenbauer J, Wakeland EK. Polygenic control of susceptibility to murine systemic lupus erythematosis. *Immunity* 1994;1:219-229.
81. Nabika T, Nara Y, Ikeda K, Endo J, Yamori Y. A new genetic locus cosegregating with blood pressure in F_2 progeny obtained from stroke-prone spontaneously hypertensive rats and Wistar-Kyoto rats. *J Hypertens.* 1993;11:13-18.
82. Nara Y, Nabika T, Ikeda K, Sawamua M, Mano M, Endo J, Yamori Y. Basal high blood pressure cosegregates with the loci on chromosome 1 in the F_2 generation from crosses between normotensive Wistar Kyoto rats and stroke-prone spontaneously hypertensive rats *Biochem Biophys Res Commun.* 1993;194:1344-1351.
83. Navis G, De Jong PE, De Zeeuw D. I/D polymorphism of the angiotensin converting enzyme gene: a clue to the heterogeneity in the progression of renal disease and in the renal response to therapy ? *Nephrol Dial Transplant.* 1997;12:1097-1100.
84. Norwood VF, Gomez RA. Bridging the gap between physiology and molecular biology: new approaches to perpetual questions. *Am J Physiol.* 1994;267:R865-R878.
85. Oliver JD III, Simons JL, Troy JL, Provoost AP, Brenner BM, Deen WM. Proteinuria and impaired glomerular permselectivity in uninephrectomized Fawn-Hooded rats. *Am J Physiol.* 1994;267:F917-F925.
86. Overstreet DH, McArthur RA, Rezvani AH, Post C. Selective inhibition of alcohol intake in diverse alcohol-preferring rat strains by the 5-HT2A antagonists amperozide and FG 5974. *Alcohol Clin Exp Res.* 1997;21:1448-1454.
87. Overstreet DH, Rezvani AH. Behavioral differences between two inbred strains of Fawn-Hooded rat: a model of serotonin dysfunction. *Psychopharmacology* 1996;128:328-330.
88. Overstreet DH, Rezvani AH, Janowsky DS. Genetic animal models of depression and ethanol preference provide support for cholinergic and serotonergic involvement in depression and alcoholism. *Biol Psychiatry* 1992;31:919-936.
89. Prieur DJ, Meyers KM. Genetics of the fawn-hooded rat strain. The coat color dilution and platelet storage pool deficiency are pleiotropic effects of the autosomal recessive red-eyed dilution gene. *J Hered.* 1984;75:349-352.
90. Provoost AP, Kouwenhoven EA, Van Dokkum RPE, De Bruin RWF, Marquet RL. Donor and recipient factors in the development of albuminuria after kidney transplantation (KTx) in rats. *J Am Soc Nephrol.* 1997;8:3096A (Abstract).
91. Provoost AP Spontaneous glomerulosclerosis: insights from the fawn-hooded rat. *Kidney Int Suppl.* 1994;45:S2-S5.
92. Provoost AP, De Keijzer MH. The Fawn-Hooded rat: A model for chronic renal failure. In: *Experimental and genetic models of chronic renal failure.* (Gretz N, Strauch M, Eds), Karger AG 1993:100-114.

93. Provoost AP, De Keijzer MH. Kidney function and volume homeostasis during the development of hypertension in the fawn-hooded rat. In: *Hypertension, Brain Catecholamines and Peptides.* Nijkamp FP, De Wied D (Eds). Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam, The Netherlands, 1989:45-50.
94. Provoost AP, Korte M, Van Dokkum RPE. Development of renal damage in the Hypertensive Fawn-Hooded rat is not affected by gonadectomy. *Am J Hypertens.* 1998;11;89A (Abstract).
95. Provoost AP, Shiozawa M, Van Dokkum RPE, Jacob HJ. Phenotype and genotype variability in four strains of fawn-hooded (FH) rats. *Clin Exp Pharmacol Physiol.* 1998;25:A33 (Abstract).
96. Provoost AP, Van Dokkum RPE, Jacob HJ. Differences in susceptibility to renal damage after unilateral nephrectomy (UNX) in rats. *Clin Exp Pharmacol Physiol.* 1998;25:A5 (Abstract).
97. Raymond SL, Dodds WJ. Characterization of the fawn-hooded (FH/Wjd) rat as a model for hemostatic studies. *Thromb Diath Haemorrh.* 1975;33:361-369.
98. Resch S, Provoost AP, Bachmann S. Morphology of the renin-angiotensin system (RAS) and the L-arginine nitric oxide (NO) pathway in Fawn-Hooded (FH) rats. *J Am Soc Nephrol.* 1995;5:590 (Abstract).
99. Rezvani AH, Overstreet DH, Janowsky DS. Drug-induced reductions in ethanol intake in alcohol preferring and fawn-hooded rats. *Alcohol Suppl.* 1991;1:S433-S437.
100. Rostand SG, Brown G, Kirk KA, Rutsky AE, Dustan HP. Racial differences in the incidence and treatment for end-stage renal disease. *N Eng J Med.* 1982;306:1276-1279.
101. Robertson CR, Deen WM, Troy JL, Brenner BM. Dynamics of glomerular ultrafiltration in the rat. III. Hemodynamics and autoregulation. *Am J Physiol.* 1972;223:1191-1200.
102. Rudofsky UH, Magro AM. Spontaneous hypertension in fawn-hooded rats. *Lab Anim Sci.* 1982;32:389-391.
103. Samani NJ, Lodwick D, Vincent M, Dubay C, Kaiser MA, Kelly MP, Lo M, Haris J, Sassard J, Lathrop M, Swales JD. A gene differentially expressed in the kidney of the spontaneously hypertensive rat cosegregates with increased blood pressure. *J Clin Invest.* 1993;92:1099-1103.
104. Sato K, Webb S, Tucker A, Rabinovitch M, O'Brien RF, McMurtry IF, Stelzner TJ. Factors influencing the idiopathic development of pulmonary hypertension in the fawn hooded rat. *Am Rev Respir Dis.* 1992;145:793-797.
105. Sato K, Tucker A, O'Brien F, McMurtry IF, Stelzner TJ. The fawn hooded rat: a model of spontaneous pulmonary hypertension. *Am Rev Respir Dis.* 1989;139:167 (Abstract).
106. Schechter MD, Meehan SM. Ethanol discrimination in fawn-hooded rats is compromised when compared to other strains. *Alcohol* 1993;10:77-81.

107. Schmidt S, Stier E, Hartung S, Stein G, Bahnisch J, Woodroffe AJ, Clarkson AR, Ponticelli C, Campise M, Mayer G. No association of converting enzyme insertion/deletion polymorphism with immunoglobulin A glomerulonephritis. *Am J Kidney Dis.* 1995;26:727-731.
108. Seaquist ER, Goetz FC, Rich S, Barbosa J. Familial clustering of diabetic kidney disease: evidence for genetic susceptibility to diabetic nephropathy. *N Eng J Med.* 1989;320:1161-1165.
109. Shiozawa M, Provoost AP, Majewski RR, Van Dokkum RPE, Jacob HJ. Interacting genes determine susceptibility to proteinuria after uninephrectomy in rats. *J Am Soc Nephrol.* 1998;9:394A (Abstract).
110. Simons JL. Pathogenesis of glomerular sclerosis in the Fawn-Hooded rat, PhD-thesis, 1994, Rotterdam, The Netherlands.
111. 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.
112. Simons JL, Provoost AP, Keijzer MH de, Anderson S, Rennke HG, Brenner BM. Pathogenesis of glomerular injury in the fawn-hooded rat: effect of unilateral nephrectomy. *J Am Soc Nephrol.* 1993;4:1362-1370.
113. Simons JL, Provoost AP, Anderson S, Troy JL, Rennke HG, Sandstrom DJ, Brenner BM. Pathogenesis of glomerular injury in the fawn-hooded rat: early glomerular capillary hypertension predicts glomerular sclerosis. *J Am Soc Nephrol.* 1993;3:1775-1782.
114. Soslau G et al. Desmopressin-induced improvement in bleeding times in chronic renal failure patients correlates with platelet serotonin uptake and ATP release. *J Med Sci.* 1991;300:372-379.
115. Starkey JR, Prieur DJ, Ristow SS. Natural killer cell activity in fawn-hooded rats. *Experientia* 1983;39:308-310.
116. Steen P van den, Lommel A van, Lauweryns JM. Presence and possible implications of open-ring centrioles, multiple basal centrioles and basal cilia in neonatal hamster bronchioles. *Acta Anat.* 1995;153:85-95.
117. Stelzner TJ, Hofmann TA, Brown DM, Jacob HJ. Genetic determinants of pulmonary hypertension in fawn-hooded rats. *Chest* 1997;111:96S (Abstract).
118. Stelzner TJ, O'Brien RF, Yanagisawa M, Sakurai T, Sato K, Webb S, Zamora M, McMurtry IF, Fisher JH. Increased lung endothelin-1 production in rats with idiopathic pulmonary hypertension. *Am J Physiol.* 1992;262:L614-L620.
119. Sterzel RB, Luft FC, Gao Y, Schnermann J, Briggs JP, Ganten D, Waldherr R, Schnabel E, Kriz W. Renal disease and the development of hypertension in salt-sensitive Dahl rats. *Kidney Int.* 1988;33:I 119-I 129.
120. Sweeney TK, Needleman P. Mechanisms underlying the defect in storage pool deficient platelets from fawn-hooded rats. *Fed Proc.* 1979;38:419 (Abstract).
121. Tiell ML, Sussmann II, Moss R, Drouet LC, Spaet TH. Production of experimental arteriosclerosis in fawn-hooded rats with platelet storage pool deficiency. *Artery* 1982;10:329-340.

122. Tobach E, DeSantis JL, Zucker MB. Platelet storage pool disease in hybrid rats. F1 Fawn-Hooded rats derived from crosses with their putative ancestors (*Rattus norvegicus*). *J Hered.* 1984;75:15-18.
123. Tobach E, Bellins JS, Das DK. Differences in bitter taste perception in three strains of rats. *Behav Genet.* 1974;4:405-410.
124. Tschopp TB, Baumgartner HR. Defective platelet adhesion and aggregation on subendothelium exposed *in vivo* or *in vitro* to flowing blood of Fawn-Hooded rats with storage pool disease. *Thrombos Haemostas.* 1977;38:620-629.
125. Tschopp TB, Weiss HJ. Decreased ATP, ADP and serotonin in young platelets of fawn-hooded rats with storage pool disease. *Thrombos Diathes Haemorrh.* 1974;32:670-677.
126. Tschopp TB, Zucker MB. Hereditary defect in platelet function in rats. *Blood* 1972;40:217-226.
127. Urizar RE, Cerda J, Dodds WJ, Raymond SL, Largent JA, Simon R, Gilboa N. Age-related renal, hematologic, and hemostatic abnormalities in FH/Wjd rats. *Am J Vet Res.* 1984;45:1624-1631.
128. Verseput GH, Koomans HA, Braam BB, Weening JJ, Provoost AP. Normalization of intraglomerular pressure by ACE-inhibition, in the presence of severe albuminuria, delays, but does not prevent development of terminal renal failure. *Kidney Int.* 1998;submitted.
129. Verseput GH, Braam BB, Provoost AP, Koomans HA. Tubuloglomerular feedback and prolonged ACE-inhibitor treatment in the hypertensive fawn-hooded rat. *Nephrol Dial Transplant.* 1998;13:893-899.
130. Verseput GH, Braam BB, Van Dokkum RPE, Weening JJ, Koomans HA, Provoost AP. Renoprotection with ACE-inhibition is only partially dependent on blood pressure reduction in hypertensive Fawn-Hooded rats. *J Am Soc Nephrol.* 1998;submitted.
131. Verseput GH, Provoost AP, Tol A van, Koomans HA, Joles JA. Hyperlipidemia is secondary to proteinuria and is completely normalized by angiotensin-converting enzyme inhibition in hypertensive fawn-hooded rats. *Nephron* 1997;77:346-352.
132. 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.
133. Viberti GC, Keen H, Wiseman MJ. Raised arterial pressure in parents of proteinuric insulin dependent diabetics. *Br Med J.* 1987; 295: 515-517.
134. Weening JJ, Beukers JJB, Grond J, Elema JD. Genetic factors in focal segmental glomerulosclerosis. *Kidney Int.* 1986;29:789-798.
135. Weening JJ, Westenend PJ, Nooyen YA, Brummelen P van. Monotherapy with captopril (CAP) prevents progressive sclerosis (GS) in fawn-hooded rats, but ketanserin (K) is ineffective. *Kidney Int.* 1990;37:523 (Abstract).
136. Westenend PJ, Nooyen YA, Krogt JA van der, Brummelen P van, Weening JJ. Functional and structural determinants of glomerulosclerosis in the fawn-hooded rat. *Eur J Clin Invest.* 1992;22:391-395.

137. Westenend PJ, Nooyen YA, Krogt JA van der, Brummelen P van, Weening JJ. The effect of a converting enzyme inhibitor upon renal damage in spontaneously hypertensive fawn-hooded rats. *J Hypertens.* 1992;10:417-422.
138. Westenend PJ, Nooyen YA, Van Brummelen P, Weening JJ. Intrinsic vasodilation protects Wistar Kyoto rats from progressive glomerulosclerosis after unilateral nephrectomy. *J Lab Clin Med.* 1991;117:25-32.
139. Wey H, Gallon L, Subbiah MTR. Prostaglandin synthesis in aorta and platelets of fawn-hooded rats with platelet storage pool disease and its response to cholesterol feeding. *Thromb Haemost.* 1982;48:94-97.
140. Ziai F, Ots M, Provoost AP, Troy JL, Rennke HG, Brenner BM, Mackenzie HS. The angiotensin receptor anatagonist, irbesartan, reduces renal injury in experimental chronic renal failure. *Kidney Int Suppl.* 1996;50:S132-S136.

CHAPTER 2

DIFFERENCE IN SUSCEPTIBILITY OF DEVELOPING RENAL DAMAGE IN NORMOTENSIVE FAWN-HOODED (FHL) AND AUGUST X COPENHAGEN IRISH (ACI) RATS AFTER N^{ω}-NITRO-L-ARGININE METHYL ESTER INDUCED HYPERTENSION

American Journal of Hypertension 1997;10:1109-1116

Richard P.E. van Dokkum, Howard J. Jacob, and Abraham P. Provoost

ABSTRACT

Previous studies using the Fawn-Hooded Hypertensive (FHH) rat have indicated that genetic factors appear to be important in determining the susceptibility to develop renal damage. This was further investigated by comparing the effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME) induced hypertension on functional and structural renal damage in two normotensive strains, the resistant August x Copenhagen Irish (ACI) rat and the Fawn-Hooded Low blood pressure (FHL) rat, which also appears to carry a susceptibility locus for renal failure. Male rats were studied during chronic treatment with L-NAME in either a low dose (LD, 75 to 100 mg/L drinking fluid) or a high dose (HD, 175 to 250 mg/L). Survival of FHL rats was adversely affected by L-NAME treatment. All FHL-HD and 6 out of 14 FHL-LD rats died before the end of the 11 weeks study period, whereas all but one of the treated ACI rats survived. In both strains, L-NAME caused a dose dependent increase in systolic blood pressure (SBP). However, at similar levels of SBP, the increase in albuminuria (UaV) was significantly higher in FHL compared with ACI, as was the incidence of focal glomerulosclerosis (FGS). Both the SBP and the blood pressure burden (SBP-Av), defined as SBP averaged over the study period, directly correlated with UaV and FGS in both strains. However, the increase in the degree of renal damage per millimeter of mercury increase in SBP or SBP-Av was significantly higher in the FHL than in the ACI rats. Our findings clearly show that FHL rats are more susceptible to renal damage after induction of hypertension by chronic L-NAME treatment. We conclude that there is an interaction between blood pressure and the genetic susceptibility to develop renal failure in the FHL rat.

INTRODUCTION

The susceptibility of a hypertensive patient to developing end stage renal failure (ESRF) seems to vary with ethnicity. Although only a minority of hypertensive patients develop ESRF,[1] hypertensives of African decent carry a 16-fold greater risk of developing renal failure than do hypertensives of Caucasian decent.[2-6] This points towards differences in susceptibility to developing renal complications that may be genetically determined.[5] A dissociation between hypertension and renal disease is also present in animal models of genetic hypertension. The Fawn-Hooded Hypertensive (FHH) rat, an inbred strain with a moderate level of systemic hypertension, develops progressive proteinuria (UpV) and focal glomerulosclerosis (FGS) by 12 months of age, leading to premature death due to ESRF.[7-9] In contrast, the Spontaneously Hypertensive Rat (SHR) develops severe hypertension but does not develop renal failure until very late in life.[10] One of the striking differences between the two strains is the presence of glomerular hypertension in FHH rats,[11] whereas glomerular pressure in SHR rats is not different from that of normotensive Wistar-Kyoto rats.[12,13]

Genetic factors influencing the development and progression of renal damage have been previously suggested, but specific genes have not been identified.[14] Recently, however, we have studied the genetics of hypertension and renal disease in the FHH rat.[15] Using a backcross of (FHH x [August x Copenhagen Irish]) F_1 x FHH rats, we localized three quantitative trait loci on chromosome 1, one for SBP and two for renal disease in the FHH rat. The locus for SBP was denoted *Bpfh-1*, and the loci for renal damage were denoted as *Rf-1* and *Rf-2*, respectively. *Bpfh-1* mapped to the S_A region, also known as an important gene in determining SBP in other crosses of genetic hypertensive rats.[16-18] The *Rf-1* locus might contain a major gene in the control of renal function and could be responsible for the high susceptibility to develop renal damage in FHH rats.

During the inbreeding of the FHH strain, we also developed a strain of Fawn-Hooded Low blood pressure (FHL) rats. Both were obtained by selective inbreeding from randomly bred strains of fawn-hooded rats. In contrast to the FHH rat, the FHL rat not only has a lower SBP level, but develops UpV, albuminuria (UaV), and FGS much more slowly, resulting in a longer survival time.[7-9] Comparison of the FHH and FHL genotypes, however, showed that the markers flanking the alleles of the *Rf-1* region were identical in both strains.[19] This indicates that the mutation in the *Rf-1* gene is likely to be also present in the FHL strain. Consequently, FHL rats should also have an increased susceptibility to renal damage when compared with other normotensive rat strains. We surmise that progressive UpV and FGS should develop once FHL rats are exposed to high

levels of SBP, and that this damage should be more prevalent in FHL rats compared with August x Copenhagen Irish (ACI) rats, which we do not expect to develop marked renal damage. To test this hypothesis, we elevated SBP in both FHL and ACI rats by chronic administration of different doses of the nitric oxide synthase-inhibitor (NOi), N^{ω}-nitro-L-arginine methyl ester (L-NAME).[20,21]

METHODS

Animals

Experiments were performed on 65 animals that were 7 weeks old at the start of the study. These animals were derived from our own breeding colonies of FHL and ACI rats. Animals were housed in standard rat cages with lights on from 8 AM to 8 PM. Standard commercial rat chow containing 56% carbohydrates, 26% digestible protein, 7% fat, 4% fiber, and 5% minerals (AM II; Hope Farms, Woerden, The Netherlands) and drinking fluid (tap water, acidified to pH 3.0) were provided *ad libitum*.

Induction of hypertension

Hypertension was induced by chronic treatment with L-NAME (Sigma Chemical Co., St. Louis, MO) dissolved in the drinking water at a concentration either of 75 to 100 mg/L (low dose, LD) or 175 to 250 mg/L (high dose, HD). Age-matched control animals were provided with normal drinking fluid.

Animals from each strain were randomly divided into three groups. The three ACI-groups initially consisted of nine control, eight LD treated, and eight HD treated rats. In about 20% of the cases, ACI rats presented with only one kidney, known as congenital unilateral renal agenesis (URA).[22,23] Animals with URA were excluded from data analysis. The three FHL groups initially consisted of 12 control, 14 LD treated, and 14 HD treated rats.

Absolute L-NAME intake of the individual rats was determined at the time of the metabolic studies. The mean dosages, calculated from fluid intake and body weight, for the various L-NAME-treated groups of rats are presented in Table 1.

Systolic blood pressure

Systolic blood pressure (SBP) was measured with the tail-cuff method using a plethysmography (IITC Life Science, Woodland Hills, CA) in awake, restrained animals. Every Thursday between noon and 2 PM, at least three consecutive measurements were recorded and averaged. To investigate the relation between SBP and renal damage over a certain period of time, we calculated the SBP average (SBP-Av), by averaging all SBP data obtained from week 3 until the end of the protocol.

Functional studies

Measurement of water and food intake and 24-h urine collections were done at 3, 7, and 11 weeks of follow-up, by placing the animals in polycarbonate metabolic cages (Tecniplast Gazzada, Buguggiate, Italy). The animals were allowed to adapt over the weekend. Actual measurements were done on two consecutive days.

Autopsy

Moribund rats (that is, rats showing a rapid decrease in body weight over several days to below 300 g) were autopsied at the time of detection, whereas surviving animals were killed after the 11-wk protocol. A laparotomy was performed under ethyl ether anesthesia and a blood sample was taken from the abdominal aorta. After bleeding the animals, heart and kidneys were removed, washed with saline, and weighed. Heart and kidneys of rats that died before the end of the 11-wk protocol were weighed and processed if possible, that is, when obtained shortly after death.

Tissue processing

Kidneys were fixed by immersion for 48 h in a 3.6% buffered formaldehyde solution (Lommerse Pharma, Oss, The Netherlands) after longitudinal bisection. Subsequently, they were dehydrated in alcohol and blocked in paraffin wax. Sections of 3 μm were stained with periodic acid Schiff (PAS) and hemotoxylin and eosin (HE) counterstain.

The kidney sections were microscopically evaluated by determining the incidence of FGS. For each animal, 50 glomeruli were examined in the inner and outer cortical region and the number of sclerotic glomeruli were examined. Criteria on which glomeruli were designated as sclerotic consisted of adhesion of the glomerulus to Bowman's capsule, thickening of Bowman's capsule, the presence of increased amounts of PAS-positive material in the mesangial region, and folding of the glomerular basement membrane with entrapment of amorphous material. Interstitial changes were not assessed. All sections were evaluated without knowledge of the group to which individual rats belonged.

Analytic procedures

Plasma and urinary samples were analyzed with the ELAN system (Eppendorf-Merck, Hamburg, Germany) using colorimetric assays for total protein with molybdate red, albumin with bromocresol green, and creatinine with the Jaffé method without deproteinization.

Statistics

Data are presented as mean ± SD in the tables in this article and as mean ± SEM in the figures. Differences in mean values between groups were compared using one way analysis of variance and a Student-Newman-Keuls test to identify the groups that were different. In case of a nonnormal distribution of UaV, groups were compared using the Mann-Whitney rank sum test. In all tests $p < 0.05$ was considered statistically significant. The relationship between SBP or SBP-Av and parameters of functional and structural renal damage was investigated by linear regression analysis.

RESULTS

Dosage

Table 1 shows the actual L-NAME intake at the time of the metabolic evaluation. No significant differences were present between the HD groups of both strains at the three time-points evaluated. However, the LD treated FHL rats received slightly, but significantly more L-NAME at 3 and 7 weeks of follow-up.

Table 1. Calculated values for L-NAME dose in mg/kg at 3, 7, and 11 weeks of study.

Group	n	Week 3	n	Week 7	n	Week 11
ACI-LD	7	8.1 ± 0.5	7	7.0 ± 0.7	7	7.1 ± 0.9
ACI-HD	7	18.0 ± 7.6	7	21.2 ± 5.3	6	18.1 ± 4.7
FHL-LD	14	9.5 ± 0.8	12	9.5 ± 0.9	6	8.9 ± 4.8
FHL-HD	14	25.6 ± 7.0	6	23.4 ± 17.1		No survivors
p1/p2		s/ns		s/ns		ns

Abbreviations: ACI, August x Copenhagen Irish rat; FHL, Fawn-Hooded Low blood pressure rat; HD, high dose; LD, low dose; p1, ACI-LD *vs.* FHL-LD; p2, ACI-HD *vs.* FHL-HD; s, $p < 0.05$; ns, not significant. Values are given as mean ± SD.

Survival

All ACI rats, except one in the HD group, survived for the duration of the experiment. In contrast, a large number of FHL rats died during the course of the study. In the LD group, only six out of 14 completed the 11-wk protocol. In the HD group, all 15 FHL rats died prematurely due to L-NAME-induced complications. These complications consisted of a decrease in body weight, severe vasoconstriction, and occasionally paralysis of the hind limbs. In general, animals showed signs of cardiac failure, *i.e.*, coronary artery constriction and scarring of the heart muscle as was evaluated macroscopically at autopsy.

Systolic blood pressure and albuminuria

Values for SBP, and UaV obtained at 3, 7 and 11 weeks are summarized in Table 2. In both ACI and FHL rats, a dose-dependent increase in SBP was observed. All SBP values in the L-NAME-treated rats were significantly different from the control ACI and FHL rats throughout the entire experiment. During the latter part of the follow-up, measurement of the SBP in FHL rats became extremely difficult due to the weak, or even lack of, pulsation in the tail arteries, caused by the severe L-NAME-induced vasoconstriction. At week 7 only two out of six in the FHL-HD group could be measured. At week 11, SBP could be determined in only one out of six rats in the FHL-LD group.

Urinary protein excretion was measured simultaneously with UaV in all rats. As changes in both parameters were almost identical; for brevity, only the analyses of the UaV findings will be presented. Both strains differed greatly in the effects of L-NAME treatment on UaV. Compared with ACI controls, the LD treated ACI rats showed hardly any increase in UaV, whereas, at the end of the follow-up in the HD treated ACI rats, UaV had increased threefold to a mean value of only 13 mg/day. In contrast, very marked increases in UaV were observed in FHL rats. Compared with FHL controls, already at 7 weeks of follow-up there was a 24-fold increase in the FHL-HD rats to 191 mg/day. At the end of follow-up, the six surviving LD treated FHL rats showed a 16-fold increase in UaV, to a mean level of 121 mg/day.

Table 2. Body weight (BW in g), systolic blood pressure (SBP in mmHg), and albuminuria (UaV in mg/day) in ACI and FHL rats at 3, 7, and 11 weeks of follow-up.

Week 3

Group	n	BW	SBP	UaV
ACI-con	8	229 ± 17	130 ± 6	5.1 ± 1.2
ACI-LD	7	235 ± 8	159 ± 7	4.0 ± 0.7
ACI-HD	7	213 ± 6	170 ± 7	5.2 ± 0.9
p1/p2/p3		ns/s/s	s/s/s	ns/ns/ns
FHL-con	12	283 ± 29	119 ± 10	7.6 ± 1.3
FHL-LD	14	263 ± 14	145 ± 10	12.1 ± 10.2
FHL-HD	14	267 ± 12	160 ± 13	13.1 ± 9.9
p1/p2/p3		s/s/ns	s/s/s	ns/ns/ns
p4/p5/p6		s/s/s	s/ns/s	s/s/s

Week 7

Group	n	BW	SBP	UaV
ACI-con	8	254 ± 22	119 ± 5	4.8 ± 1.4
ACI-LD	7	259 ± 12	169 ± 9	5.2 ± 1.1
ACI-HD	7	242 ± 7	172 ± 9	10.1 ± 3.7
p1/p2/p3		ns/ns/ns	s/s/ns	ns/s/s
FHL-con	12	317 ± 6	122 ± 9	7.9 ± 2.4
FHL-LD	12	301 ± 17	177 ± 7	26.5 ± 20.6
FHL-HD	6	235 ± 21	199 ± 11†	191 ± 72
p1/p2/p3		ns/s/s	s/s/s	s/s/s
p4/p5/p6		s/ns/s	ns/s/ns	s/s/s

Week 11

Group	n	BW	SBP	UaV
ACI-con	8	276 ± 24	128 ± 7	3.8 ± 0.7
ACI-LD	7	278 ± 15	165 ± 9	6.3 ± 2.3
ACI-HD	6	264 ± 7	206 ± 18	12.7 ± 9.0
p1/p2/p3		ns/ns/ns	s/s/s	s/s/ns
FHL-con	9	331 ± 19	121 ± 10	7.5 ± 2.3
FHL-LD	6	270 ± 24	207*	121 ± 60
FHL-HD		No survivors		
p1/p2/p3		s/-/-	s/-/-	s/-/-
p4/p5/p6		ns/-/ns	-/-/-	s/-/s

p1, LD *vs.* con; p2, HD *vs.* con; p3, LD *vs.* HD; p4, ACI-LD *vs.* FHL-LD; p5, ACI-HD *vs.* FHL-HD; p6, FHL-LD *vs.* ACI-HD; s, $p < 0.05$; ns, not significant; con, control. Other abbreviations as in Table 1; * SBP could be obtained in one rat only; † SBP could be obtained in two rats only; Values are given as mean ± SD.

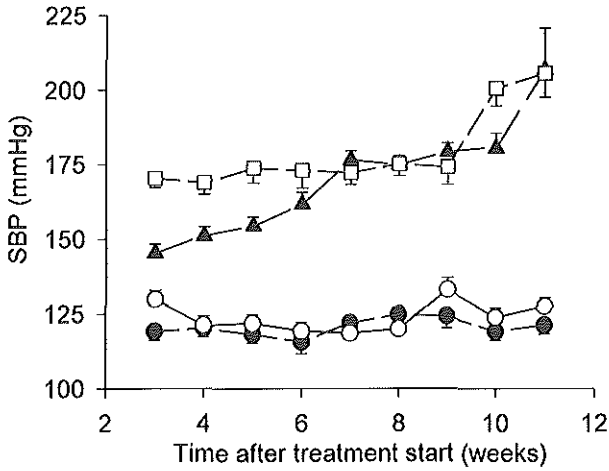

Figure 1. Tail cuff systolic blood pressure (SBP) in millimeters of mercury (mmHg) in awake August x Copenhagen Irish control (ACI-con, ○) and high dose (ACI-HD, □) rats, Fawn-Hooded Low blood pressure control (FHL-con, ●) rats and low-dose (FHL-LD, ▲) rats from 3 to 11 weeks of follow-up. Values are given as mean ± SEM (error bars).

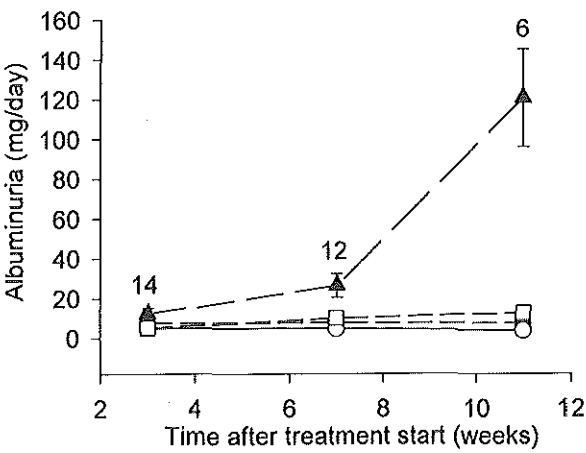

Figure 2. Urinary albumin excretion (UaV) in mg/day in ACI-con (○), ACI-HD (□), FHL-con (●), and FHL-LD (▲) rats with comparable systolic blood pressure (SBP) at 3, 7 and 11 weeks of follow-up. Values are given as mean ± SEM. Numbers at markers represent the number of animals measured.

Autopsy findings

Body and organ weights are summarized in Table 3. In the ACI rat, treatment with L-NAME had little effect on body and wet kidney weight. Both absolute and relative heart weight in the ACI-HD group were significantly increased when compared with the ACI-LD and ACI control groups.

Body weight of the L-NAME-treated FHL rats was significantly reduced when compared with that of the FHL control rats. Total kidney weight was about the same in all three groups. However, due to the differences in body weight, relative kidney weight was higher in FHL-LD and FHL-HD rats compared with that of FHL control rats. In combination with the lower body weights of the treated rats, the differences in relative heart weight were even more pronounced.

Table 3. Body, kidney and heart (absolute and relative) weights and incidence of glomerulosclerosis at autopsy at week 11 or after premature death.

Group	n	BW	TKW	TKW/100g	FGS (%)	HW	HW1
ACI-con	8	279±12	1953±100	701±27	0.9±0.6	736±34	265±14
ACI-LD	7	272±16	1784±74	658±21	3.7±1.3	756±42	279±11
ACI-HD	7	261±18	1856±136	717±88	7.0±1.5	835±37	321±17
p1/p2/p3		ns/ns/ns	ns/ns/ns	ns/s/s	s/s/s	ns/s/s	ns/s/s
FHL-con	12	329±19	2329±155	703±24	1.9±1.6	978±64	298±15
FHL-LD	11	256±17	2467±328	958±169	17.2±4.0	1045±89	410±40
FHL-HD	13	231±18	2519±225	1074±159	25.4±5.1	1132±89	495±62
p1/p2/p3		s/s/s	ns/ns/ns	s/s/s	s/s/s	s/s/s	s/s/s

BW, body weight in g; TKW, total wet kidney weight in mg; TKW/100g, total wet kidney weight per 100 g BW; FGS%, focal glomerulosclerosis incidence; HW, wet heart weight in mg; HW1, wet heart weight per 100 g BW; p1, LD *vs.* con; p2, HD *vs.* con; p3, LD *vs.* HD; s, $p<0.05$; ns, not significant. Other abbreviations as in Table 1. Values are given as mean ± SD.

Focal glomerulosclerosis

The incidence of FGS, presented in Table 3 and Figure 3, indicates a dose dependent increase in structural renal damage in both strains. However, the increase in L-NAME-treated ACI rats was much less marked than that observed in both groups of treated FHL rats.

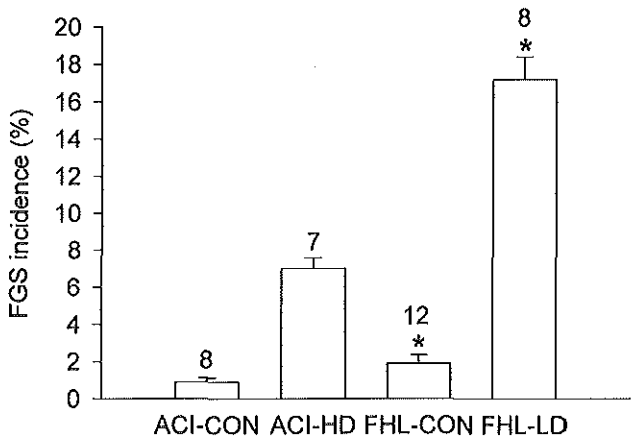

Figure 3. The incidence of focal glomerulosclerosis (FGS) given as percentage of affected glomeruli in ACI and FHL rats. Values are given as mean ± SE. Numbers at the different bars indicate the number of animals that were evaluated. * $p < 0.05$, FHL-LD *vs.* ACI-HD, and FHL-con *vs.* ACI-con.

Clinical chemistry

Plasma creatinin levels in ACI-control (n=8), ACI-LD (n=7), and ACI-HD (n=6) rats were 46 ± 4, 52 ± 5, and 49 ± 7 μmol/L, respectively. The values of the L-NAME-treated ACI rats were not significantly different from those of ACI control rats. In contrast, when compared with FHL control rats, elevated levels of plasma creatinin were present at autopsy in both groups of L-NAME-treated FHL rats, indicative of a deteriorating glomerular filtration rate. The values for FHL-control (n=12), FHL-LD (n=5) and FHL-HD (n=2) rats, were 41 ± 3, 75 ± 36, and 76 ± 8 μmol/L, respectively.

ACI rats with unilateral renal agenesis

At autopsy we found that one rat in each of the ACI groups had URA. Values for SBP, UaV, and FGS of these rats were similar to those of two-kidney rats in the same group. Albeit with a small sample size, this indicates that, even with one kidney, L-NAME-treated ACI rats do not develop severe functional and structural renal damage.

Comparison of FHL and ACI rats with similar SBP levels

Because we were interested in detecting differences in the effects of a similar increase in SBP on the development of renal damage between the two inbred strains, we directly compared the ACI-HD and FHL-LD groups. As shown in Figure 1, these rats developed similar levels of blood pressure throughout the follow-up period. Although the SBP was initially higher in ACI-HD than in FHL-LD rats, there were no significant differences from week 6 onward.

As shown in Figure 2, the FHL-LD rats developed significantly higher levels of UaV than the ACI-HD rats during the study period. Figure 3 shows that at autopsy, the incidence of FGS was significantly higher in the FHL-LD rats compared with the ACI-HD rats. This indicates that the structural renal damage after L-NAME-induced hypertension is also more prevalent in FHL than in ACI rats, even though SBP levels were equivalent.

Comparison of FHL and ACI rats by regression analysis

We also compared the two strains by examining the relationships between SBP and the parameters for functional and structural renal damage. The regression equations for FHL and ACI rats obtained by the linear regression analyses are summarized in Table 4.

In the ACI rats, the correlation between SBP and UaV became significant only at week 11. In contrast, for the FHL rats, statistically significant correlations between SBP and UaV were present at all time-points. Comparing the equations obtained at week 11 shows that in the ACI rats the increase in UaV per millimeter of mercury (mmHg) increase in SBP was 0.12 mg/day. For the FHL rats, the slope of this relationship is about 15 times higher, *i.e.*, the increase in UaV is 1.80 mg/day per mmHg. When correlating the UaV data obtained at week 11 with the SBP-Av (*i.e.*, the systolic blood pressure averaged over the whole follow-up period), a similar picture emerges. For each mmHg increase in SBP-Av, the

increase in UaV was 2.40 mg/day in FHL rats and 0.16 mg/day in ACI rats. This again indicates that the increase per mmHg increase in SBP is 15 times higher in FHL rats than in ACI rats.

At autopsy, the incidence of FGS correlated with SBP-Av in both strains. However, the increase in FGS per mmHg was about fourfold larger in FHL (0.38 % FGS incidence per mmHg) than in ACI (0.10 % FGS incidence per mmHg). At week 11 in the FHL rats, there was a significant correlation between UaV and FGS ($r = 0.836$, $p < 0.001$). In contrast, such a correlation was not present in the ACI rats ($r = 0.321$, $p = 0.156$).

Relative heart weight was also directly related to SBP-Av in both FHL ($r = 0.810$, $p < 0.001$) and ACI ($r = 0.751$, $p < 0.001$) rats. Again the effects were more pronounced in FHL than in ACI rats. For each mmHg increase in SBP-Av, the increase in relative heart weight was 3.06 mg/100 g body weight (BW) in FHL versus 0.88 mg/100 g BW in ACI rats.

Table 4. Equations obtained from linear regression analysis between blood pressure and morphological parameters in ACI and FHL rats.

Group	n	Week	Equation	r	p
SBP and UaV					
ACI	22	3	UaV = -0.01 x SBP + 6.0	-0.129	0.566^{ns}
	22	7	UaV = 0.04 x SBP + 0.8	0.298	0.177^{ns}
	21	11	UaV = 0.12 x SBP - 12.4	0.655	0.001
FHL	41	3	UaV = 0.13 x SBP - 6.8	0.283	0.073^{ns}
	31	7	UaV = 1.44 x SBP - 173	0.543	0.002
	15	11	UaV = 1.80 x SBP - 212	0.892	<0.001
SBP-Av and UaV at week 11					
ACI	21	11	UaV11 = 0.16 x (SBP-Av) - 17.0	0.612	0.003
FHL	15	11	UaV11 = 2.40 x (SBP-Av) - 277	0.800	<0.001
SBP-Av and FGS					
ACI	22	11	FGS = 0.10 x (SBP-Av) - 11.3	0.842	<0.001
FHL	22	11	FGS = 0.38 x (SBP-Av) - 43.5	0.891	<0.001
SBP-Av and HW/100g BW (HW1)					
ACI	22	11	HW1 = 0.88 x (SBP-Av) + 152	0.751	<0.001
FHL	36	11	HW1 = 3.06 x (SBP-Av) - 66.5	0.810	<0.001

n, total number of animals included in the analysis; Week, time of measurement; r, correlation coefficient; p, p-value. Other abbreviations as in Table 1.

DISCUSSION

The present study was performed to compare the susceptibility to develop renal damage in two normotensive rat strains during chronic L-NAME-induced increases in blood pressure. Because the genetic markers flanking a known susceptibility gene in the FHH strain are identical in the FHL strain, we surmised that the FHL rat would be more susceptible to develop renal damage than would the rather renal damage resistant ACI rat after an increase in blood pressure.[15,23] Our data clearly support this hypothesis. It was demonstrated that, at similar levels of elevated blood pressure, FHL rats developed significantly more functional and structural renal damage than did the ACI rats. Furthermore, the FHL rats showed correlations between SBP and UaV or FGS that were present earlier and had steeper slopes than those calculated for the ACI rats. Using the SBP-Av instead of SBP at each time point, similar differences were observed, indicating that the same blood pressure burden caused more renal damage in the FHL than in the ACI rat.

A major limitation of this study was the high mortality of the L-NAME treated FHL rats. This is in contrast to studies using Sprague-Dawley (SD) rats, in which even higher doses of L-NAME were used.[24,25] In a previous study, we observed that unilaterally nephrectomized FHH rats also died prematurely during chronic L-NAME treatment,[26] whereas others reported a very high mortality rate in L-NAME-treated spontaneously hypertensive rats.[27] As indicated by the moderately elevated plasma creatinin levels, none of the screened FHL rats suffered from terminal renal failure at the time of death. We think that cardiac or central nervous damage due to the severe L-NAME-induced vasoconstriction in combination with the very high blood pressure was the most likely cause of premature death. Therefore it appears that, apart from the kidney, other organ systems of the FHL rat are also more sensitive to the adverse effects of nitric oxide synthase inhibition. Relative heart weight was increased in treated rats of both strains, but heart weights were significantly higher in treated FHL rats compared with treated ACI rats. Cardiac hypertrophy after chronic L-NAME treatment has also been reported by others,[28,29] although some authors claim the cardiac hypertrophy to be less than expected from the degree of blood pressure elevation.[30,31]

An increase in systemic blood pressure appears to be a universal characteristic of chronic L-NAME treatment. Since the first studies in Munich-Wistar (MW) rats,[20] a relatively large, dose dependent increase in blood pressure has been reported to occur in various strains of rats.[24-37] Micropuncture studies have shown that the elevation of systemic pressure is accompanied by an increase in intraglomerular capillary pressure.[20,26,33,37] The effects of the L-NAME-induced

hypertension on parameters of functional or structural renal damage have only occasionally been reported. In animals with two intact kidneys a relatively mild increase in UpV, UaV, or FGS, indicating a low degree of renal damage similar to that observed by us in the ACI rat, has been reported for MW[20,21,33,36] and SD rats.[24,32] However, it should be noted that a study similar to ours (*i.e.*, directly comparing various rats strains with regard to effects of L-NAME-induced hypertension on renal damage) has not been reported.

The adverse renal effects of chronic treatment with L-NAME appears to manifest when the development of renal damage is accelerated by other procedures. We reported that L-NAME worsens the renal damage in the unilaterally nephrectomized FHH rat,[26] whereas others reported similar findings in MW rats with subtotal nephrectomy.[37] In addition, it has been reported that in MW rats sodium excess aggravates both the L-NAME-induced systemic and glomerular hypertension, leading to more severe renal parenchymal injury.[33]

We conclude that, in general, FHL rats are more vulnerable than are ACI rats to L-NAME-induced complications. At similar levels of hypertension, FHL rats develop more severe functional and structural renal damage than do ACI rats. Accordingly, the present study supports our previous observation that susceptibility to renal damage in rats appears to be genetically determined.[15]

Acknowledgments

We thank Mrs. Luhrman-Schlomski, Mr. Ton Boijmans, and Mrs. Coby Peekstok for their excellent technical assistance.

This study was supported in part by Grant R01-HL56294-01 (to Drs. Jacob and Provoost) from the National Institutes of Health.

A part of this study was presented at the 11th Scientific Meeting of the American Society of Hypertension, New York, New York, May 15-18, 1996, and has been published in abstract form (*Am J Hypertens.* 1996;9:20A).

REFERENCES

1. Perneger TV, Klag MJ, Feldman HI, Whelton PK. Projections of hypertension-related renal disease in middle-aged residents of the United Kingdom. *J Am Med Assoc.* 1993;269:1272-1277.
2. Brancatie FL, Whelton PK, Whittle JC, Klag MJ. Epidemiologic analysis of existing data to investigate hypertensive renal disease: an example from the Maryland End-Stage Renal Disease Registry. *Am J Kidney Dis.* 1993;21:S15-S24.
3. Jones CA, Agodoa L. Kidney disease and hypertension in blacks: scope of the problem. *Am J Kidney Dis.* 1993;21:S6-S9.
4. McClellan W. Hypertensive end-stage renal disease in blacks: the role of end-stage disease surveillance. *Am J Kidney Dis.* 1993;21:S25-S30.
5. Freedman BI, Spray BJ, Tuttle AB, Buckalew VM. The familial risk of end-stage renal disease in African Americans. *Am J Kidney Dis.* 1993;21:387-393.
6. Freedman BI, Iskandar SS, Appel RG. The link between hypertension and nephrosclerosis. *Am J Kidney Dis.* 1995;25:207-221.
7. De Keijzer MH, Provoost AP, Molenaar JC. Proteinuria is an early marker in the development of progressive renal failure in hypertensive fawn-hooded rats. *J Hypertens.* 1989;7:525-528.
8. Provoost AP, De Keijzer MH. The fawn-hooded rat: a model of chronic renal failure, in Gretz N, Strauch M (eds): *Experimental and genetic rat models of chronic renal failure.* Basel, Karger, 1993; pp 100-114.
9. Provoost AP. Spontaneous glomerulosclerosis: Insights from the fawn-hooded rat. *Kidney Int.* 1994;45:S2-S5.
10. Feld LG, Van Liew JB, Galaske RG, Boylan JW. Selectivity of renal injury and proteinuria in the spontaneously hypertensive rat. *Kidney Int.* 1977;12:332-343.
11. Simons JL, Provoost AP, Anderson S, Troy JL, Rennke HG, Sandstrom DJ, Brenner BM. Pathogenesis of glomerular injury in the fawn-hooded rat; Early glomerular capillary hypertension predicts glomerular sclerosis. *J Am Soc Nephrol.* 1993;3:1775-1782.
12. Azar S, Johnson MA, Scheinman J, Bruno L, Tobian L. Regulation of glomerular capillary pressure and filtration rate in young Kyoto hypertensive rats. *Clin Sci.* 1979;56:203-209.
13. Dworkin LD, Feiner HD. Glomerular injury in uninephrectomized spontaneously hypertensive rats. A consequence of glomerular capillary hypertension. *J Clin Invest.* 1986;77:797-809.
14. Weening JJ, Beukers JJB, Grond J, Elema JD. Genetic factors in focal segmental glomerulosclerosis. *Kidney Int.* 1986;29:789-798.
15. 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 Gen.* 1996;12:44-51.

16. Lindpaintner K, Hilbert P, Ganten D, Nadal-Ginard B, Inagami T, Iwai N. Molecular genetics of the S_A-gene: cosegregation with hypertension and mapping to rat chromosome 1. *J Hypertens.* 1993;11:19-23.
17. Nara Y, Nabika T, Ikeda K, Sawamua M, Mano M, Endo J, Yamori Y. Basal high blood pressure cosegregates with the loci on chromosome 1 in the F_2 generation from crosses between normotensive Wistar-Kyoto rats and stroke-prone spontaneously hypertensive rats. *Biochem Biophys Res Commun.* 1993;194:1344-1351.
18. Samani NJ, Lodwick D, Vincent M, Dubay C, Kaiser MA, Kelly MP, Lo M, Haris J, Sassard J, Lathrop M, Swales JD. A gene differentially expressed in the kidney of the spontaneously hypertensive rat cosegregates with increased blood pressure. *J Clin Invest.* 1993;92:1099-1103.
19. Provoost AP, Shiozawa M, Van Dokkum RPE, Jacob HJ. Differences in phenotype and genetic make-up between four strains of fawn-hooded rats. *J Am Soc Nephrol.* 1998;9:A2003 (Abstract).
20. Baylis C, Mitruka B, Deng A. Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest.* 1992;90:278-281.
21. Ribeiro MO, Antunes E, De Nucci G, Lovisolò SM, Zatz R. Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. *Hypertension* 1992;20:298-303.
22. Marshall FF, Carcia-Bunuel R, Beisel DS. Hydronephrosis, renal agenesis and associated genitourinary anomalies in ACI rats. *Urology* 1978;11:58-61.
23. Provoost AP, Van Aken M. Renal function in aging ACI rats with congenital unilateral renal agenesis. *Pediat Nephrol.* 1991;5:C55 (Abstract).
24. Tojo A, Kobayashi N, Kimura K, et al. Effects of antihypertensive drugs on nitric oxide synthase activity in rat kidney. *Kidney Int.* 1996;49:S138-S140.
25. Garcia-Estan J, Navarro, J, Atucha N, et al. Chronic effects of nitric oxide and prostaglandin inhibition on pressure diuresis and natriuresis in rats. *Kidney Int.* 1996;49:S141-S143.
26. 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.
27. Arnal JF, Battle T, Ménard J, Michel JB. The vasodilatory effect of endogenous nitric oxide is a major counter-regulatory mechanism in the spontaneously hypertensive rat. *J Hypertens.* 1993;11:945-950.
28. Jover B, Herizi A, Ventre F, et al. Sodium and angiotensin in hypertension induced by long-term nitric oxide blockade. *Hypertension* 1993;21:944-948.
29. Böger RH, Bode-Böger SM, Gerecke U, Frölich JC. Long term administration of L-arginine, L-NAME, and exogenous NO donor molsidomine modulates urinary nitrate and cGMP excretion in rats. *Cardiovasc Res.* 1994;28:494-499.
30. Arnal JF, El Amrani AI, Chatellier G, Ménard J, Michel JB. Cardiac weight in hypertension induced by nitric oxide blockade. *Hypertension* 1993;22:380-387.

31. Lantelme P, Lo M, Sassard J. Decreased cardiac baroreflex sensitivity is not due to cardiac hypertrophy in N^G-nitro-L-arginine methyl ester-induced hypertension. *J Hypertens.* 1994;12:791-795.
32. Pollock DM, Polakowski JS, Divish BJ, Opgenorth TJ. Angiotensin blockade reverses hypertension during long-term nitric oxide synthase inhibition. *Hypertension* 1993;21:660-666.
33. Fujihara CK, Michellazo SM, De Nucci G, Zatz R. Sodium excess aggravates hypertension and renal parenchymal injury in rats with chronic NO inhibition. *Am J Physiol.* 1994;266:F697-F705.
34. Matsuoka H, Nishida H, Nomura G, Van Vliet BN, Toshima H. Hypertension induced by nitric oxide synthesis inhibition is renal nerve dependent. *Hypertension* 1994;23:971-975.
35. Bank N, Aynedjian S, Khan GA. Mechanism of vasoconstriction induced by chronic inhibition of nitric oxide in rats. *Hypertension* 1994;24:322-328.
36. Erley CM, Rebmann S, Strobel U, Schmidt T, Wehrmann M, Osswald H, Risler T. Effects of antihypertensive therapy on blood pressure and renal function in rats with hypertension due to chronic blockade of nitric oxide synthesis. *Exp Nephrol.* 1995;3:293-299.
37. Fujihara CK, DeNucci G, Zatz R. Chronic nitric oxide synthase inhibition aggravates glomerular injury in rats with subtotal nephrectomy. *J Am Soc Nephrol.* 1995;5:1498-1507.

CHAPTER 3

GENETIC DIFFERENCES DEFINE SEVERITY OF RENAL DAMAGE AFTER L-NAME-INDUCED HYPERTENSION IN RATS

Journal of the American Society of Nephrology 1998;9:363-371

Richard P.E. van Dokkum, Howard J. Jacob, and Abraham P. Provoost

ABSTRACT

Genetic factors are important in determining the susceptibility to develop renal damage. In a backcross of hypertensive and proteinuric Fawn-Hooded (FHH/EUR) with the normotensive, non-proteinuric August x Copenhagen Irish (ACI/EUR) rat, two genes were genetically mapped for parameters of functional and structural renal damage, denoted *Rf-1* and *Rf-2*. The aim of the present study was to investigate the susceptibility to develop functional and structural renal damage in heterozygous (FHH x ACI) F_1 rats compared with the parental FHH and ACI strains at similar levels of systolic blood pressure (SBP). Blood pressure elevation was induced by chronic treatment with N^{ω}-nitro-L-arginine methyl ester (L-NAME) in either a low dose (LD, 75 to 100 mg/L) or a high dose (HD, 175 to 250 mg/L) in the drinking fluid. Survival of FHH rats and, to a lesser extent, F_1 rats, was adversely affected by L-NAME treatment. All ACI rats except for one ACI-HD animal survived. In all strains, L-NAME caused a dose-dependent increase in SBP. At similar levels of SBP, the increase in functional renal damage, as indicated by the level of albuminuria (UaV), was higher in F_1 compared with ACI, but lower compared with FHH. The same differences were found for the level of structural renal damage, as indicated by the incidence of focal glomerulosclerosis (FGS). Both the SBP and the average BP burden (SBP-Av), defined as SBP averaged over the period of follow-up, directly correlated with the level of UaV and the FGS incidence in all strains. However, the increase in the degree of renal damage per mmHg increase in SBP or SBP-Av was significantly higher in the F_1 rats compared with ACI, but lower compared with FHH rats. Values for these F_1 rats were closer to the ACI rats than to values for the FHH rats and increased above an SBP level of 180 mmHg. The F_1 rats, being heterozygous for *Rf-1* and *Rf-2*, as well as for other potential genes responsible for renal disease, were largely, but not completely protected from hypertension-induced renal damage. It is concluded that complete susceptibility to hypertension-associated renal damage in rats primarily depends on the presence of predisposing genes for renal failure even after a significant increase in blood pressure.

INTRODUCTION

Genetic factors appear to be important in determining the susceptibility to renal damage. In humans, the susceptibility of a hypertensive patient to end-stage renal failure (ESRF) shows racial differences. Although African-Americans make up only 12% of the U.S. population, 28% of patients on hemodialysis are African-Americans,[1] and the rate of developing ESRF is four times greater for African-Americans than for Caucasians.[2-6] This suggests that differences in susceptibility to develop renal complications may be genetically determined.[5] The increased susceptibility to renal failure in African-American hypertensive patients is not accounted for solely by a higher prevalence of hypertension or severity of hypertension.[1]

A dissociation between hypertension and renal disease is also present in animal models of genetic hypertension. The Fawn-Hooded Hypertensive (FHH) rat, an inbred strain with a moderate level of systemic hypertension, develops progressive proteinuria and focal glomerulosclerosis (FGS) at a relatively young age, leading to premature death due to ESRD.[7-9] In contrast, the Spontaneously Hypertensive Rat (SHR) develops severe systemic hypertension, but remains without significant renal damage until late in life.[10] A striking difference between the two strains is the presence of glomerular hypertension in FHH rats,[11] whereas in SHR rats glomerular pressure is normal.[12,13]

Genetic factors influencing the development and progression of renal damage have been suggested earlier, but specific genes have not yet been found.[14] However, we have recently studied the genetics of hypertension and renal damage in the FHH rat.[15] Using a backcross of (FHH x August Copenhagen Irish [ACI]) F_1 x FHH rats, we genetically mapped three genes on chromosome 1: one for SBP, and two for renal damage in the FHH rat. The gene for SBP, denoted *Bpfh-1*, maps to the S_A region, which is also known as an important gene in determining SBP in other crosses of genetically hypertensive rats.[16-18] The genes for renal damage, denoted *Rf-1* and *Rf-2*, were found to be separate and distinct from each other. The *Rf-1* gene with the highest logarithm-of-odds scores appears to be a major gene responsible for the high susceptibility to renal damage in FHH rats.

Cross-breeding the FHH with the ACI rat results in an F_1 rat, which is heterozygous for all genes, including *Rf-1* and *Rf-2*. In the present study, we wanted to investigate the susceptibility of the heterozygous F_1 rat to hypertension-induced renal damage compared with both parental strains. Hypertension was induced by chronic administration of different doses of the nitric oxide synthase-inhibitor (NOi) N^{ω}-nitro-L-arginine methyl ester (L-NAME).[19-21]

METHODS

Animals

Experiments were conducted in accordance with Dutch laws on animal experimentation. A total of 93 animals was used, 7 wk old at the start of the study. FHH and ACI rats were derived from our own breeding colonies at Erasmus University Rotterdam (EUR). F_1 (FHH x ACI) rats were obtained by cross breeding FHH/EUR and ACI/EUR rats. Animals were housed in macrolon cages with lights on from 8 AM to 8 PM. Standard commercial rat chow containing 56% carbohydrates, 26% digestible protein, 7% fat, 4% fiber, and 5% minerals (AM II, Hope Farms, Woerden, The Netherlands) and drinking fluid (tap water, acidified to pH 3.0) was provided *ad libitum*.

Experimental groups

Animals from each strain were divided at random into three groups, *i.e.*, control rats and rats treated with either a low dose (LD) or high dose (HD) (see below). The three ACI-groups consisted initially of eight controls, seven LD-treated, and seven HD-treated rats. Data from the ACI rats were obtained from the same groups in a previous study.[21] ACI rats which presented with unilateral renal agenesis at autopsy were off line excluded from data analysis. The three FHH groups initially consisted of nine control rats, 17 LD-treated, and six HD-treated rats. From a total of 36 F_1 (FHH x ACI) rats, nine were used as controls, 17 were LD-treated, and 10 were HD-treated.

Induction of hypertension

Hypertension was induced by chronic treatment with L-NAME (Sigma-Aldrich Chemicals, Zwijndrecht, The Netherlands) dissolved in the drinking water at a concentration of either 75 to 100 mg/L (LD) or 175 to 250 mg/L (HD). Age-matched control animals were provided with normal drinking fluid. Absolute L-NAME intake of the individual rats was determined at the time of the metabolic studies. The mean doses, calculated from fluid intake and body weight, are presented in Table 1.

Systolic blood pressure

Systolic blood pressure (SBP) was measured by the tail-cuff method using a plethysmograph (IITC Life Science, Woodland Hills, CA) in awake, restrained animals, which were prewarmed for approximately 30 min by ceramic lamps to obtain proper dilation of the tail vessels. Every Thursday between noon and 2 PM, at least three consecutive measurements were recorded and averaged. To investigate the relationship between SBP over time and renal damage, we calculated the SBP average (SBP-Av) by averaging all SBP data obtained from 3 weeks until the end of follow-up.

Metabolic studies

Measurement of water and food intake and 24 hour urine collection were done at 3, 7, and 11 wk of follow-up, by placing the animals in polycarbonate metabolic cages (Tecniplast Gazzada, Buguggiate, Italy). The animals were allowed to adapt over the weekend.

Autopsy

Moribund rats, *i.e.*, rats showing a rapid decrease in body weight over several days to below 300 g, were autopsied at the time of detection, whereas surviving animals were sacrificed after the 11 week protocol. A laparotomy was performed under ethyl ether anesthesia, and a blood sample was taken from the abdominal aorta. After bleeding the animals, heart and kidneys were removed, washed with saline, and weighed. Rats that died before the end of the study period were weighed and processed if possible, *i.e.*, when obtained shortly after death.

Final glomerular filtration rate (GFR)

The creatinine clearance (C_{Cr}) calculated from the creatinine concentrations in plasma from blood obtained at autopsy and in urine from the last metabolic measurement shortly before autopsy was used as measure for GFR. C_{Cr} (ml/min) was calculated using the formula:
$C_{Cr} = (U_{Cr} \times V) / (P_{Cr} \times 1.44)$, where U_{Cr} is the creatinine level in urine (mmol/L), V is the urine excretion rate (ml/24h), and P_{Cr} the plasma creatinine level (mmol/L). To correct for differences in body weight between strains, C_{Cr} was also calculated per 100 gram body weight (C_{Cr}/100g).

Tissue processing

Kidneys were fixed by immersion for 48 hours in buffered formaldehyde solution (3.6% M/V, Lommerse Pharma, Oss, The Netherlands, pH 7.4) after longitudinal bisection. Subsequently, pieces were dehydrated in alcohol and blocked in paraffin wax. Sections of 3 μm were stained with periodic acid-Schiff and hemotoxylin and eosin counterstain.

The kidney sections were microscopically evaluated by determining the incidence of FGS as a semiquantitative injury score. For each animal, 50 glomeruli were examined in both the cortical and juxtaglomerular region, and the number of sclerotic glomeruli were counted, giving the incidence of FGS. Criteria on which glomeruli were designated sclerotic consisted of adhesion of the glomerulus to Bowman's capsule, thickening of Bowman's capsule, the presence of increased amounts of periodic acid-Schiff-positive material in the mesangial region, and folding of the glomerular basement membrane with entrapment of amorphous material. Interstitial changes and vascular damage were not assessed at that time. All sections were evaluated without knowledge of the group to which individual rats belonged.

Analytical procedures

Plasma and urinary samples were analyzed with the ELAN system (Eppendorf/Merck, Darmstadt, Germany) for the following compounds, using colorimetric assays for total protein with molybdate red, albumin with bromocresol green, and creatinine with the Jaffé method without deproteinisation.

Statistical analysis

Data are presented as mean ± SD in tables and as mean ± SE in figures. Differences in mean values between groups were compared using one-way ANOVA and a 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, differences were considered statistically significant for $p < 0.05$. The relationship between SBP or SBP-Av and parameters of functional and structural renal damage was assessed by linear regression analysis.

RESULTS

Survival

All ACI animals except one in the HD group survived throughout the experiment. In contrast, a large number of FHH rats died during the protocol. In the LD group, only three out of 15 completed the 11 wk follow-up. In the HD group, all six FHH rats died prematurely due to L-NAME-induced complications. From the F_1-LD group, all animals completed the follow-up, but from the HD group, five of ten rats died prematurely. The L-NAME-induced complications consisted of a decrease in body weight, severe vasoconstriction, and occasionally paralysis of the hind limbs, probably due to constriction of nervous system arteries. In general, animals showed signs of cardiac failure, *i.e.*, constriction of the coronary arteries and scarring of heart muscle as observed macroscopically.

Table 1. Calculated L-NAME intake in mg/kg at 3, 7, and 11 wk of treatment.

Group	n	Week 3	n	Week 7	n	Week 11
ACI-LD	7	8.1 ± 0.5	7	7.0 ± 0.7	7	7.1 ± 0.9
ACI-HD	7	18.0 ± 7.6	7	21.2 ± 5.3	6	18.1 ± 4.7
F_1-LD	17	9.1 ± 1.9	17	8.4 ± 2.6	17	7.9 ± 2.7
F_1-HD	10	16.5 ± 1.6	10	19.5 ± 3.7	5	27.2 ± 4.6
FHH-LD	17	10.8 ± 2.2	15	8.9 ± 4.2	1	16.2
FHH-HD	6	22.5 ± 5.9	4	15.4 ± 7.4		No survivors
p1/p2/p3		ns/ns/ns		ns/ns/ns		ns/-/-
p4/p5/p6		ns/s/s		ns/ns/ns		s/-/-

Values are given as mean ± SD. L-NAME, N^{ω}-L-arginine methyl ester; ACI, August x Copenhagen Irish; LD, low dose; HD, high dose; FHH, Fawn-Hooded Hypertensive; ns, not significant; s, significant ($p < 0.05$); p1, ACI-LD *vs.* F_1-LD; p2, ACI-LD *vs.* FHH-LD; p3, F_1-LD *vs.* FHH-LD; p4, ACI-HD *vs.* F_1-HD; p5, ACI-HD *vs.* FHH-HD; p6, F_1-HD *vs.* FHH-HD.

Body weight

Body weights, shown in Table 2, progressed normally over time in the ACI groups and showed no effect of L-NAME treatment. In F_1 rats, there was mild growth retardation but no deterioration in body weight in the HD-treated group over time. Body weight in control and LD-treated rats progressed normally. Body weight of the L-NAME-treated FHH rats were significantly reduced compared with control rats, although the decrease in body weight was only mild in the LD-treated group.

Systolic blood pressure and albuminuria

Values for SBP and albuminuria (UaV) obtained at 3, 7, and 11 wk are summarized in Table 2. In all three strains, a dose-dependent increase in SBP was observed. All SBP values in the L-NAME-treated rats were significantly different from the control ACI, F_1, and FHH rats throughout the entire experiment. In the F1-HD group, two of five rats could not be measured at week 11 due to vasoconstriction. In the FHH rats, measurement of the SBP became extremely difficult during the latter part of the follow-up due to the weak, or even lack of, pulsation in the tail arteries caused by severe L-NAME-induced vasoconstriction. At week 7, only eight of 15 in the FHH-LD group and none in the HD group could be measured. At week 11, two of the three remaining FHH-LD animals could not have their SBP measured for the same reason. In Table 2 and Figures 3 and 4, we have used the last measured SBP, usually obtained 1 to 2 wk earlier, to calculate the mean values and the regression equations.

Urinary protein excretion was measured simultaneously with UaV in all rats. Because changes in both parameters were almost identical, for brevity only the UaV data will be presented. All strains differed greatly in the effects of L-NAME treatment on UaV. The LD-treated ACI rats showed hardly any increase in UaV, while at the end of the follow-up in the HD-treated rats, UaV had increased to a mean value of 13 mg/day. In the F_1 rats, UaV was moderately elevated to 30 mg/day at week 7 and further increased to 94 mg/day at week 11. In contrast, a very marked increase in UaV was observed in FHH rats already after 7 wk of follow-up. At that time, the mean UaV in the LD- and HD-treated FHH rats had increased to more than 200 mg/day. After 11 wk of follow-up, the three surviving FHH-LD rats had a mean level of 269 mg/day.

Table 2. Body weight (g), systolic BP (mm Hg), and albuminuria (mg/day) in ACI, F1, and FHH rats at 3, 7, and 11 weeks of follow-up.[a]

Week 3

Group	n	BW	SBP	UaV
ACI-con	8	229 ± 17	130 ± 6	5.1 ± 1.2
ACI-LD	7	235 ± 8	159 ± 7	4.0 ± 0.7
ACI-HD	7	213 ± 6	170 ± 7	5.2 ± 0.9
p1/p2/p3		ns/s/s	s/s/s	ns/ns/ns
F_1-con	9	284 ± 28	126 ± 2	5.5 ± 1.0
F_1-LD	17	287 ± 24	155 ± 7	6.6 ± 2.8
F_1-HD	10	274 ± 22	172 ± 4	6.0 ± 1.4
p1/p2/p3		ns/ns/ns	s/s/s	ns/ns/ns
FHH-con	9	284 ± 26	144 ± 9	25 ± 18
FHH-LD	17	282 ± 23	170 ± 8	64 ± 47
FHH-HD	6	267 ± 31	187 ± 12	46 ± 45
p1/p2/p3		ns/ns/ns	s/s/s	ns/ns/ns
p4/p5/p6		s/s/ns	ns/s/s	ns/s/s
p7/p8/p9		s/s/ns	ns/s/s	ns/ns/ns
p10/p11/p12		s/s/ns	ns/s/s	ns/ns/ns

Week 7

Group	n	BW	SBP	UaV
ACI-con	8	254 ± 22	119 ± 5	4.8 ± 1.4
ACI-LD	7	259 ± 12	169 ± 9	5.2 ± 1.1
ACI-HD	7	242 ± 7	172 ± 9	10.1 ± 3.7
p1/p2/p3		ns/ns/ns	s/s/ns	ns/s/s
F_1-con	9	315 ± 41	125 ± 5	5.6 ± 1.3
F_1-LD	17	327 ± 26	186 ± 13	9.5 ± 5.2
F_1-HD	10	300 ± 19	201 ± 16	30 ± 25
p1/p2/p3		ns/ns/ns	s/s/s	ns/s/s
FHH-con	9	330 ± 25	143 ± 8	43 ± 25
FHH-LD	15	278 ± 41	209 ± 20[c]	211 ± 89
FHH-HD	4	241 ± 15	210 ± 10[e]	223 ± 27
p1/p2/p3		s/s/s	s/s/-	s/s/ns
p4/p5/p6		s/ns/s	s/s/s	ns/s/s
p7/p8/p9		s/ns/s	s/-/-	ns/s/s
p10/p11/p12		s/s/s	ns/s/s	ns/ns/ns

Table 2 (continued)

Week 11

Group	n	BW	SBP	UaV
ACI-con	8	276 ± 24	128 ± 7	3.8 ± 0.7
ACI-LD	7	278 ± 15	165 ± 9	6.3 ± 2.3
ACI-HD	6	264 ± 70	206 ± 18	12.7 ± 9.0
p1/p2/p3		ns/ns/ns	s/s/s	s/s/ns
F_1-con	9	356 ± 31	134 ± 5	6.9 ± 1.1
F_1-LD	17	359 ± 25	187 ± 16	31 ± 40
F_1-HD	5	299 ± 21	210 ± 16[b]	94 ± 31
p1/p2/p3		ns/s/s	s/s/s	ns/s/s
FHH-con	9	348 ± 25	151 ± 5	54 ± 32
FHH-LD	3	267 ± 35	216 ± 1[d]	269 ± 46
FHH-HD		No survivors		
p1/p2/p3		s	s	s
p4/p5/p6		s/ns/s	s/s/s	ns/s/s
p7/p8/p9		ns/-/-	ns/-/-	s/-/-
p10/p11/p12		s/s/ns	ns/s/s	ns/s/s

[a] Values are given ± SD. p1, LD *vs.* con; p2, HD *vs.* con; p3, LD *vs.* HD; p4, ACI-LD *vs.* F_1-LD; p5, ACI-LD *vs.* FHH-LD; p6, F_1-LD *vs.* FHH-LD; p7, ACI-HD *vs.* F_1-HD; p8, ACI-HD *vs.* FHH-HD; p9, F_1-HD *vs.* FHH-HD; p10, ACI-con *vs.* F_1-con; p11, ACI-con *vs.* FHH-con; p12, F_1-con *vs.* FHH-con; s, $p < 0.05$; ns, not significant ($p > 0.05$).

[b] At week 11, SBP could be obtained in 2 rats, only; for the other three rats, the last measured SBP obtained 1 wk earlier was used

[c] At week 7, SBP could be obtained in 8 rats, only; for the other seven rats, the last measured SBP (1 to 2 wk earlier) was used.

[d] At week 11, SBP could be obtained in one rat, only; for the other two rats, the last measured SBP obtained 3 wk earlier was used.

[e] At week 7, SBP could not be measured; data obtained at week 6 were used.

Autopsy findings

Body and organ weights of autopsied animals are summarized in Table 3. In ACI rats, treatment with L-NAME had little effect on body weight and wet kidney weight. Both absolute (not shown) and relative heart weight in the ACI-HD group were significantly increased when compared with those of the ACI-LD and ACI control rats. Total kidney weight (not shown) was about the same in all three groups. In the F_1 rats, body weights were significantly lower in HD- *vs.* LD-treated and controls. The same applies for the relative total kidney weight. The relative heart weight was significantly increased in both treated groups compared with controls. In the FHH rats, body weight was severely reduced by L-NAME treatment. Relative kidney weight was significantly increased in FHH-LD and FHH-HD rats compared with FHH control rats. In combination with the lower body weights of the treated rats, the differences in relative heart weight were even more pronounced.

Table 3. Body, kidney and heart weights and incidence of glomerulosclerosis at autopsy after 11 weeks of follow-up or at premature death.[a]

Group	n	BW (g)	TKW/100g	FGS (%)	HW/100g
ACI-con	8	279 ± 12	701 ± 27	0.9 ± 0.6	265 ± 14
ACI-LD	7	272 ± 16	658 ± 21	3.7 ± 1.3	279 ± 11
ACI-HD	7	261 ± 18	717 ± 88	7.0 ± 1.5	321 ± 17
p1/p2/p3		ns/s/ns	s/ns/ns	s/s/s	ns/s/s
F_1-con	9	362 ± 30	719 ± 31	2.0 ± 1.6	261 ± 11
F_1-LD	17	365 ± 32	698 ± 33	7.3 ± 4.4	295 ± 25
F_1-HD	8	265 ± 27	940 ± 98	19.5 ± 3.8	394 ± 31
p1/p2/p3		ns/s/s	ns/s/s	s/s/s	s/s/s
FHH-con	9	342 ± 25	714 ± 26	4.2 ± 1.5	321 ± 59
FHH-LD	16	245 ± 24	1016 ± 42	31.4 ± 7.0	466 ± 55
FHH-HD	4	240 ± 12	1123 ± 98	51.0 ± 5.7	451 ± 10
p1/p2/p3		s/s/ns	s/s/s	s/s/s	s/s/ns
p4/p5/p6		s/ns/s	ns/s/s	ns/s/s	ns/s/s
p7/p8/p9		ns/ns/ns	s/s/s	s/s/s	s/s/s
p10/p11/p12		s/s/ns	ns/ns/ns	ns/ns/ns	ns/s/s

[a] Values are given as mean ± SD. TKW/100g, TKW per 100 g BW; HW/100g, wet HW per 100 g BW; p1, LD *vs.* con; p2, HD *vs.* con; p3, LD *vs.* HD; p4, ACI-LD *vs.* F_1-LD; p5, ACI-LD *vs.* FHH-LD; p6, F_1-LD *vs.* FHH-LD; p7, ACI-HD *vs.* F_1-HD; p8, ACI-HD *vs.* FHH-HD; p9, F_1-HD *vs.* FHH-HD; p10, ACI-con *vs.* F_1-con; p11, ACI-con *vs.* FHH-con; p12, F_1-con *vs.* FHH-con; s, $p < 0.05$; ns, not significant ($p > 0.05$)

Focal glomerulosclerosis

The incidence of FGS presented in Table 3 indicates a dose-dependent increase in structural renal damage in all strains. However, the increase in L-NAME-treated ACI rats was much less marked than that observed in both groups of treated F_1 and FHH rats. The most severe structural damage was observed in the treated FHH rats. It must be stated that FGS of FHH controls was also higher compared with ACI and F_1 control rats.

Creatinine clearance

The final plasma creatinine and calculated C_{Cr} values, as measure for GFR, are presented in Table 4. The mean C_{Cr} values in L-NAME-treated ACI rats were not significantly different from controls. In contrast, mean C_{Cr} was higher in control and treated F_1 rats compared with ACI rats. However, C_{Cr} in the F_1-HD was severely decreased compared with control and LD-treated rats, indicative of a deteriorating GFR. In the FHH control rats, C_{Cr} was higher compared with F_1 and ACI control and treated rats. FHH rats treated with L-NAME all showed a significantly lower C_{Cr} compared with control rats and ACI and F_1 control and treated rats. Differences are even greater when C_{Cr} per 100 gram body weight (C_{Cr}/100g, Table 4) is considered.

Table 4. Final plasma creatinine and calculated creatinine clearances. [a]

Group	n	P_{Cr}	U_{Cr} x V	C_{Cr}	C_{Cr}/100g
ACI-con	8	46.3 ± 4.4	91.5 ± 21.1	1.39 ± 0.35	0.50 ± 0.11
ACI-LD	7	51.7 ± 5.3	103.2 ± 9.7	1.40 ± 0.19	0.52 ± 0.06
ACI-HD	6	49.3 ± 6.7	99.4 ± 5.9	1.44 ± 0.32	0.54 ± 0.11
p1/p2/p3		ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns
F_1-con	9	46.4 ± 2.2	125.9 ± 15.9	1.88 ± 0.20	0.52 ± 0.02
F_1-LD	17	51.3 ± 8.0	137.8 ± 17.2	1.91 ± 0.35	0.52 ± 0.06
F_1-HD	5	124.0 ± 62.9	111.4 ± 21.6	0.78 ± 0.34	0.28 ± 0.10
p1/p2/p3		ns/s/s	ns/ns/s	ns/s/s	ns/s/s
FHH-con	9	40.0 ± 5.7	123.8 ± 8.5	2.18 ± 0.26	0.64 ± 0.07
FHH-LD	9	83.1 ± 24.4	87.8 ± 21.6	0.80 ± 0.29	0.31 ± 0.09
FHH-HD	2	129.5 ± 17.5	75.2 ± 4.1	0.41 ± 0.04	0.17 ± 0.03
p1/p2/p3		s/s/s	s/s/ns	s/s/ns	s/s/ns
p4/p5/p6		ns/s/s	s/ns/s	s/s/s	ns/s/s
p7/p8/p9		s/s/ns	ns/ns/ns	s/s/ns	s/s/ns
p10/p11/p12		ns/ns/ns	s/s/ns	s/s/ns	ns/s/s

[a] Values are given as mean ± SD. P_{Cr}, plasma creatinine level (μmol/L), U_{Cr} x V, urine creatinine excretion (mmol/24 h); C_{Cr}, creatinine clearance (ml/min); C_{Cr}/100g, creatinine clearance per 100 gram body wt. Other abbreviations as in Tables 1 and 2. p1, LD *vs.* con; p2, HD *vs.* con; p3, LD *vs.* HD; p4, ACI-LD *vs.* F_1-LD; p5, ACI-LD *vs.* FHH-LD; p6, F_1-LD *vs.* FHH-LD; p7, ACI-HD *vs.* F_1-HD; p8, ACI-HD *vs.* FHH-HD; p9, F_1-HD *vs.* FHH-HD; p10, ACI-con *vs.* F_1-con; p11, ACI-con *vs.* FHH-con; p12, F_1-con *vs.* FHH-con; s, $p < 0.05$; ns, not significant ($p > 0.05$).

Comparison of groups with similar SBP levels

Because we wanted to relate the development of renal damage in groups with approximately the same blood pressure levels, we compared the UaV and GS in the ACI-HD and F_1-HD, and the FHH-LD groups. All three groups developed hypertension, the final SBP level being approximately 210 mmHg. The ACI rats, however, showed a more gradual rise than the F_1 and FHH rats (Table 2).

Figure 1 shows the changes in UaV in the three groups. FHH-LD rats already developed UaV after 3 wk of treatment, progressing with time to approximately 270 mg/day at week 11. ACI-HD rats developed no increase in UaV during the study. F_1 rats showed intermediate responses. At week 7, there was an increase from 30 to 95 mg/day at week 11.

Figure 2 shows that at autopsy, the incidence of FGS was significantly higher in the FHH-LD rats compared with the ACI- and F_1-HD rats. Hypertensive ACI-HD rats showed only a moderate degree of FGS, with F_1-HD rats being intermediate.

This indicates that the structural renal damage at similar SBP levels is also more pronounced in FHH rats than in ACI and F_1 rats.

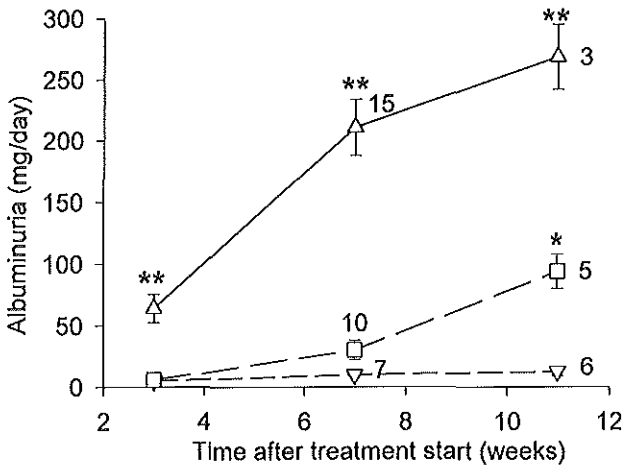

Figure 1. Urinary albumin excretion (UaV, mg/day) of August x Copenhagen Irish-high dose (ACI-HD, ∇) rats, Fawn-Hooded Hypertensive low-dose (FHH-LD, Δ) rats, and F_1-HD (□) rats at 3, 7, and 11 weeks of follow-up. Numbers at the different markers represent the number of rats that were measured. Values are given as mean ± SE. * $p < 0.05$, F_1-HD rats *vs.* ACI-HD rats, ** $p < 0.05$, FHH-LD rats *vs.* ACI and F_1-HD rats.

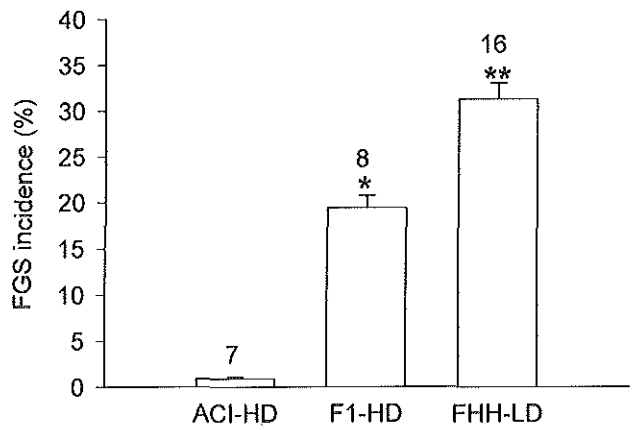

Figure 2. The incidence of focal glomerulosclerosis (FGS) in ACI-HD, F_1-HD, and FHH-LD rats. Values are given as mean ± SEM. Numbers above the bars indicate the number of animals that were evaluated. * $p < 0.05$, F_1-HD rats *vs.* ACI-HD rats, ** $p < 0.05$, FHH-LD rats *vs.* ACI and F_1-HD rats.

Comparison of the three strains by regression analysis

We also compared the three strains by examining the relationships between SBP and the parameters for functional (*i.e.*, UaV) and structural (*i.e.*, incidence of FGS) renal damage. Figures 3 and 4 show the scatter plots of the relationship between SBP and UaV in the three strains at weeks 7 and 11 of the follow-up, respectively. In the ACI rats, the correlation between SBP and UaV became significant only at week 11. In the FHH and F_1 rats, statistically significant correlations between SBP and UaV were present at weeks 7 and 11 of the follow-up. Slopes of the regression lines differed between the three strains. At week 7, the rise in UaV was 0.04 mg/day per mmHg in the ACI rats, 0.2 mg in the F_1 rats, and 2.0 mg in the FHH rats (Figure 3). Thus, already at week 7, increase in UaV per mmHg increase in SBP in FHH rats was approximately 50 times higher than in ACI and approximately 10 times higher than in F_1 rats. The F_1 rats showed an increase in albumin loss five times higher than the ACI rats.

When comparing the equations obtained at week 11, as shown in Figure 4, it is clear that the rise in UaV per mmHg increase in SBP was highest in the FHH rats, *i.e.*, 3.1 mg/day. The rise was only 0.12 in the ACI rats. The F_1 rats showed a biphasic distribution pattern. Up to an SBP level of 180 mmHg, UaV increased only with 0.2 mg per mmHg increase in SBP, which was similar to the situation at week 7. Above the SBP level of 180 mmHg, the increase was eight times more, up to 1.7 mg/day. These data indicate that F_1 rats were protected against the development of severe UaV up to an SBP level of 180 mmHg.

Comparing the UaV obtained at week 11 with SBP-Av, *i.e.*, the SBP averaged over the entire follow-up period, showed a similar pattern. For each mmHg increase in SBP-Av, the increase in UaV was 3.4 mg/day in FHH, 0.9 mg/day in F_1, and 0.2 mg/day in ACI rats.

At autopsy, the incidence of FGS correlated with SBP-Av in all strains. However, the increase in FGS per mmHg in FHH (FGS = 0.7 x SBP-Av - 92, $n = 29$, $r = 0.886$, $p < 0.001$) was sevenfold larger than in ACI (FGS = 0.1 x SBP-Av - 11, $n = 22$, $r = 0.842$, $p < 0.001$) and approximately fourfold larger than in F_1 (FGS = 0.2 x SBP-Av - 25, $n = 34$, $r = 0.727$, $p < 0.01$). The increase was twice as much in the F_1 rats compared with the ACI rats. Significant correlations were also present between the incidence of FGS and UaV at week 11 in FHH ($r = 0.851$, $p < 0.001$), and F_1 rats ($r = 0.836$, $p < 0.001$). Such a correlation was absent in the ACI rats ($r = 0.321$, $p = 0.156$).

Relative heart weight was also directly related to SBP-Av in FHH ($r = 0.668$, $p < 0.001$), F_1 ($r = 0.741$, $p < 0.001$), and ACI ($r = 0.751$, $p < 0.001$) rats. For each mmHg increase in SBP-Av, the increase in relative heart weight was 2.5 mg/100 g BW in FHH rats, 1.5 mg/100 g BW in F_1 rats, and 0.9 mg/100 g BW in ACI rats.

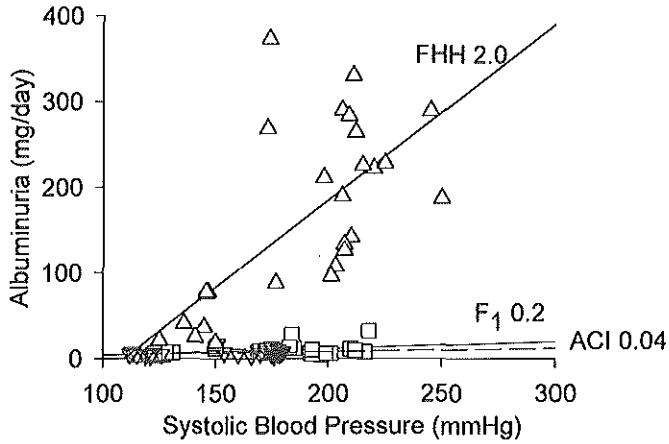

Figure 3. Relationship between systolic blood pressure (SBP) and UaV at week 7. Slopes of the regression lines are indicated next to the different lines. ACI-HD (▽); F_1-HD (□); FHH-LD (△). See also the legend of Table 2. Equations: ACI, UaV = 0.04 x SBP + 0.8 (n = 22, r = 0.298, p = 0.177); F_1, UaV = 0.2 x SBP - 15.9 (n = 36, r = 0.329, p = 0.05); FHH, UaV = 2.0 x SBP - 215 (n = 28, r = 0.660, p < 0.001).

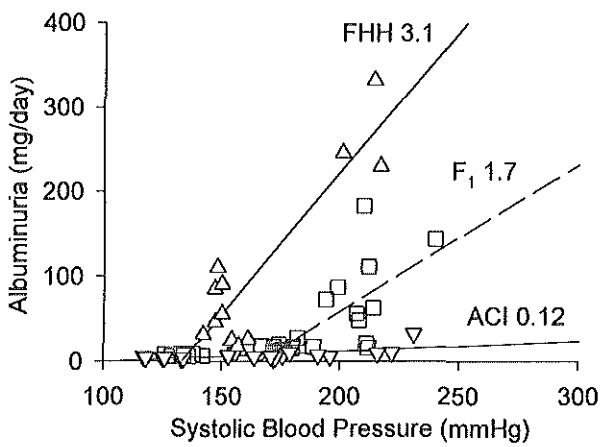

Figure 4. Relationship between systolic blood pressure (SBP) and UaV at week 11. Slopes of the regression lines are indicated next to the different lines. ACI-HD (▽); F_1-HD (□); FHH-LD (△). See also the legend of Table 2. Equations: ACI, UaV = 0.12 x SBP - 12.4 (n = 21, r = 0.655, p = 0.001); F_1, UaV = 0.2 x SBP - 22.0 (first part, n = 21, r = 0.814, p < 0.001), and UaV = 1.7 x SBP - 228 (last part, n = 22, r = 0.708, p < 0.001); FHH, UaV = 3.1 x SBP - 411 (n = 12, r = 0.896, p < 0.001).

DISCUSSION

The present study was performed to compare the development of renal damage in the presence of L-NAME-induced increases in blood pressure in two rat strains differing in susceptibility and in the progeny of a cross between these strains. The presented data clearly support the hypothesis that in the rats studied, the susceptibility to develop renal damage depends on genetic background. It was demonstrated that at similar blood pressure levels, FHH rats developed more severe functional and structural renal damage than did the ACI rats. (FHH x ACI) F_1 rats showed intermediate responses, but phenotypically closer to ACI than to FHH rats. The ACI rats were largely protected from developing marked UaV up to an SBP level of 225 mmHg. The F_1 rats were protected to a level of approximately 180 mmHg, whereas FHH rats were not protected at all. Furthermore, the FHH and F_1 rats showed correlations between SBP and UaV or FGS that were present earlier and had steeper slopes than those in the ACI rats, with the F_1 rats again being intermediate. Using the SBP-Av instead of SBP at each time point, similar differences were observed, indicating that a similar blood pressure burden over the treatment period caused more renal damage in the FHH and the F_1 than in the ACI rat.

A major drawback was the high mortality of the L-NAME treated FHH rats. In previous studies we observed that unilaterally nephrectomized FHH rats and FHL rats with two kidneys also died prematurely during chronic L-NAME treatment.[21,22] Others reported a very high mortality rate in L-NAME treated SHR rats.[23] A decrease in GFR, indicated by a decrease in C_{Cr}, was found in the F_1-HD rats and both treated FHH groups. However, none of the screened FHH and F_1 rats that died before the end of the study did so because of terminal renal failure. We think that ischemic cardiac or central nervous damage due to the severe L-NAME induced vasoconstriction, in combination with the high blood pressure, would be the most likely cause of the premature death. Thus, apart from the kidney, other organ systems of heterozygous rats, are also more sensitive to the adverse effects of nitric oxide synthase inhibition. Relative heart weight was increased in treated rats of all strains, but heart weights were higher in treated FHH and F_1 rats compared with treated ACI rats, being most pronounced in FHH rats. Cardiac hypertrophy after chronic L-NAME treatment has also been reported by others[24,25], although some authors found the increase in cardiac weight to be relatively mild.[26,27]

An increase in SBP appears to be a universal characteristic of chronic L-NAME treatment, because it has been reported to occur in various rat strains.[19-33] Micropuncture studies have shown that the elevation of systemic pressure is accompanied by an increase in intraglomerular capillary pressure.[19,22,29,33] The effects of the L-NAME-induced increase in blood pressure on urinary protein or

albumin excretion or on structural renal damage have only occasionally been reported. However, in our previous study in Fawn-Hooded Low blood pressure (FHL) rats, we showed that renal damage occurs upon blood pressure elevation.[21] This strain is also susceptible to renal damage, and multiple gene interaction is likely to be involved. A relatively mild increase in proteinuria, UaV, or FGS, indicating a relative mild degree of renal damage similar to that observed by us in the ACI rat, has been reported after investigating Munich-Wistar (MW)[19,20,29,32] or Sprague-Dawley rats[28] with two intact kidneys. However, it should be noted that a study directly comparing various rats strains with regard to effects of L-NAME-induced hypertension on renal damage, such as the one presented here, has not been reported.

The effects of chronic treatment with L-NAME appear to be more distinct in rats with reduced renal mass or other models of renal damage. It has been reported that L-NAME worsens renal damage in the unilaterally nephrectomized FHH rat[22] and in MW rats after subtotal nephrectomy.[33] In addition, it has been reported that in MW rats, sodium excess aggravates both the L-NAME-induced systemic and glomerular hypertension, leading to more severe renal parenchymal injury.[29]

In conclusion, F_1 rats, which are heterozygous for renal failure genes, are partially protected from hypertension-associated renal damage. At similar levels of hypertension, (FHH x ACI) F_1 rats are more vulnerable than ACI rats to L-NAME-induced complications, but less susceptible than the parental FHH rats. The present data support our previous observation that susceptibility to renal damage in rats is genetically determined.[15]

Acknowledgments

This work was supported in part by the National Institutes of Health Grant RO1-HL56284-01 (to Drs. H.J. Jacob and A.P. Provoost). Parts of this study were presented at the 29th Scientific Meeting of the *American Society of Nephrology*, New Orleans, LA, November 1996, and the 12th Scientific Meeting of the *American Society of Hypertension*, San Francisco, CA, May 1997, and have been published in abstract form (*J Am Soc Nephrol* 1996;7:1866 and *Am J Hypertens* 1997;10:13A.

REFERENCES

1. The USRDS 1996 Annual Report (Excerpts): Incidence and prevalence of ESRD. *Am J Kidney Dis.* 1996;28:S34-S47.
2. Brancatie FL, Whelton PK, Whittle JC, Klag MJ. Epidemiologic analysis of existing data to investigate hypertensive renal disease: an example from the Maryland End-Stage Renal Disease Registry. *Am J Kidney Dis.* 1993;21:S15-S24.
3. Jones CA, Agodoa L. Kidney disease and hypertension in blacks: scope of the problem. *Am J Kidney Dis.* 1993;21:S6-S9.
4. McClellan W. Hypertensive end-stage renal disease in blacks: the role of end-stage disease surveillance. *Am J Kidney Dis.* 1993;21:S25-S30.
5. Freedman BI, Spray BJ, Tuttle AB, Buckalew VM. The familial risk of end-stage renal disease in African-Americans. *Am J Kidney Dis.* 1993;21:387-393.
6. Freedman BI, Iskandar SS, Appel RG. The link between hypertension and nephrosclerosis. *Am J Kidney Dis.* 1995;25:207-221.
7. De Keijzer MH, Provoost AP, Molenaar JC. Proteinuria is an early marker in the development of progressive renal failure in hypertensive fawn-hooded rats. *J Hypertens.* 1989;7:525-528.
8. Provoost AP, De Keijzer MH. The fawn-hooded rat: a model of chronic renal failure. In: Experimental and genetic rat models of chronic renal failure, edited by Gretz N, Strauch M, Basel, Karger, 1993, pp 100-114
9. Provoost AP. Spontaneous glomerulosclerosis: Insights from the fawn-hooded rat. *Kidney Int.* 1994;45:S2-S5.
10. Feld LG, Van Liew JB, Galaske RG, Boylan JW. Selectivity of renal injury and proteinuria in the spontaneously hypertensive rat. *Kidney Int.* 1977;12:332-343.
11. Simons JL, Provoost AP, Anderson S, Troy JL, Rennke HG, Sandstrom DJ, Brenner BM. Pathogenesis of glomerular injury in the fawn-hooded rat; Early glomerular capillary hypertension predicts glomerular sclerosis. *J Am Soc Nephrol.* 1993;3:1775-1782.
12. Azar S, Johnson MA, Scheinman J, Bruno L, Tobian L. Regulation of glomerular capillary pressure and filtration rate in young Kyoto hypertensive rats. *Clin Sci.* 1979;56:203-209.
13. Dworkin LD, Feiner HD. Glomerular injury in uninephrectomized spontaneously hypertensive rats. A consequence of glomerular capillary hypertension. *J Clin Invest.* 1986;77:797-809.
14. Weening JJ, Beukers JJB, Grond J, Elema JD. Genetic factors in focal segmental glomerulosclerosis. *Kidney Int.* 1986;29:789-798.
15. 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 Gen.* 1996;12:44-51.
16. Lindpaintner K, Hilbert P, Ganten D, Nadal-Ginard B, Inagami T, Iwai N. Molecular genetics of the S_A-gene: cosegregation with hypertension and mapping to rat chromosome 1. *J Hypertens.* 1993;11:19-23.

17. Nara Y, Nabika T, Ikeda K, Sawamua M, Mano M, Endo J, Yamori Y. Basal high blood pressure cosegregates with the loci on chromosome 1 in the F_2 generation from crosses between normotensive Wistar-Kyoto rats and stroke-prone spontaneously hypertensive rats. *Biochem Biophys Res Commun.* 1993;194:1344-1351.
18. Samani NJ, Lodwick D, Vincent M, Dubay C, Kaiser MA, Kelly MP, Lo M, Haris J, Sassard J, Lathrop M, Swales JD. A gene differentially expressed in the kidney of the spontaneously hypertensive rat cosegregates with increased blood pressure. *J Clin Invest.* 1993;92:1099-1103.
19. Baylis C, Mitruka B, Deng A. Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest.* 1992;90:278-281.
20. Ribeiro MO, Antunes E, De Nucci G, Lovisolo SM, Zatz R. Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. *Hypertension* 1992;20:298-303.
21. Van Dokkum RPE, Jacob HJ, Provoost AP. Difference in susceptibility of developing renal damage in normotensive Fawn-Hooded (FHL) and August x Copenhagen Irish (ACI) rats after N^{ω}-L-arginine methyl ester induced hypertension. *Am J Hypertens.* 1997;10:1109-1116.
22. 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.
23. Arnal JF, Battle T, Ménard J, Michel JB. The vasodilatory effect of endogenous nitric oxide is a major counter-regulatory mechanism in the spontaneously hypertensive rat. *J Hypertens.* 1993;11:945-950.
24. Jover B, Herizi A, Ventre F, Dupont M, Mimran A. Sodium and angiotensin in hypertension induced by long-term nitric oxide blockade. *Hypertension* 1993;21:944-948.
25. Böger RH, Bode-Böger SM, Gerecke U, Frölich JC. Long term administration of L-arginine, L-NAME, and exogenous NO donor molsidomine modulates urinary nitrate and cGMP excretion in rats. *Cardiovasc Res.* 1994;28:494-499.
26. Arnal JF, El Amrani AI, Chatellier G, Ménard J, Michel JB. Cardiac weight in hypertension induced by nitric oxide blockade. *Hypertension* 1993;22:380-387.
27. Lantelme P, Lo M, Sassard J. Decreased cardiac baroreflex sensitivity is not due to cardiac hypertrophy in N^{G}-nitro-L-arginine methyl ester-induced hypertension. *J Hypertens.* 1994;12:791-795.

28. Pollock DM, Polakowski JS, Divish BJ, Opgenorth TJ. Angiotensin blockade reverses hypertension during long-term nitric oxide synthase inhibition. *Hypertension* 1993;21:660-666.
29. Fujihara CK, Michellazo SM, De Nucci G, Zatz R. Sodium excess aggravates hypertension and renal parenchymal injury in rats with chronic NO inhibition. *Am J Physiol.* 1994;266:F697-F705.
30. Matsuoka H, Nishida H, Nomura G, Van Vliet BN, Toshima H. Hypertension induced by nitric oxide synthesis inhibition is renal nerve dependent. *Hypertension* 1994;23:971-975.
31. Bank N, Aynedjian S, Khan GA. Mechanism of vasoconstriction induced by chronic inhibition of nitric oxide in rats. *Hypertension* 1994;24:322-328.
32. Erley CM, Rebmann S, Strobel U, Schmidt T, Wehrmann M, Osswald H, Risler T. Effects of antihypertensive therapy on blood pressure and renal function in rats with hypertension due to chronic blockade of nitric oxide synthesis. *Exp Nephrol.* 1995;3:293-299.
33. Fujihara C, DeNucci G, Zatz R. Chronic nitric oxide synthase inhibition aggravates glomerular injury in rats with subtotal nephrectomy. *J Am Soc Nephrol.* 1995;5:1498-1507.

CHAPTER 4

BLOOD PRESSURE AND THE SUSCEPTIBILITY TO RENAL DAMAGE AFTER UNILATERAL NEPHRECTOMY AND L-NAME-INDUCED HYPERTENSION IN RATS

Submitted for publication

Richard P.E. van Dokkum, Howard J. Jacob, and Abraham P. Provoost

ABSTRACT

Fawn-Hooded Hypertensive (FHH) rats carry several genes which determine the susceptibility to develop renal damage in contrast to the renal damage resistant August x Copenhagen Irish (ACI) rats. Kidneys from F_1 (FHH x ACI) rats, being heterozygous for the renal susceptibility genes, appeared to be largely, but not completely protected when blood pressure was increased with N^{ω}-nitro-L-arginine methyl ester (L-NAME). In the present experiment, we examined the role of an increased hemodynamic burden on the development of renal damage by 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. Therefore, the rats were simultaneously treated with L-NAME and the angiotensin converting enzyme inhibitor, lisinopril.

Surgery was performed at the age of 15 weeks and L-NAME treatment (50 or 150 mg/L) started immediately thereafter. 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). A dose-dependent hypertension developed in the L-NAME treated rats of both strains. At similar L-NAME intake, F_1 rats developed more severe hypertension and more UaV than ACI rats. Furthermore, the increase in UaV per mmHg increase in SBP at was fivefold higher in F_1 compared with ACI rats. A significant relationship between functional and structural renal damage was present in both strains. However, in F_1 rats, the increase in UaV per % incidence increase in FGS was three times higher. In lisinopril 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 depending on the activation of the renin-angiotensin system.

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.

INTRODUCTION

The Fawn-Hooded Hypertensive (FHH) rat is a unique model of hypertension-associated renal failure.[1,2] 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.[3,4] In the FHH rat, renal damage is greatly enhanced by unilateral nephrectomy (UNX),[5,6] a procedure that will further increase the hemodynamic burden to the glomeruli. A linear relationship was found between systolic blood pressure (SBP) 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.[4]

Genetic factors influencing the development and progression of renal damage are apparent in FHH rats. Using a backcross of (FHH x August Copenhagen Irish [ACI]) F_1 x FHH rats, we mapped two quantitative trait loci (QTLs) on chromosome 1, influencing proteinuria and structural renal damage.[7] 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 x ACI) F_2 progeny.[8] 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 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 inhibition (NOi) with L-NAME on the development of renal damage in F_1 (FHH x ACI) rats and in both parental strains with two kidneys. It was shown that despite similar SBP levels, severe UaV and FGS were present in FHH rats, while ACI rats hardly developed any renal damage.[9] 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, 6 and 12 weeks after UNX and L-NAME-induced hypertension were assessed in F_1 (FHH x ACI) and ACI rats. At

12 weeks after surgery, rats were sacrificed, creatinine clearance was measured, and morphologic 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[9], especially in combination with UNX.[6]

A second study was carried out under a similar protocol to exclude the renal damage from 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 level. The SBP and UaV levels were measured 12, 18, and 24 weeks after UNX in control and treated groups.

METHODS

Experimental animals

A total of 55 animals was used in study 1, and a total of 25 animals was 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 x ACI) rats were specially bred for the study. All rats were housed under standard conditions in the Animal Facilities of the Erasmus University Medical School, which is approved by the Dutch Veterinary Inspection. They had free access to food and water throughout the study. The protocol received approval from the animal ethical committee of the Erasmus University.

Unilateral nephrectomy

Laparotomy was carried out under ethyl ether (Sigma Chemical Co., St. Louis, MO) anesthesia, administered through an evaporation system connected to an airtight box. The right kidney was carefully separated from the adrenal gland and the surrounding connective tissue. The renal artery and vein, as well as the ureter, were ligated with a 4.0 silk suture (Braun AG, Melsungen, Germany) and cut. As previously reported, ACI rats presented with congenital unilateral agenesis (URA), in about 20% of the cases.[10] All ACI rats were checked for this anomaly and the rats that presented with URA were excluded from analysis. For analgetic purposes, rats post-operatively received a subcutaneous injection of 1:1 diluted fentanyl (Hypnorm®, Janssen Cilag Ltd., Saunderton, England) at a dose of 5 mg/kg and were allowed to recover from surgery in a warmed cage for 1 to 2 hours. Treatment was started after the rats had recovered for 3 to 4 days.

Study 1

Chronic nitric-oxide inhibition was induced by treatment with N^{ω}-nitro-L-arginine methyl ester (L-NAME, Sigma-Aldrich Chemicals, Zwijndrecht, The Netherlands) dissolved in the drinking water at a concentration of 50 or 150 mg/L, based on previous experiments.[9,11] 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, ten rats treated with 50 mg L-NAME, and ten rats treated with 150 mg L-NAME. The ACI groups (n=26) consisted of ten control rats, six rats treated with 50 mg L-NAME, and ten 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.

Study 2

Blood pressure was maintained at low levels by chronic ACEi, using lisinopril (Novatec®, Merck Sharp & Dome, Haarlem, The Netherlands) which 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.[12] 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 control (con) 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 lisinopril directly after UNX 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 (Table 1).

SBP and metabolic measurements

SBP was measured by the tail-cuff method, using a photoelectric oscillatory detection device (IITC Life Science, Woodland Hills, CA), as previously described.[9,11] The week before the measurement, animals were allowed to adapt to the equipment and the procedure to minimize stress-induced artifacts. Although SBP was measured every three weeks after UNX, only the values obtained at the time of the metabolic measurements will be presented. Urine excretion, food and water intake were determined gravimetrically at 6 and 12 weeks after UNX in study 1 and 12, 18, and 24 weeks after UNX in study 2, by housing the animals in metabolic cages (Tecniplast Gazzada, Buggugiate, Italy), as described previously.[9,11]

Terminal procedure

Shortly after the last metabolic measurement, rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (Nembutal®, Sanofi Sante BV, Maassluis, The Netherlands, 50 mg/kg). A 21 gauge needle (B. Braun AG, Melsungen, Germany) was introduced into the dorsal aorta at the bifurcation of the right and left iliac artery and a blood sample was taken. After sampling of the blood, the abdominal aorta was ligated above the renal arteries and the kidney was flushed with sterile 0.9% NaCl (Baxter BV, Utrecht, The Netherlands) containing 50 IU/L heparin (Leo Pharmaceutical Products, Weesp, The Netherlands) for 2 min after cutting the renal vein. The kidney was subsequently perfused for approximately 5 min with buffered formaldehyde solution (3.6 % M/V Lommerse Pharma, Oss, The Netherlands, pH 7.4). Both solutions were perfused at pressures similar to the SBP that was obtained at the last tail-cuff measurement. Both the heart and the kidney were removed and weighed. Animals that died prematurely were necropsied within 12 h and included in the structural damage analysis.

Terminal analysis

Processing of kidneys and the criteria for the assessment of the incidence of FGS, and methods and calculations for final creatinine clearance as a measure of glomerular filtration rate (GFR) were performed as previously described.[9,11]

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 ± SE in the figures and as mean ± SD in the 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 done using the Primer of Biostatistics (S.E. Glantz) software package.

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 study 1 and 2.

Group	n	Week 6	Week 12	Week 18	Week 24
F_1-50	10	3.3 ± 0.6	3.0 ± 0.7	nm	nm
F_1-150	10	9.4 ± 1.8	9.4 ± 2.1	nm	nm
ACI-50	6	3.1 ± 0.4	2.7 ± 0.7	nm	nm
ACI-150	10	8.6 ± 1.5	7.9 ± 3.1	nm	nm
p1/p2		ns/ns	ns/ns		
F_1-NAME	8	nm	8.3 ± 1.5	8.1 ± 2.9	8.7 ± 4.7
F_1-NAME + LIS	9	nm	8.6 ± 2.8	7.3 ± 3.3	6.3 ± 2.7
p3		-	ns	ns	ns

Values are given as mean ± SD. L-NAME, N^{ω}-nitro-L-arginine methyl ester; LIS, lisinopril; F_1, F_1 (FHH x ACI) rats; ACI, August x Copenhagen Irish rats; 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, not significant ($p > 0.05$).

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 both strains at the measured time-points was detected.

The values for SBP in both F_1 and ACI control rats and after L-NAME treatment are summarized in Table 2. SBP in control F_1 rats was significantly higher compared with control ACI 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 levels 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 more steeply increased 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.

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

Week 6

Group	n	BW	SBP	UaV
F_1-con	9	349 ± 41	129 ± 3	9.6 ± 3.8
F_1-50	10	345 ± 39	160 ± 9	12.3 ± 8.6
F_1-150	10	351 ± 38	182 ± 10	18.1 ± 11.8
p1/p2/p3		ns/ns/ns	s/s/s	ns/s/ns
ACI-con	10	262 ± 19	109 ± 6	3.4 ± 1.6
ACI-50	6	254 ± 19	133 ± 10	5.6 ± 1.8
ACI-150	10	262 ± 20	158 ± 14	9.2 ± 3.0
p1/p2/p3		ns/ns/ns	s/s/s	ns/ns/ns
p4/p5/p6		s/s/s	s/s/s	ns/ns/s

Week 12

Group	n	BW	SBP	UaV
F_1-con	9	387 ± 38	134 ± 7	16.5 ± 8.6
F_1-50	10	382 ± 45	170 ± 13	27.1 ± 14.8
F_1-150	10	366 ± 38	212 ± 12	109 ± 48
p1/p2/p3		ns/ns/ns	s/s/s	ns/s/s
ACI-con	10	286 ± 21	113 ± 6	5.3 ± 2.3
ACI-50	6	272 ± 31	148 ± 11	10.9 ± 3.0
ACI-150	10	276 ± 24	165 ± 15	21.6 ± 8.9
p1/p2/p3		ns/ns/ns	s/s/s	ns/ns/ns
p4/p5/p6		s/s/s	s/s/s	ns/ns/s

Values are given as mean ± SD. BW, body weight; SBP, systolic blood pressure; UaV, albuminuria; con, control; Other abbreviations as in Table 1. p1, con *vs.* 50 mg; p2, con *vs.* 150 mg; p3, 50 mg *vs.* 150 mg; p4, ACI-con *vs.* F_1-con; p5, ACI-50 *vs.* F_1-50; p6, ACI-150 *vs.* F_1-150; s, $p < 0.05$; ns, not significant ($p > 0.05$).

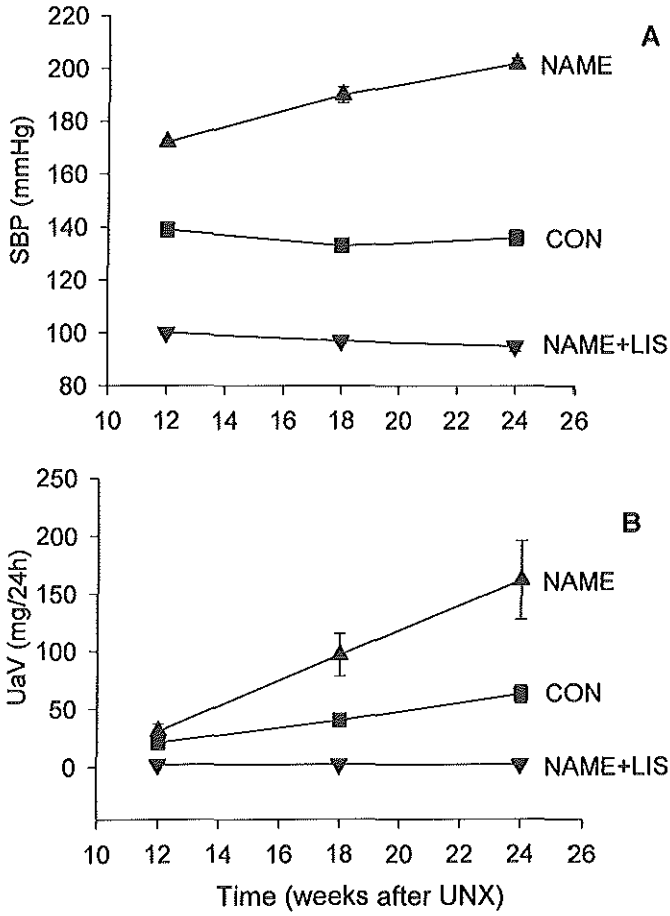

Figure 1A. Tail-cuff systolic blood pressure (SBP) and **B.** urinary albumin excretion (UaV) in F_1 (FHH x ACI) rats at 12, 18, and 24 weeks after UNX. Groups are: untreated controls (con, ■, n=8), rats treated with 150 mg/L L-NAME (NAME, ▲, n=8), and rats treated with 50 mg/L lisinopril and 150 mg/L L-NAME directly after UNX (NAME + LIS, ▼, n=9). Values are given as mean ± SE.

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 control and treated rats, as shown in Table 4. In contrast, GFR was significantly lower in the 150 mg treated groups of both strains compared with the 50 mg treated groups. By comparing the creatinine clearance in the present study with those obtained in a previous study performed in F_1 and ACI rats with two kidneys (2K) after similar L-NAME intake,[9] we were able to calculate the compensatory increases after UNX in both strains. 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 rats.

Table 3. Body, kidney, and heart weights and incidence of glomerulosclerosis at autopsy after 12 weeks of follow-up.

Group	n	BW (g)	LKW	LKW/g	FGS (%)	HW	HW/g
F_1-con	9	388±47	1920±214	495±18	6.2±3.1	988±145	254±14
F_1-50	10	398±60	1908±243	481±27	9.6±3.7	980±103	248±15
F_1-150	10	358±48	1944±313	533±40	23.4±13.3	1138±110	314±16
p1/p2/p3		ns/ns/ns	ns/ns/ns	ns/ns/s	ns/s/s	ns/s/s	ns/s/s
ACI-con	10	289±25	1385±134	480±24	3.2±2.7	715±57	248±10
ACI-50	6	259±37	1302±116	509±65	8.5±2.1	685±71	266±21
ACI-150	10	279±26	1343±129	481±15	10.9±6.0	745±73	267±20
p1/p2/p3		ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/s/ns
p4/p5/p6		s/s/s	s/s/s	ns/ns/s	ns/ns/s	s/s/s	ns/ns/s

n, number of rats; BW, body weight; LKW, left kidney weight in mg; LKW/100g, LKW per 100 g BW; FGS, focal glomerulosclerosis; HW, heart weight in mg; HW/100g, HW per 100 g BW. Other abbreviations as in Table 1. p1, con *vs.* 50 mg; p2, con *vs.* 150 mg; p3, 50 mg *vs.* 150 mg; p4, ACI-con *vs.* F_1-con; p5, ACI-50 *vs.* F_1-50; p6, ACI-150 *vs.* F_1-150; s, $p < 0.05$; ns, not significant ($p > 0.05$).

Renal damage in groups with similar SBP levels

The groups that develop similar levels of SBP appeared to be the F_1-con and the ACI-50, and the F_1-50 and the ACI-150 groups, respectively. The SBP level in these groups averaged 132 ± 2 and 139 ± 5, and 165 ± 7 and 160 ± 6 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. The incidence of FGS in the F_1-150 group was also significantly higher compared with all other groups.

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 treated rats were included in this analysis. 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 ± 0.07 ($r = 0.397$, $p = 0.033$) and 0.10 ± 0.02 ($r = 0.709$, $p < 0.01$) mg/24h 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 ± 0.18 and 0.25 ± 0.05 mg/24h 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 percent increase in FGS incidence was three times higher ($p < 0.001$) in F_1 compared with ACI rats and averaged 3.98 ± 0.49, and 1.31 ± 0.27 mg/24h, respectively. Furthermore, per percent increase in FGS, F_1 rats showed an increase in LKW/100 g BW of 0.25 ± 0.03, whereas ACI rats did not show a significant correlation between those parameters.

Study 2

Blood pressure and albuminuria

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

Longitudinal values for the tail-cuff SBP and UaV at 12,18, and 24 weeks after UNX are shown in Figure 1A and 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 rats that were simultaneously treated with L-NAME and lisinopril 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 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 and lisinopril did not develop any significant UaV during the entire period, the average level being around 2 mg/24h.

Final GFR and structural renal damage

Final creatinine clearance as an indicator of final GFR is summarized in Figure 2A. Final GFR in the control 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, is shown in Figure 2B, and was significantly higher in the NAME group compared with all other groups. 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 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.

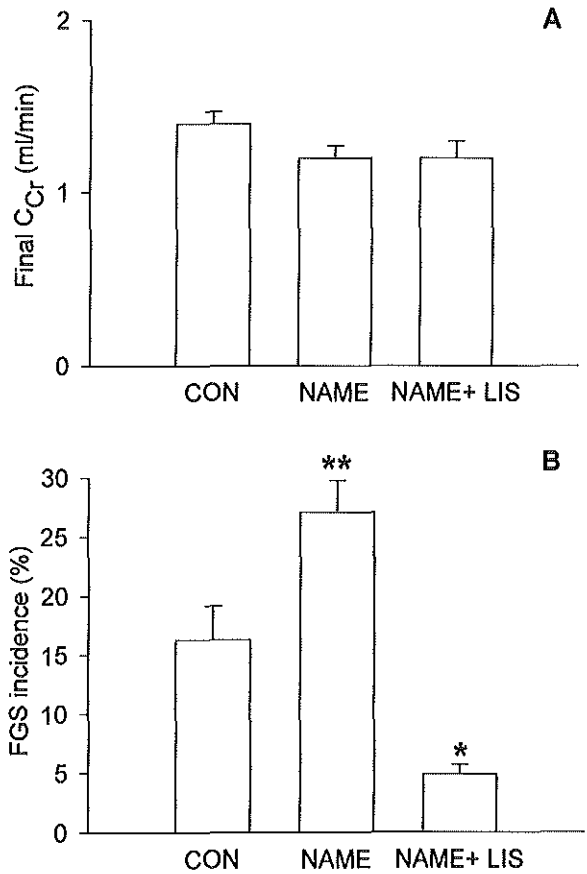

Figure 2A. Final creatinine clearance (C_{Cr}), and **B.** incidence of focal glomerulosclerosis (FGS, % of damaged glomeruli) in F_1 (FHH x ACI) rats. Values are given as mean ± SE. * $p < 0.05$, NAME + LIS *vs.* con and NAME, ** $p < 0.05$, NAME *vs.* con and NAME + LIS.

Table 4. Final plasma creatinine levels and calculated creatinine clearances.

Group	n	P_{Cr}	C_{Cr}	C_{Cr}/100 g
F_1-con	9	59.9 ± 7.3	1.49 ± 0.23	0.39 ± 0.08
F_1-50	10	57.7 ± 3.2	1.61 ± 0.14	0.42 ± 0.06
F_1-150	10	70.1 ± 6.4	1.20 ± 0.21	0.33 ± 0.05
p1/p2/p3		ns/s/s	ns/s/s	ns/ns/s
ACI-con	10	66.0 ± 6.2	0.81 ± 0.12	0.28 ± 0.04
ACI-50	6	64.2 ± 3.2	0.88 ± 0.18	0.32 ± 0.06
ACI-150	10	68.9 ± 8.1	0.80 ± 0.13	0.28 ± 0.04
p1/p2/p3		ns/ns/ns	ns/ns/ns	ns/ns/ns
p4/p5/p6		ns/ns/ns	s/s/s	s/s/ns

Values are given as mean ± SD. P_{Cr}, plasma creatinine (μmol/L); C_{Cr}, creatinine clearance (ml/min); C_{Cr}/100 g, creatinine clearance per 100 g BW. Other abbreviations as in Tables 1 and 2. p1, con *vs.* 50 mg; p2, con *vs.* 150 mg; p3, 50 mg *vs.* 150 mg; p4, ACI-con *vs.* F_1-con; p5, ACI-50 *vs.* F_1-50; p6, ACI-150 *vs.* F_1-150; s, $p < 0.05$; ns, not significant ($p > 0.05$).

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, even a 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 lisinopril completely prevented the development of functional and structural renal damage.

In rats, the susceptibility to renal damage with aging or after renal mass reduction differs among strains.[13] Based on studies in Munich Wistar (MW) rats after renal ablation, Brenner and coworkers postulated in the early 1980s, that the intraglomerular circulatory adaptation to nephron loss and/or injury, notably an increase in glomerular capillary pressure (P_{GC}), is the main driving force for continuous progressive glomerular damage.[14,15] The impact of an increased P_{GC} was thought to be more important than that of a systemic blood pressure elevation. Since, this glomerular hypertension hypothesis has been confirmed in numerous additional studies with renal ablation,[16,17] as well as in other experimental models, such as the FHH rat and experimental diabetes.[4-6,12,18]

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.[1-4] Glomerular hypertension and the severity of renal damage in the FHH is further increased by UNX.[5,6] The P_{GC} level in UNX-FHH rats was found to be higher than those reported following UNX in MW or Wistar Kyoto (WKY) rats,[19,20] strains that are less susceptible to the development of renal damage than the FHH rat. However, the glomerular hypertension in UNX-FHH is comparable to that reported for MW rats with remnant kidneys.[14,16,17] In general, normotensive rat strains appear less susceptible than hypertensive ones. We have previously studied the normotensive WAG,[21] in which survival time after UNX is only slightly reduced compared with the two-kidney WAG.[21] In the present study, the normotensive ACI rat, the other parental strain of the F_1 rats, hardly develops any renal damage even after a substantial elevation of systemic blood pressure. However, normal blood pressure is no guarantee for low susceptibility. Rats of the Milan Normotensive Strain

(MNS), in contrast to the rats of the Milan Hypertensive Strain (MHS) spontaneously develop renal damage without hypertension.[22,23]

The relative resistance of the MNS already indicates that systemic hypertension *per se*, is not sufficient to induce to renal damage. Similar to male FHH rats, male Munich-Wistar-Fromter (MWF) rats spontaneously develop glomerular and systemic hypertension.[24] Again, the development of renal damage in MWF rats is greatly worsened by UNX.[25] In contrast to the FHH and MWF rat, systemic hypertension in the Spontaneously Hypertensive Rat (SHR) is much higher. However, the high level of systemic blood pressure in SHR is not transmitted to the glomerular capillary network, and glomerulosclerosis and renal failure are not early findings in this rat strain.[20,26] 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.[17] Subsequent proteinuria develops albeit much slower than in FHH and MWF rats following UNX.[20] Even a further increase in systemic pressure using L-NAME, alone or in combination of both, does not severely impair renal function in SHR rats, due to adequate control of afferent arteriolar resistance.[27] 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 such an impaired renal autoregulation leading to hyperperfusion, glomerular hypertension, and hyperfiltration in the FHH rat.[28] These studies are also described in chapters 5 and 6 of this thesis.

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 backcross of both strains, but also revealed the presence of three additional loci for renal failure.[7,8] These findings underscore the complexity of the genetics to progressive renal failure and might point towards additional genetically regulated mechanisms related to changes induced by UNX.

Alterations in the glomerular permeability of proteins, allowing the development of UpV, can occur while glomerular filtration rate (GFR) remains within normal limits in different renal diseases both in animals and humans.[29] 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. This phenomenon was also found in other studies.[30] 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 NOi. When we compare the present GFR data with those obtained in 2K F_1 and ACI rats, the compensatory increase in creatinine clearance after UNX in F_1 rats is much higher than in ACI rats after UNX.[9] This might indicate differences in renal hemodynamic regulatory mechanisms between both strains.

When 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.[31-34] Although the role of the renin-angiotensin system (RAS) in chronic NOi remains somewhat controversial, the development of hypertension in this model is though to be renin-dependent and may result partly from increased vascular Ang II receptor expression.[35,36] Various reports suggest that the endothelial NO synthase system and the RAS interact as regulators of the glomerular microcirculation and are under the influence of different genetic control mechanisms.[37-39]

In conclusion, after UNX and chronic NOi, the F_1 (FHH x 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.

Acknowledgments

The authors would like to thank Mrs. C. Luhrman-Schlomski, Mrs. D.R. Tulling, Mrs. A.L. van den Bosch, Mrs. C. Peekstok, Mr. T. Boijmans, and Mr. W.P. van Schalkwijk for their technical assistance.

This work was supported in part by the National Institutes of Health Grant RO1-HL56284-01 (to Drs. H.J. Jacob and A.P. Provoost).

A part of this study was presented at the 9th International Symposium on SHR and Cardiovascular Genetics, Montréal, Québec, Canada, November 13-16, 1997, and has been published in abstract form (*Clin Exp Pharmacol Physiol.* 1998;25:A20).

REFERENCES

1. Provoost AP, De Keijzer MH. The fawn-hooded rat: a model for chronic renal failure. In: *Experimental and genetic rat models of chronic renal failure.* (Gretz N, Strauch M, eds.) Basel, Karger, 1993;100-114.
2. Provoost AP. Spontaneous glomerulosclerosis. Insights from the fawn-hooded rat. *Kidney Int.* 1997;45:S2-S5.
3. De Keijzer MH, Provoost AP, Molenaar JC. Glomerular hyperfiltration in hypertensive Fawn-Hooded rats. *Renal Physiol Biochem.* 1988;11:103-108.
4. Simons JL, Provoost AP, Anderson S, Troy JL, Rennke HG, Sandstrom DJ, Brenner BM. Pathogenesis of glomerular injury in the fawn-hooded rat: early glomerular capillary hypertension predicts glomerular sclerosis. *J Am Soc Nephrol.* 1993;3:1775-1782.
5. 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.
6. 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.
7. 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. *Nat Gen.* 1996;12:44-51.
8. Provoost AP, Shiozawa M, Majewski RR, Van Dokkum RPE, Jacob HJ. Genetic analysis of the suceptibility to develop renal damage after unilateral nephrectomy in rats. *Am J Hypertens.* 1998;11:5A (Abstract).
9. 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.
10. Marshall FF, Carcia-Bunuel R, Beisel DS. Hydronephrosis, renal agenesis and associated genitourinary anomalies in ACI rats. *Urology.* 1978;11:58-61.
11. Van Dokkum RPE, Jacob HJ, Provoost AP. Difference in susceptibility to develop renal damage in normotensive Fawn-Hooded (FHL) and ACI rats after L-NAME-induced hypertension. *Am J Hypertens.* 1997;10:1109-1116.
12. 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.
13. 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.
14. 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-F93.

15. Brenner BM: Nephron adaptation to renal injury or ablation. *Am J Physiol.* 1985;249:F324-F337.
16. 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.
17. Meyer TW, Anderson S, Rennke HG, Brenner BM. Reversing glomerular hypertension stabilizes established glomerular injury. *Kidney Int.* 1987;31:752-759.
18. Anderson S, Rennke HG, Garcia DL, Brenner BM. Short and long term effects of antihypertensive therapy in the diabetic rat. *Kidney Int.* 1989;36:526-536.
19. 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.
20. Dworkin LD, Feiner HD. Glomerular injury in uninephrectomized spontaneously hypertensive rats: a consequence of glomerular hypertension. *J Clin Invest.* 86;77:796-809.
21. 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.
22. 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.
23. Floege J, Hackmann B, Kliem V, Kriz W, Alpers CE, Johnson RJ, Kühn KW, Koch K-M, Brunkhorst R. Age-related glomerulosclerosis and interstitial fibrosis in Milan normotensive rats: A podocyte disease. *Kidney Int.* 1997;51:230-243.
24. 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.
25. Remuzzi A, Benigni 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.
26. Feld LG, Van Liew JB, Brentjens JR, Boylan JW: Renal lesions and proteinuria in the spontaneously hypertensive rat made normotensive by treatment. *Kidney Int.* 1981;20:606-614.
27. Reverte M, Flores O, Gallego B, Leston A, Lopez-Novoa JM. Effect of chronic NG-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.
28. Van Dokkum RPE, Provoost AP, Jacob HJ, Roman RJ. Intraglomerular hypertension and hyperfiltration due to impaired renal autoregulation in the fawn-hooded hypertensive (FHH) rat. *Am J Hypertens.* 1998;11:1A (Abstract).

29. Kaysen GA, Meyers BD, Couser WG. Mechanisms and consequences of proteinuria. *Lab Invest.* 1986;54:479-484.
30. Ruilope LM, Rodicio JL. Clinical relevance of proteinuria and microalbuminuria. *Curr Opin Nephrol Hypertens.* 1993;2:962-967.
31. Bidani AK, Mitchell KD, Schwartz MM, Navar LG, Lewis EJ. Absence of glomerular injury or nephron loss in a normotensive rat remnant kidney model. *Kidney Int.* 1990;38:28-38.
32. Grond J, Beukers JYB, Schilthuis MS, Weening JJ, Elema JD. Analysis of renal structural and functional features in two rat strains with a different susceptibility to glomerular sclerosis. *Lab Invest.* 1986;54:77-83.
33. Fujihara CK, Michellazo SM, De Nucci G, Satz R. Sodium excess aggravates hypertension and renal parenchymal injury in rats with chronic NO inhibition. *Am J Physiol.* 1994;266:F697-F705.
34. Lafayette RA, Mayer G, Park SK, Meyer TW. Angiotensin II receptor blockade limits glomerular injury in rats with reduced renal mass. *J Clin Invest.* 1992;90:776-771.
35. Miller PL, Rennke HG, Meyer TW. Glomerular hypertrophy accelerates hypertensive glomerular injury in rats. *Am J Physiol.* 1991;F459-F465.
36. Yang Y, Macdonald GJ, Duggan KA. A study of angiotensin II receptors after chronic inhibition if nitric oxide synthase in the spontaneously hypertensive rat. *Clin Exp Pharmacol Physiol.* 1996;23:441-443.
37. Morton JJ, Beattie EC, Speirs A, Gullliver F. Persistent hypertension following inhibition of nitric oxide formation in the young Wistar rat: role of renin and vascular hypertrophy. *J Hypertens.* 1993;11:1083-1088.
38. Ohishi K, Carmines PK, Inscho EW, Navar LG. EDRF-angiotensin II interaction in rats juxtamedullary afferent and efferent arterioles. *Am J Physiol.* 1992;263:F900-F906.
39. Raij L. Nitric oxide and the kidney: *Circulation.* 1993;87:V26-V29.

CHAPTER 5

IMPAIRED AUTOREGULATION OF RENAL BLOOD FLOW IN THE FAWN-HOODED RAT

American Journal of Physiology 1999;276(1):R189-R196

Richard P.E. van Dokkum, Magdalena Alonso-Galicia,
Abraham P. Provoost, Howard J. Jacob, and Richard J. Roman

ABSTRACT

The responses to changes in renal perfusion pressure (RPP) were compared in 12-wk-old Fawn-Hooded Hypertensive (FHH) rats, Fawn-Hooded Low blood pressure (FHL) rats, and August x Copenhagen Irish (ACI) rats to determine whether autoregulation of renal blood flow (RBF) is altered in the FHH rat. Mean arterial pressure was significantly higher in conscious, chronically instrumented FHH rats compared with FHL rats (121 ± 4 vs. 109 ± 6 mmHg). Baseline arterial pressures measured in ketamine-Inactin-anesthetized rats averaged 147 ± 2 mmHg (n=9) in FHH rats, 132 ± 2 mmHg (n=10) in FHL rats, and 123 ± 4 mmHg (n=9) in ACI rats. Baseline RBF was significantly higher in FHH rats than in FHL and ACI rats and averaged 9.6 ± 0.7, 7.4 ± 0.5 and 7.8 ± 0.9 ml/min/gkwt, respectively. RBF was autoregulated in ACI and FHL rats but not in FHH rats. Autoregulatory indexes in the range of RPPs from 100 to 150 mmHg averaged 0.96 ± 0.12 in FHH rats *vs.* 0.42 ± 0.04 in FHL rats and 0.30 ± 0.02 in ACI rats. Glomerular filtration rate was 20-30% higher in FHH rats than in FHL and ACI rats at every RPP studied. Elevations in RPP from 100 to 150 mmHg increased urinary protein excretion in FHH rats from 27 ± 2 to 87 ± 3 μg/min, whereas it was not significantly altered in FHL or ACI rats. The incidence of glomeruli exhibiting histological evidence of injury was not significantly different in the three rat strains. These results indicate that autoregulation of RBF is impaired in FHH rats before the development of glomerulosclerosis and suggest that an abnormality in the control of renal vascular resistance may contribute to the development of proteinuria and renal failure in this strain of rats.

INTRODUCTION

The Fawn-Hooded Hypertensive (FHH) rat is a model of hypertension-associated renal damage.[19,20] This strain develops mild hypertension and severe proteinuria and glomerulosclerosis at a young age. A second inbred control strain has been developed from the same ancestry. This Fawn-Hooded Low blood pressure (FHL) strain remains normotensive and does not develop proteinuria or glomerular damage until late age.[20]

Recently, independent genes for hypertension and renal disease have been identified on chromosome one in a backcross of FHH and August x Copenhagen Irish (ACI) rats.[3] One of the renal failure genes, *Rf-1*, lies between markers D1Mgh12 and D1Mit6. Another region, *Bpfh-1*, which exhibits positive linkage with systolic blood pressure (SBP), is located near the S_A-region which has been linked to the development of hypertension in a variety of genetic rat models.[10,17,18] However, the functional significance of the genes residing in this region and the gene products involved in the development of hypertension and proteinuria remain to be determined.[14,25,27] Previous observations that glomerular filtration rate (GFR), renal blood flow (RBF), and P_{GC} are elevated in FHH rats suggest that control of renal vascular resistance may be altered in this strain.[6,7,24,25] If true, one might expect to find an impaired ability to autoregulate RBF and GFR, which could contribute to the elevated P_{GC} and the development of glomerulosclerosis.

The present study compared autoregulation of RBF and the pressure natriuresis responses in FHH, FHL, and ACI rats. FHL rats were chosen as a closely related control strain of fawn-hooded rats that do not develop hypertension or renal damage. ACI rats were used as a second control strain because they have been previously used in cosegregation studies and in the development of congenic strains.[3] Furthermore, ACI rats are known to be one of the strains most resistant to the development of proteinuria and renal failure.[28]

METHODS

General

Experiments were performed on 52 male FHH, FHL, and ACI rats. The rats were studied at 12 weeks of age, when none of the strains show evident functional and structural renal damage. Body weights averaged 263 ± 29, 260 ± 21, and 194 ± 8 g in FHH, FHL, and ACI rats, respectively. The FHH and FHL rats were obtained from colonies maintained at the Medical College of Wisconsin that were derived from the original colony maintained by Dr. Provoost at the Erasmus University in Rotterdam (FHH/EUR and FHL/EUR). ACI rats were purchased from Harlan Sprague-Dawley Laboratories (Indianapolis, IN). The rats were housed in an animal care facility at the Medical College of Wisconsin, which is approved by the American Association for the Accreditation of Laboratory Animal Care, and had free access to food and water throughout the study. The day before the acute experiments were performed, food was withdrawn overnight to facilitate surgical procedures.

Acute studies

Rats were anesthetized with a 10 mg/kg i.m. injection of ketamine (Ketajet; Phoenix Scientific; St. Joseph, MO) and a 30 mg/kg i.p. injection of 5-*sec*-butyl-5-ethyl-2-thiobarbituric acid (Inactin; Byk-Gulden, Konstanz, Germany). The animals were placed on a thermostatically controlled warming table to maintain body temperature at 37°C. A PE-50 cannula was placed in the femoral artery, and arterial pressure was recorded with a model P23 Gould Statham pressure transducer (Gould, Cleveland, OH) connected to a model RPS 7C8A Grass amplifier (Grass Instruments, Quincy, MA). A PE-50 cannula was also placed in the left carotid artery to allow for measurement of arterial pressure when the aorta below the renal arteries was ligated to raise renal perfusion pressure (RPP). The trachea was cannulated with PE-240 tubing to facilitate breathing. The left external jugular vein was catheterized for the infusion of 1% bovine serum albumin in a 0.9% NaCl solution at a rate of 100 µl/min throughout the experiment. Both ureters were cannulated using PE-50 tubing pulled to a tip diameter of 200 µm for timed urine collections. A 1.5 or 2.0-mm flow probe was placed around the left renal artery to measure RBF using an electromagnetic flowmeter (Carolina Medical Electronics, King, NC). A micro-Blalock clamp (Medical College of Wisconsin, Milwaukee, WI) was placed on the aorta above

the renal arteries, and ligatures were placed around the superior mesenteric and celiac artery to allow for manipulation of RPP.

Neural and hormonal influences on the kidney were controlled as follows. The kidney was denervated by stripping the visible renal nerves and by coating the renal artery with a 5% solution of phenol in ethanol. Circulating levels of vasopressin and norepinephrine were fixed at high levels by i.v. infusion (vasopressin: 2.4 U/ml/min, norepinephrine: 100 ng/min). [^{3}H]Inulin (2 μCi/ml) was also included in the infusion solution to allow for the measurement of GFR.

Experimental protocol

After surgery, 30 min were allowed for stabilization of urine flow and arterial pressure, and RPP was lowered to 100 mmHg by tightening the aortic clamp above the renal arteries. After a 20-min equilibration period, urine and plasma samples were collected during a 20-min clearance period. The aortic clamp was then released to allow RPP to return to ~120 mmHg, and urine and plasma samples were collected during an additional 20-min clearance period. RPP was then increased to 150 mmHg by tightening the ligatures around the superior mesenteric and celiac arteries and by tightening the clamp on the lower aorta below the renal arteries when necessary. After a 10-min equilibration period, urine and blood samples were collected during a 20-min clearance period. In most, but not all rats, a RBF autoregulatory curve was generated after the pressure diuresis experiment. However, this experiment could not be completed in every fawn-hooded rat because RPP often could not be maintained at a high level, caused by a bleeding disorder in these rats. Therefore, additional rats had to be included in the blood flow autoregulation studies only to obtain enough animals for adequate statistics.

In these experiments, the renal artery was briefly occluded distal to the flow probe to obtain a zero-flow signal. RBF was then continuously recorded as RPP was lowered from 150 to 50 mmHg in steps of 10-20 mmHg. The kidney was perfused at each pressure step for 3 min until a steady-state level of RBF was recorded.

Measurement of arterial pressure in conscious rats

Arterial pressure was measured in conscious 12-wk-old FHH (n=5) and FHL (n=5) rats fed a normal (1% NaCl) diet. Studies were also performed in a group of 36-wk-old FHH rats (n=5) that had advanced glomerulosclerosis. These rats were also measured after they were fed a high salt (8% NaCl) diet for 7 days. Animals were anesthetized with ketamine, xylazine, and acepromazine (56, 3.2, 0.8 mg/kg, i.m.), and a catheter (MRE-025/Tygon) was implanted in the femoral artery. The catheter was routed subcutaneously to the scapular region, exteriorized through a Dacron mesh button (Instech), and protected with a stainless-steel spring that also served to tether the rats to a swivel.

After surgery, the rats were housed in individual cages in a quiet, air-conditioned room with a 12:12-h light-dark cycle and were allowed 1 wk to fully recover from surgery. The catheter was flushed daily with 0.2 ml of heparinized saline solution (100 USP/ml).

Mean arterial pressure (MAP) and heart rate (HR) were measured for 24 h on 3-4 consecutive days. Pulsatile arterial pressure signals were amplified, digitized, and analyzed with a computerized Apollo software system. The analog signal was sampled at 30 Hz, and the minute averages were used for calculation of daily MAP, SBP, diastolic blood pressures (DBP), and HR.

Analytic techniques

[^{3}H]inulin concentrations of urine and plasma samples were determined using a liquid scintillation counter (Delta 300 model 6891 Liquid Scintillation System; Tracor Analytic, Elk Grove Village, IL). Urine flow rate was determined gravimetrically. Sodium and potassium concentrations of the samples were determined using a flame photometer (model 480; Ciba Corning Diagnostics, Medfield, MA). Plasma and urine protein concentrations were measured spectrophotometrically using the Bradford method (Bio-Rad Protein Assay; Bio-Rad Laboratories, Hercules, CA) and bovine serum albumin as a standard. GFR and sodium excretion were calculated using standard formulas described previously.[21,22] Urinary excretion data, RBF, and GFR were factored per gram kidney weight to normalize for strain differences in kidney size. RBF autoregulatory indexes over the range of pressures from 100 to 150 mmHg were calculated by the method of Semple and de Wardener[23] using the following formula:

RBF autoregulatory index = $[(RBF_2 - RBF_1)/(RBF_1)]/[(RPP_2 - RPP_1)/(RPP_1)]$.

The autoregulatory indexes were calculated assuming that RPP was reduced in a single step from a high pressure (RPP_1) to a lower pressure (RPP_2). According to this analysis, an autoregulatory index of 0 indicates perfect autotegulation of RBF. An index of 1 is characteristic of a circulation with a fixed vascular resistance. An autoregulatory index > 1 is indicative of a compliant system in which vascular resistance decreases as RPP increases.[23]

Assessment of glomerular injury

At the end of each study, both kidneys from each rat were collected and weighed. Coronal sections of the kidneys were immersed in 3% Formalin. After fixation, 2- to 3-mm slices of the tissue were embedded in paraffin and prepared for light microscopy. The extent of glomerular damage was determined in 3-µm sections stained with periodic acid-Schiff reagent. In each animal, 50 glomeruli were evaluated for the presence of sclerotic lesions, *i.e.*, segmental glomerular scarring, obliteration of glomerular capillaries, mesangial matrix expansion, and adhesion formation between tuft and Bowman's capsule. The extent of glomerular damage is expressed as the percentage of the glomeruli exhibiting one or more of these features.

Statistical analysis

Differences in mean values measured at different perfusion pressures between and within groups were compared using a two-factor ANOVA for repeated measures followed by Duncan's multiple range test.[11] Linear regression analysis was used to calculate the relationship between urine flow, sodium excretion, and RPP in each group. Differences in the slopes of these relationships were compared using a one-way ANOVA. Throughout the study, a p value < 0.05 was considered significant.

RESULTS

Autoregulation of RBF

A comparison of the RBF autoregulatory curves for FHH, FHL, and ACI rats is presented in Figure 1A. Baseline MAP measured under ketamine-Inactin anesthesia was significantly higher in these animals than that measured in conscious FHH and FHL rats and averaged 147 ± 3 mmHg (n=9) in FHH rats, 132 ± 2 mmHg (n=10) in FHL rats, and 123 ± 4 mmHg (n=9) in ACI rats. Baseline RBF was 35% higher in FHH rats compared with FHL and ACI rats (Figure 1A). There was no significant difference in RBF between FHL and ACI rats. RBF was autoregulated over a range of pressures from 100 to 150 mmHg in FHL and ACI rats. Autoregulatory indexes over the pressure range between 100 and 150 mmHg averaged 0.42 ± 0.04 in FHL and 0.30 ± 0.02 in ACI rats. In contrast, RBF was not autoregulated in FHH rats. The autoregulatory index averaged 0.96 ± 0.12 in FHH rats, and it was significantly higher than the corresponding values measured in FHL and ACI. A similar picture emerges when the data are evaluated in 25-mmHg pressure steps (Figure 1B). Below a RPP of 100 mmHg, none of the three strains exhibited any autoregulation of RBF (autoregulatory indexes were ~1). However, in the range of RPP from 100 to 125 mmHg, the RBF autoregulatory index in the FHH rats was significantly greater than that seen in FHL and ACI rats.

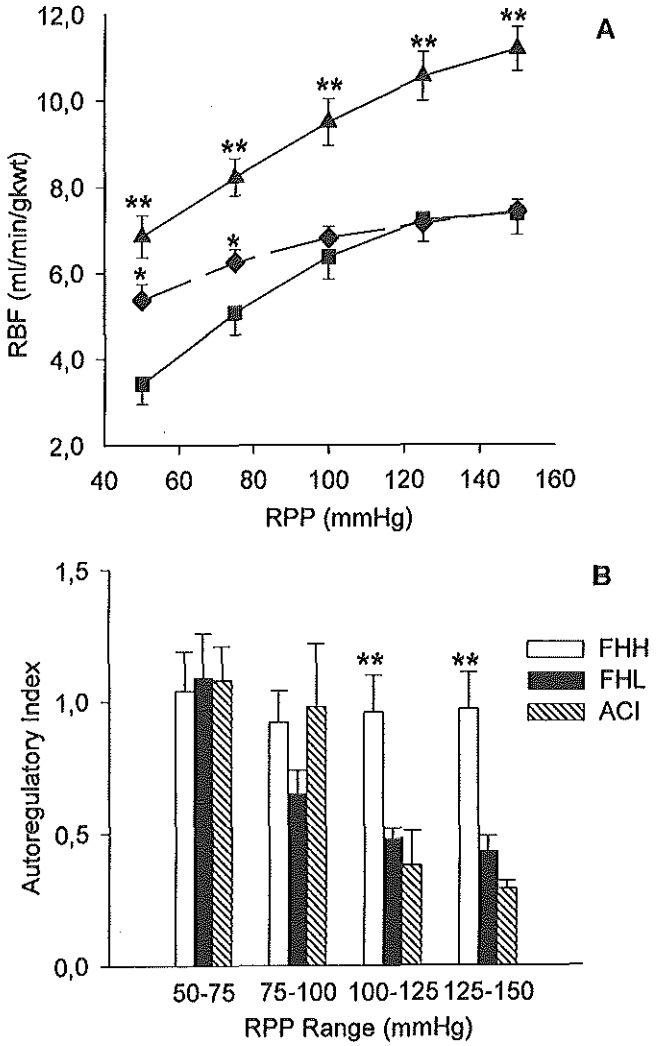

Figure 1A. Relation between renal blood flow (RBF) and renal perfusion pressure (RPP) in 3-mo-old Fawn-Hooded Hypertensive (FHH, ▲, n=9) rats, Fawn-Hooded Low blood pressure (FHL, ◆, n=10) rats, and August x Copenhagen Irish (ACI, ■, n=9) rats. Kidney weights (kwt) averaged 2.50 ± 0.03 g in FHH, 2.51 ± 0.06 g in FHL, and 1.94 ± 0.02 g in ACI rats. Values are means ± SE (error bars). * $p < 0.05$, FHL compared with ACI. ** $p < 0.05$, FHH compared with FHL and ACI. **B.** Renal blood flow (RBF) autoregulatory indexes for several RPP ranges in 3-mo-old FHH (n=9), FHL (n=10), and ACI rats (n=9) rats. Values are means ± SE (error bars). ** $p < 0.05$, FHH compared with FHL and ACI.

Pressure diuretic and natriuretic responses

The relations between water and sodium excretion and RPP in FHH, FHL, and ACI rats are summarized in Figure 2 A and B, respectively. The diuretic and natriuretic responses to changes in RPP were not significantly different among the strains. Urine flow and sodium excretion increased 6- to 12-fold in these rats when RPP was increased from 100 to 150 mmHg. The slopes of the best-fit linear regression line relating urine flow and RPP averaged 0.85 ± 0.32, 0.79 ± 0.10, and 0.56 ± 0.12 ml/min/gkwt/mmHg in FHH, FHL, and ACI rats, respectively. The slopes of these lines were not significantly different. The slopes of the regression line relating sodium excretion and RPP were also not significantly different among the strains and averaged 0.13 ± 0.06, 0.12 ± 0.03, and 0.10 ± 0.05 mmol/min/gkwt/mmHg in FHH, FHL, and ACI rats, respectively. The pressure diuretic and natriuretic responses in the 36-wk-old FHH rats with advanced glomerular disease were markedly weaker compared with those seen in the younger animals. The slope of the regression line relating sodium excretion and RPP in old FHH rats was significantly lower than those observed in the other three groups and averaged only 0.014 ± 0.002 mmol/min/gkwt/mmHg

Elevating RPP from 100 to 150 mmHg increased fractional sodium excretion similarly from 0.6 ± 0.1 to 2.6 ± 0.5 % and from 0.5 ± 0.08 to 3.1 ± 0.8 % of the filtered sodium load in FHH and FHL rats, respectively (Fig. 3). These responses were significantly less than the corresponding response seen in ACI rats. In old FHH rats, the rise in fractional excretion of sodium was markedly blunted when RPP was elevated from 100 to 150 mmHg and it just increased from 0.38 ± 0.13 to 1.41 ± 0.37% of the filtered load (not shown). Moreover, fractional excretion of sodium was reduced in old and young FHH and FHL rats compared with values in ACI rats at an elevated level of RPP (150 mmHg).

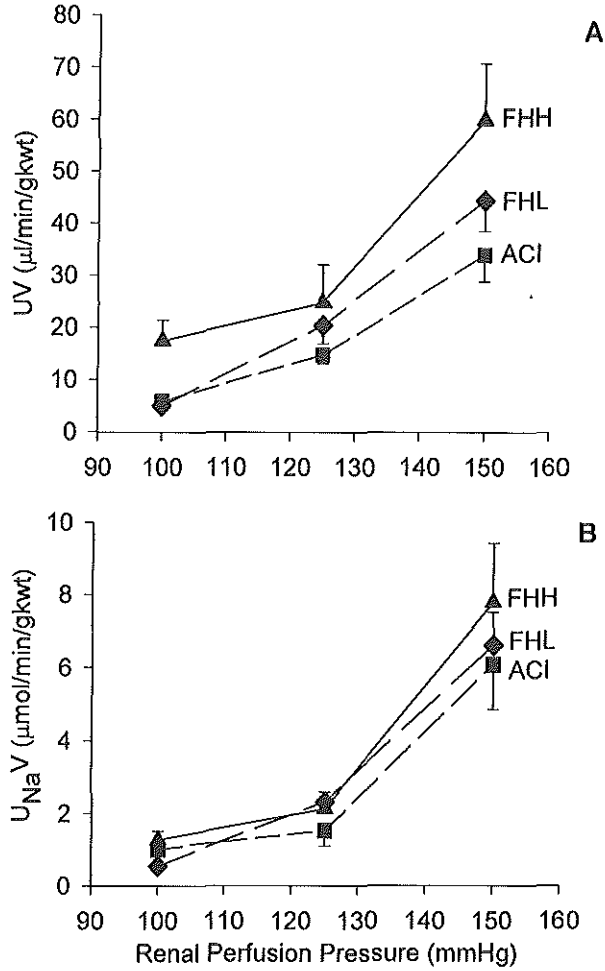

Figure 2. Relationships between urine flow (UV; A) and sodium excretion ($U_{Na}V$; B) and RPP in FHH (▲, n=9), FHL (◆, n=10) and ACI (■, n=9) rats. Kidney weights (kwt) averaged 2.50 ± 0.03 g in FHH, 2.51 ± 0.06 g in FHL, and 1.92 ± 0.02 g in ACI rats. Values are means ± SE (error bars).

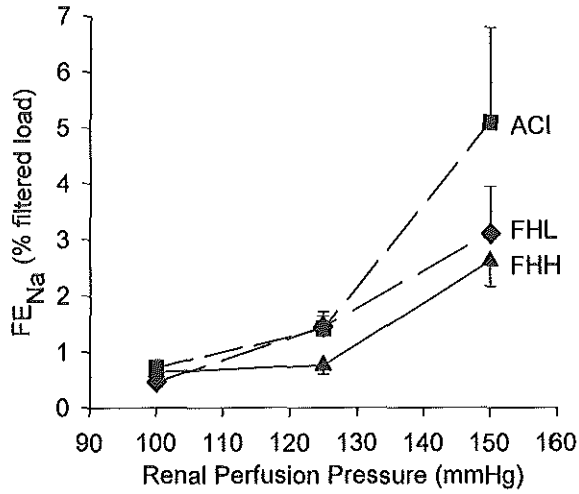

Figure 3. Relationship between fractional sodium excretion (FE_{Na}) in percentage of filtered load and RPP in FHH (▲, n=9), FHL (◆, n=10) and ACI (■, n=9) rats. Values are means ± SE (error bars).

Glomerular filtration rate

Whole kidney GFR was significantly higher in young FHH than in FHL and ACI at every RPP studied and reached 2.37 ± 0.10 ml/min/gkwt at an 150 mmHg RPP (Figure 4A). GFR was also greater in FHL than in ACI rats at an RPP of 125 mmHg and averaged 1.23 ± 0.03 vs. 0.56 ± 0.07 ml/min/gkwt, respectively. In old FHH rats, GFR was markedly reduced compared with values seen in all three groups of young animals. Similarly, RBF was lower in old FHH rats than the corresponding values observed in the other groups (not shown). The GFR autoregulatory indexes (Figure 5A) showed no significant differences between the three strains in the pressure range of 125 and 150 mmHg, whereas these indexes were significantly higher in FHH and ACI rats compared with FHL rats. The RBF autoregulatory indexes during the pressure natriuresis study (Figure 5B) were similar to those in the autoregulatory studies between RPPs of 125 and 150 mmHg.

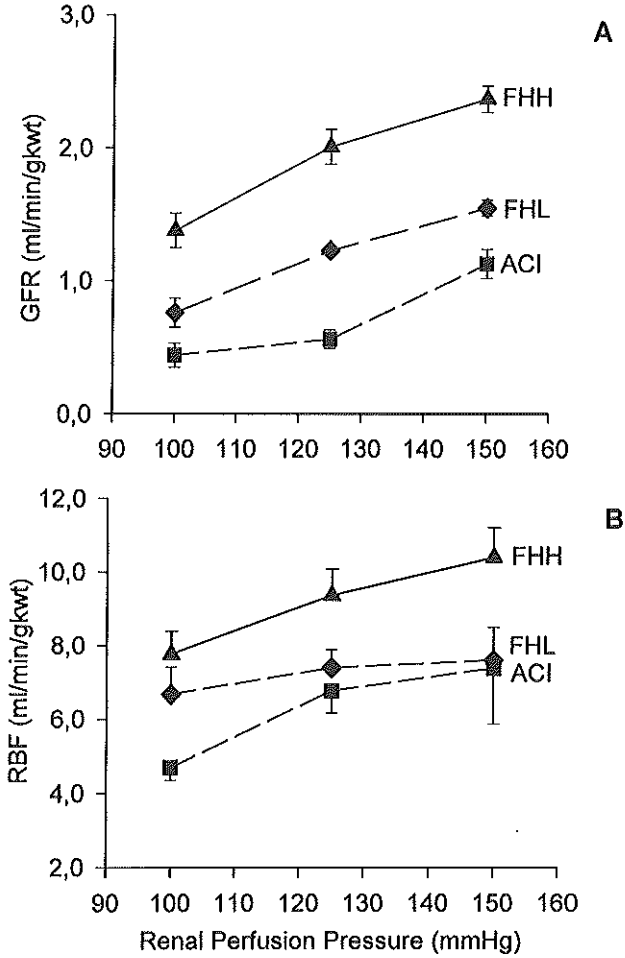

Figure 4. Relationships between glomerular filtration rate (GFR; A) and renal blood flow (RBF) (B) and renal perfusion pressure (RPP) in FHH (▲, n=9), FHL (◆, n=10) and ACI (■, n=9) rats. Kidney weights averaged 2.50 ± 0.03 g in FHH, 2.51 ± 0.06 g in FHL, and 1.92 ± 0.02 g in ACI rats. Values are means ± SE. All differences were statistically significant ($p < 0.05$), except for RBF differences at 125 mmHg between FHL and ACI.

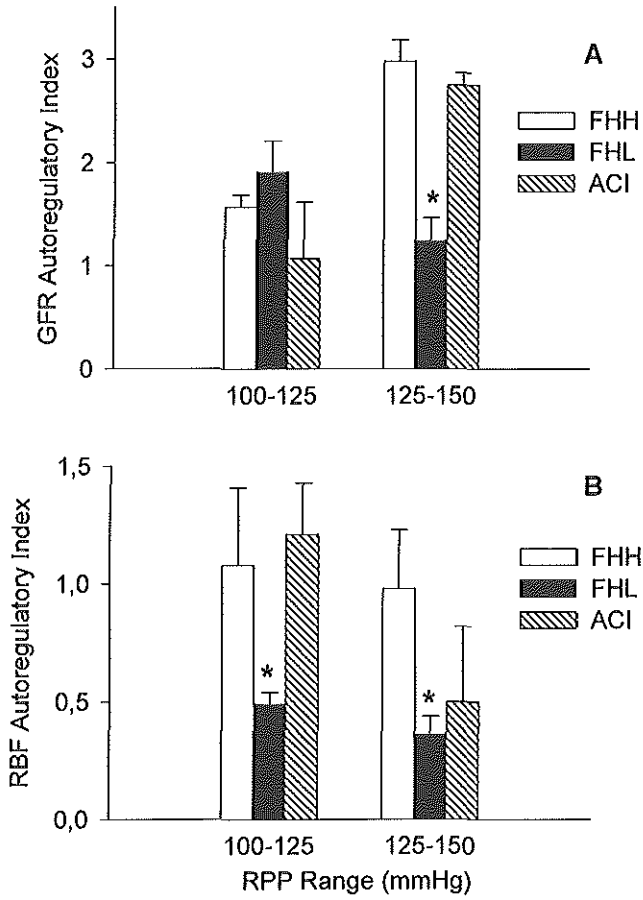

Figure 5. GFR (A) and RBF (B) autoregulatory indexes in the range of RPP from 100 to 125 and from 125 to 150 mmHg in 3-mo-old FHH (n=9), FHL (n=10) and ACI (n=9) rats. Values are means ± SE. * $p < 0.05$, FHL rats compared with FHH and ACI rats.

Pressure-proteinuric responses

A comparison of urinary protein excretion as a function of RPP in FHH, FHL, and ACI rats is presented in Figure 6. Basal protein excretion at an RPP of 100 mmHg was greater in FHH rats than in FHL and ACI rats and averaged 27 ± 2, 16 ± 2, and 11 ± 1 µg/min, respectively. An elevation of RPP to 150 mmHg increased protein excretion to 87 ± 3 µg/min in FHH rats, whereas protein excretion was not significantly altered in FHL and ACI rats.

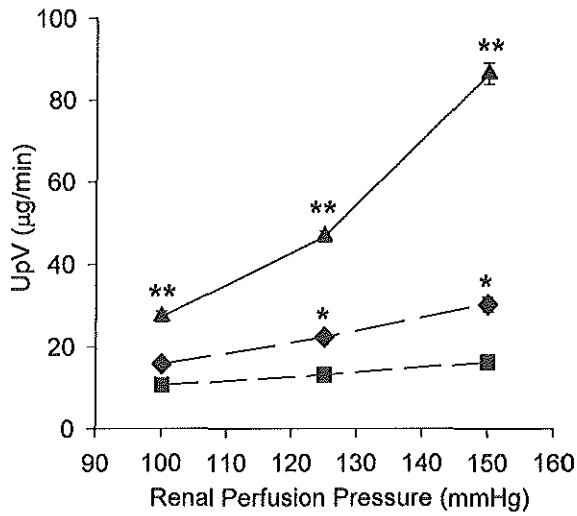

Figure 6. Relationship between urinary protein excretion (UpV) in 3-mo-old FHH (▲, n=9), FHL (◆, n=10), and ACI (■, n=9) rats. Values are means ± SE. ** $p < 0.05$, FHH compared with FHL and ACI. * $p < 0.05$, FHL compared with ACI.

Measurement of arterial pressure

MAPs measured in conscious 12-wk-old FHH rats were slightly but significantly higher than those obtained in FHL rats. In FHH rats (n=5) the values for MAP, SBP, and DBP were 121 ± 4, 153 ± 3, and 98 ± 6 mmHg vs. 109 ± 6, 141 ± 6, and 89 ± 6 mmHg measured in FHL rats, respectively. Mean pulse pressures averaged 55 ± 7 mmHg in FHH and 54 ± 3 mmHg in FHL rats; mean heart rate 411 ± 25 beats/min in FHH and 436 ± 17 beats/min in FHL, and they were not significantly different. In 36-wk-old FHH rats, MAP was not significantly different from that measured in the younger FHH rats. However, MAP rose in the old FHH rats from 125 ± 4 to 142 ± 4 mmHg (n=4) when they were fed a high-salt diet (8% NaCl) for 7 days.

Assessment of glomerular injury

Kidney weights were similar in FHH and FHL rats (2.50 ± 0.03 vs. 2.51 ± 0.06 g), and both were higher than those seen in ACI rats (1.92 ± 0.02 g). The percentage of glomeruli exhibiting any signs of injury were not significantly different in the three strains and averaged 1.8 ± 0.5 % in FHH, 1.1 ± 0.5 % in FHL, and 0.9 ± 0.6 % in ACI rats. This indicates that the rats in these groups were studied at an age before the development of significant glomerular injury. In contrast, the kidneys of 36-wk-old FHH rats exhibited severe glomerulosclerosis and the percentage of injured glomeruli averaged 66 ± 8%.

DISCUSSION

The present study compared renal hemodynamics and the pressure natriuretic responses in relatively young (12-wk-old) FHH with FHL and in ACI rats in order to determine whether an abnormality in the autoregulation of RBF might precede the development of proteinuria and glomerular damage in the FHH rat. Our major findings are that RBF and GFR were markedly elevated in FHH rats compared with values observed in FHL and ACI rats and that FHH rats did not autoregulate RBF as well as the control strains in the range of pressures from 100 to 150 mmHg. Indeed, the RBF autoregulatory index in FHH rats was significantly higher than those measured in both control strains, and averaged 0.96 ± 0.12, which is indicative of a system with a fixed vascular resistance.[23] These results are consistent with previous reports indicating that GFR and P_{GC} are elevated in the kidney of FHH rats and indicate that these animals probably exhibit a failure of the afferent arteriole to constrict in response to elevations in RPP.[6,25] Presumably this could involve an abnormality in myogenic mechanisms, tubuloglomerular feedback (TGF), or both. Because TGF has recently been reported to be relatively normal in the FHH rat, the most likely explanation for the impairment in RBF autoregulation in FHH rats in the present study is an altered myogenic response.[31] In this regard, we have recently obtained direct evidence that the myogenic response of isolated perfused preglomerular arterioles to elevations in transmural pressure is absent in vessels obtained from the kidneys of FHH rats.[29] These studies are also described in chapter 6 of this thesis.

We also observed that urinary protein excretion was directly dependent on the changes in RPP in FHH rats. The mechanism involved in this unusual pressure proteinuric response may be dependent on the GFR and the impaired autoregulation of RBF and GFR in response to elevations in RPP. Thus the high baseline filtered load of protein and the increment seen when RPP is elevated, likely increased the delivered load of protein to a level that exceeds the transport maximum for reabsorption of protein in the proximal tubule. The present observation that protein excretion is directly dependent on the level of RPP in FHH rats is also consistent with previous reports mentioning that pharmacologic agents that lower blood pressure reduce the degree of proteinuria and delay the onset of glomerular disease in FHH rats.[30] Similarly, pharmacological agents that raise systemic blood pressure in the Fawn-Hooded rat worsen the severity of glomerular damage and proteinuria.[27,28]

In the present study, we also compared the pressure natriuretic responses in the three strains of rats. Previous studies have indicated that the pressure natriuretic relationship is blunted or reset to a higher level of RPP in every genetic model of hypertension examined to date.[5,21] However, in the present study the relationship

between urine flow, sodium excretion, and RPP was not altered in 12-wk-old FHH rats relative to values seen in FHL and ACI rats primarily because GFR is elevated in these rats. The slope of the relationship between the fractional excretion of sodium and RPP was significantly reduced in FHH rats relative to ACI rats; however, it was not different from the levels seen in FHL rats that do not develop hypertension. From this data we have to conclude that the pressure natriuresis relationship is not blunted in 12-wk-old FHH rats. We also measured arterial pressure and the pressure natriuretic response in an additional group of 36-wk-old FHH rats with severe proteinuria and glomerular damage. As would be expected, RBF and GFR were markedly reduced and the pressure natriuresis response was blunted in these rats.

The lack of resetting of the pressure natriuresis relationship in young FHH rats initially was surprising, but is consistent with the chronic measurements of arterial pressure obtained in the present study. Although we found that MAP was significantly greater in FHH than FHL rats, the level of MAP in FHH rats (121 mmHg) can be considered being in the normal range of pressures that we have previously reported in normotensive strains of rats.[14,21,26] Moreover, in this study, we found that MAPs measured in old FHH rats with established glomerular disease on a normal-salt diet were also in this range. However, as would be expected from the low GFR and blunted pressure natriuresis response, these rats were salt-sensitive and MAP rose from 125 ± 4 to 142 ± 4 mmHg during a 7-day period when the rats were fed a high-salt diet (8% NaCl).

The present study provides the first long-term (24 h) measurements of arterial pressure in conscious FHH rats recorded in their home cage under unstressed conditions. All previous measurements of pressure with this strain were done using the tail cuff in restrained rats. Actually, the measurements of systolic pressure in our study are very similar to those previously reported, although diastolic pressure is rather low in this strain. The FHH rat may also be more responsive to stress and may be able to transmit a greater percentage of the systolic pressure to the tail. This would be consistent with a strain of rat that exhibits impaired myogenic tone not only in the kidney but throughout the systemic circulation.

Previous studies have documented that urinary protein excretion rates for these strains at 3 mo of age average 100, 30, and 15 mg/24h for FHH, FHL, and ACI rats, respectively.[24,25,27,28] As confirmed in the present study, FHH rats do not exhibit pronounced histological evidence of glomerulosclerosis at 12 wk of age. These findings in combination with the present measurement of arterial pressure indicate that the development of proteinuria in FHH rats precedes the development of outspoken hypertension and histological development of glomerular injury. These data also fit with previous observations that the severity

of hypertension in FHH is relatively modest: (a) until the animal is quite old, or (b) when a reduced mass-like form of hypertension due to severe glomerulosclerosis develops, or (c) when the progression of glomerular injury is accelerated by uninephrectomy, or (d) when blood pressure is pharmacologically elevated using N^{ω}-nitro-L-arginine methyl ester.[24,27] These studies are also described in chapters 2 through 4 of this thesis.

Glomerular hyperfiltration and P_{GC} have been implicated as factors determining the susceptibility to develop glomerulosclerosis in hypertension. An impairment in the efficiency of autoregulation obviously could contribute to a greater transmission in pressure to the glomerulus and therefore may contribute to the differences in the incidence of glomerular disease seen in different models of hypertension. For example, autoregulation of RBF and GFR is not impaired in spontaneously hypertensive rats but is highly efficient and is shifted to higher pressures. These animals do not exhibited elevated P_{GC} or develop glomerulosclerosis until very late in the disease process. In contrast, autoregulation of RBF and GFR is impaired in DOCA-salt and reduced renal mass models of hypertension.[2,13] P_{GC} is elevated in both models, and they rapidly develop severe proteinuria and glomerulosclerosis. Autoregulation of RBF and GFR is also impaired and P_{GC} is elevated in the streptozocin-induced insulin-dependent model of diabetes, which develops a severe form of glomerulosclerosis. The only exception is the Dahl Salt-sensitive model of hypertension. These animals also rapidly develop glomerulosclerosis, but P_{GC} is still relatively normal and they exhibit no impairment of RBF autoregulation.[21]

Perspectives

In summary, the present study indicates that autoregulation of RBF is impaired in FHH rats, which leads to hyperfiltration, proteinuria, and the subsequent development of glomerulosclerosis. The mechanisms involved remain to be determined, but may be due to an impairment in the myogenic response in preglomerular renal arterioles. Further experiments need to be performed to try and link these functional abnormalities in renal hemodynamics observed in the FHH rat with the renal failure genes previously identified on chromosome one and other chromosomes. A better understanding of the physiology and genetics in this unique genetic animal model of end-stage renal failure (ESRF) may provide insight into the pathogenesis and treatment of ESRF in humans.

Acknowledgments

The authors thank Lisa M. Geiger for technical assistance. Studies were performed with financial support from the National Institutes of Health (R01-HL 36279-01 to R.J. Roman and R01-HL56284-01 to H.J Jacob and A.P. Provoost). The work of R.P.E. van Dokkum was performed at the Medical College of Wisconsin and was supported by the Royal Dutch Academy of Sciences (VWF97/SCW/42), the Trustfund of Erasmus University Rotterdam (97030.35/96.0311), and by the Three Lights Foundation (96/54 AH/rvv) in The Netherlands.

Parts of this study were presented in May 1998 at the *13th Scientific Meeting of the American Society of Hypertension*, New York, NY, and in June 1998 at the *17th Scientific Meeting of the International Society of Hypertension*, Amsterdam, The Netherlands and have been published in abstract form (*Am J Hypertens.* 1998;11:1A and *J Hypertens.* 1998;16:S189).

REFERENCES

1. Azar S, Johnson MA, Scheinman J. Regulation of glomerular capillary pressure and filtration rate in young Kyoto hypertensive rats. *Clin Sci.* 1979;56:203-209.
2. Bidani AL, Schwartz MM, Lewis EJ. Renal autoregulation and vulnerability to hypertensive injury in remnant kidney. *Am J Physiol.* 1993;252:F1003-F1010.
3. 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. *Nat Genet.* 1996;12:44-51.
4. Chen YM, Holstein-Rathlou NS. Differences in dynamic autoregulation of renal blood flow between SHR and WKY rats. *Am J Physiol.* 1993;264:F166-F174.
5. Cowley, AW Jr, Roman RJ. The role of the kidney in hypertension. *JAMA.* 1996;275:1581-1589.
6. De Keijzer MH, Provoost AP, Molenaar JC. Glomerular hyperfiltration in hypertensive Fawn-Hooded rats. *Renal Physiol Biochem.* 1988;11:103-108.
7. De Keijzer MH, Provoost AP, Molenaar JC. Proteinuria is an early marker in the development of progressive renal failure in hypertensive fawn-hooded rats. *J Hypertens.* 1989;7:525-528.
8. Dworkin LD, Feiner HD. Glomerular injury in uninephrectomized spontaneously hypertensive rats. A consequence of glomerular capillary hypertension. *J Clin Invest.* 1986;77:797-809.
9. Feld LG, Van Liew JB, Galaske RG, Boylan JW. Selectivity of renal injury and proteinuria in the spontaneously hypertensive rat. *Kidney Int.* 1997;12:332-343.
10. Freedman BI, Iskandar SS, Appel RG. The link between hypertension and nephrosclerosis. *Am J Kidney Dis.* 1995;25:207-221.
11. Glantz SA. *Primer of Biostatistics.* New York: McGraw-Hill, 1981, p. 87-88.
12. Haberle DA, Konigbauer B, Davis JM, Katawa T, Mast C, Metz C, Dahlheim H. Autoregulation of the glomerular filtration rate and the single-nephron glomerular filtration rate despite inhibition of tubuloglomerular feedback in rats chronically volume-expanded by deoxycorticosterone acetate. *Pflügers Arch.* 1990;416:548-553.
13. Hilbert P, Lindpaintner K, Beckmann JS, Serikawa T, Soubrier F, Dubay C, Cartwright P, De Gouyon B, Julier C, Takahasi S, Vencent M, Ganten D, Georges M, Lathrop GM. Chromosomal mapping of two genetic loci associated with blood-pressure regulation in hereditary hypertensive rats. *Nature* 1991;353:521-529.
14. Jiang J, Stec DE, Drummond H, Simon JS, Koike G, Jacob HJ, Roman RJ. Transfer of a salt-resistant renin allele raises blood pressure in Dahl salt-sensitive rats. Hypertension 1997;29:619-627.
15. Karlsen FM, Andersen CB, Leyssac PP, Holstein-Rathlou NH. Dynamic autoregulation and renal injury in Dahl rats. *Hypertension* 1997;30:975-983.
16. Kuijpers MHM, De Jong W. Spontaneous hypertension in the Fawn-Hooded rat: a cardiovascular disease model. *J Hypertens.* 1986;4:S41-S44.

17. Lindpaintner K, Hilbert P, Ganten D, Nadal-Ginard B, Inagami T, Iwai N. Molecular genetics of the S_A-gene: cosegregation with hypertension and mapping to rat chromosome 1. *J Hypertens.* 1996;11:19-23.
18. Nabika T, Nara Y, Ikeda K, Endo J, Yamori Y. A new genetic locus cosegregating with blood pressure in F_2 progeny obtained from stroke-prone spontaneously hypertensive rats and Wistar-Kyoto rats. *J Hypertens.* 1993;11:13-18.
19. Provoost AP. Spontaneous glomerulosclerosis: Insights from the fawn-hooded rat. *Kidney Int.* 1994;45:S2-S5.
20. Provoost AP, De Keijzer MH. The Fawn-Hooded Rat: a Model of Chronic Renal Failure. In: *Experimental and Genetic Rat Models of Chronic Renal Failure.* (Gretz N, Strauch M, eds.) Basel, Karger, 1993;100-114.
21. Roman RJ. Abnormal renal hemodynamics and pressure-natriuresis relationship in Dahl salt-sensitive rats. *Am J Physiol.* 1986;251:F57-F65.
22. Roman RJ, Kauker ML. Renal effects of prostaglandin synthetase inhibition in rats: micropuncture studies. *Am J Physiol.* 1978;235:F111-F118.
23. Semple SJ, DeWardener HE. Effect of increased renal venous pressure on circulatory autoregulation of isolated dog kidneys. *Circ Res.* 1959;7:643-648.
24. Simons JL, Provoost AP, Anderson S, Troy JL, Rennke HG, Sandstrom DJ, Brenner BM. Pathogenesis of glomerular injury in the fawn-hooded rat: early glomerular capillary hypertension predicts glomerular sclerosis. *J Am Soc Nephrol.* 1993;3:1775-1782.
25. 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.
26. Stec DE, Mattson DL, Roman RJ. Inhibition of renal outer medullary 20-HETE production produces hypertension in Lewis rats. *Hypertension* 1997;29:315-319.
27. 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.
28. Van Dokkum RPE, Jacob HJ, Provoost AP. Difference in susceptibility of developing renal damage in normotensive Fawn-Hooded (FHL) and August x Copenhagen Irish (ACI) rats after N^{ω}-Nitro-L-arginine methyl ester induced hypertension. *Am J Hypertens.* 1997;10:1109-1116.
29. Van Dokkum RPE, Sun C-W, Provoost AP, Jacob HJ, Roman RJ. Impaired autoregulation of renal blood flow and myogenic response in the fawn-hooded hypertensive (FHH) rat. *Hypertension* 1998;32:602 (Abstract).
30. 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.
31. Verseput GH, Braam BB, Provoost AP, Koomans HA. Tubuloglomerular feedback and prolonged ACE-inhibitor treatment in the hypertensive fawn-hooded rat. *Nephrol Dial Transplant.* 1998;13:893-899.

32. Yoshioka T, Rennke HG, Salant DJ, Deen WH, Ichikawa I. Role of abnormally high transmural pressure in the permselectivity defect of glomerular capillary wall: a study in early passive Heymann nephritis. *Circ Res.* 1987;61:531-538.

CHAPTER 6

ALTERED RENAL HEMODYNAMICS AND IMPAIRED MYOGENIC RESPONSES IN THE FAWN-HOODED RAT

American Journal of Physiology 1999;276(3):R855-R863

Richard P.E. van Dokkum, Cheng-Wen Sun, Richard J. Roman,
Howard J. Jacob, and Abraham P. Provoost

ABSTRACT

The present study examined whether an abnormality in the myogenic response of renal arterioles impairing autoregulation of renal blood flow (RBF) and glomerular capillary pressure (P_{GC}) contributes to the development of renal damage in Fawn-Hooded Hypertensive (FHH) rats. Autoregulation of whole kidney, cortical and medullary blood flow and P_{GC} were compared in young (12-wk-old) FHH and Fawn-Hooded Low blood pressure (FHL) rats in volume-replete and volume-expanded conditions. Baseline RBF, cortical and medullary blood flow, and P_{GC} were significantly greater in FHH than in FHL rats. Autoregulation of renal and cortical blood flow was significantly impaired in FHH rats compared with results obtained in FHL rats. Myogenically mediated autoregulation of P_{GC} was significantly greater in FHL than in FHH rats. In FHH rats, P_{GC} rose from 46 ± 1 to 71 ± 2 mmHg in response to an increase in renal perfusion pressure from 100 to 150 mmHg, whereas it only increased from 39 ± 2 to 53 ± 1 mmHg in FHL rats. Isolated perfused renal interlobular arteries from FHL rats constricted by 10% in response to elevations in transmural pressure from 70 to 120 mmHg. In contrast, the diameter of vessels from FHH rats increased by 15%. These results indicate that the myogenic response of small renal arteries is altered in FHH rats, and that this contributes to impaired autoregulation of renal blood flow and elevations in P_{GC} in this strain.

INTRODUCTION

The Fawn-Hooded Hypertensive (FHH) rat is a genetic model of hypertension-associated renal failure that develops systolic hypertension, severe albuminuria, and focal glomerulosclerosis (FGS).[23] A closely related control strain of Fawn-Hooded Low blood pressure (FHL) rats does not develop hypertension or renal failure. Previous studies have indicated that elevations in renal blood flow (RBF), glomerular filtration rate (GFR) and glomerular capillary pressure (P_{GC}) precede the development of glomerular disease in FHH rats.[10,25,26] Recently, we reported that autoregulation of RBF and GFR is impaired in FHH rats.[11] This study is also described in chapter 5 of this thesis. We surmised that afferent autoregulation is impaired in FHH rats. Previous studies by Simons *et. al,*[26] indicating that P_{GC} is related to the level of arterial pressure in FHH treated with various antihypertensive agents, indeed suggest that these animals fail to regulate afferent arteriolar resistance appropriately in response to changes in arterial pressure. Potentially, this could be due to an abnormality in tubuloglomerular feedback (TGF) and/or in the myogenic mechanisms that regulate renal vascular tone in the preglomerular vasculature of the kidney.

In this regard, Verseput *et. al.*[28] have recently reported that TGF responses are intact in FHH. Therefore, in the present study, we examined whether the myogenic response of the renal vasculature is altered before the development of FGS in young (12-wk-old) FHH rats and whether this abnormality might contribute to an impairment in autoregulation of RBF and elevations in P_{GC} in these animals. Such a defect should be especially evident after acute volume expansion or in rats fed a high-salt diet, both of which diminish the contribution of TGF to autoregulation of RBF and GFR. Changes in RBF, cortical and medullary blood flow, and P_{GC} in response to elevations in renal perfusion pressure (RPP) were therefore compared in volume-expanded and volume-replete 12-wk-old male FHH and FHL rats. In addition, the myogenic response of renal interlobular arteries microdissected from the kidneys of these animals was directly studied *in vitro*.

METHODS

General

Experiments were performed in male FHH and FHL rats matched for age and body weight. They were all 12-wk-old and weighed ~300 gram at the time of the acute experiments. FHH rats at this age do not yet exhibit significant FGS. The rats were obtained from colonies maintained at the Medical College of Wisconsin, which were derived from the original colony at Erasmus University Rotterdam (FHH/EUR and FHL/EUR) maintained by Dr. Provoost. The rats were housed in an American Association for the Accreditation of Laboratory Animal Care approved animal care facility at the Medical College of Wisconsin, and had free access to food and water throughout the study. On the night before the acute experiments, food intake was restricted to facilitate surgical procedures.

General surgical procedures.

Rats were anesthetized with an intramuscular injection of ketamine (Ketaset, Fort Dodge Laboratories, Fort Dodge, IA) in a dose of 10 mg/kg, followed by a 30 mg/kg intraperitoneal injection of 5-*sec*-butyl-5-ethyl-2-thiobarbituric acid (Inactin, Byk-Gulden, Konstanz, Germany). The animals were placed on a servocontrolled heated surgical table to maintain body temperature at 37°C. The trachea was cannulated using PE-240 tubing to facilitate breathing, and cannulas were placed in the carotid and femoral arteries for measurement of arterial pressure above and below the left renal artery, using a model P23 Gould Statham pressure transducer (Recording System Division; Gould, Cleveland, OH) connected to a model RPS 7C8A Grass amplifier (Grass Instruments, Quincy, MA). Another catheter was placed in the left external jugular vein for constant intravenous infusion, and 1% BSA in 0.9% NaCl was infused at a rate of 100 µl/min throughout the experiment. Because the FHH and FHL rat strains have a bleeding disorder, we had to use a higher infusion rate to replace surgical and fluid losses and maintain a volume-replete state. The left ureter was cannulated for urine collections, and the left kidney was immobilized for micropuncture by placing it in a stainless steel kidney cup. A 1.5- or 2.0-mm flow probe was placed around the left renal artery to allow for measurement of RBF using an electromagnetic flowmeter (Carolina Medical Electronics, King, NC). The left kidney was denervated by stripping all visible nerves from the renal artery and the artery was coated with a 5% solution of phenol in ethanol. A micro-Blalock clamp was placed on the aorta above the renal arteries, and ligatures were placed

around the superior mesenteric and celiac artery to allow for control of RPP. Circulating levels of vasopressin and norepinephrine were fixed at high levels by intravenous infusion (vasopressin, 2.4 U/ml/min; norepinephrine, 100 ng/min; obtained from Sigma, St. Louis, MO).

Protocol 1: Autoregulation of whole kidney, cortical and medullary blood flow

After surgery and a 30-min equilibration period, the relationships between whole kidney, cortical and papillary blood flow and RPP were determined. These studies were performed in volume-replete FHH and FHL rats prepared as described above and in other rats that were volume-expanded by intravenous infusion of 6 ml of a 0.9% NaCl solution containing 6% BSA. The degree of volume expansion was similar in all rats. In each animal, systemic arterial pressure was first increased ~25 mmHg by ligating the celiac and mesenteric arteries. Next, RBF and laser-Doppler red blood cell (RBC) flux signals obtained from the renal cortex and the inner medulla were recorded as RPP was lowered from 150 to 50 mmHg in 10 mmHg steps by tightening the clamp on the aorta above the renal arteries. The kidney was perfused at each RPP for 5 min or until steady-state blood flow signals were recorded.

RBF was measured using an electromagnetic flowmeter and laser-Doppler RBC flux in the renal cortex was measured using a special large-diameter fiber-optic integrating probe (Pf 342) and a dual-channel laser-Doppler flowmeter (model Pf3; Perimed, Stockholm, Sweden). Medullary RBC flux was simultaneously measured using a second Pf3 laser-Doppler flowmeter and a F316 fiber-optic probe coupled to an optical fiber that was implanted at a depth of 5 mm in the kidney and secured using a drop of cyanoacrylic adhesive as previously described.[21] The exact location of the implanted fiber at the junction of the outer and inner medulla was verified at the end of each experiment by dissecting the kidney and viewing the regions surrounding the tip of the fiber. To allow for comparisons of laser-Doppler flow signals between instruments, both laser-Doppler flowmeters were calibrated by placing the probes in a standard solution containing a colloidal suspension of 10-μm latex microspheres (Pf100; Perimed, Stockholm, Sweden) to read a flux value of 2.5 V, and the shifted light intensity was adjusted to read a normalized value of 7.75 V. This calibration is necessary for these instruments to produce a signal proportional to RBC flux rather than velocity alone.

Protocol 2: Micropuncture experiments

These experiments were performed in volume-replete FHH and FHL rats, which were surgically prepared as described above, and the left kidney was placed in a stainless steel kidney cup and surrounded with 2.5% agar (Sigma). The surface of the kidney was constantly bathed with warm (37°C) 0.9% NaCl solution. In each animal, RPP was adjusted to 100, 125, and 150 mmHg by adjusting the resistance of the clamp on the aorta, and hydrostatic pressures were measured in three to five peritubular capillaries, proximal tubules [proximal tubular pressure (P_T)], and star vessels [efferent arteriolar pressure (P_E)] using a 7-µm-OD glass micropipette filled with 2 M NaCl containing Fast Green (Sigma). The pressures were measured using a model 900 servo-null micropressure system (World Precision Instruments, Sarasota, FL) and recorded using a polygraph (Grass Instruments, Quincy, MA). Values for P_{GC} were estimated in four to six different nephrons at each level of RPP using the proximal stop-flow technique. In these experiments, an early proximal tubule was blocked with Sudan-black stained bone wax (Ethicon, W31-G) using a 10- to 12-µm-OD micropipette connected to a hydraulic microdrive unit (Stoelting Instruments). The servo-null pressure-sensing pipette was then introduced upstream of the wax block, and the stop-flow hydrostatic pressure measured.

Protocol 3: Isolated perfused vessels

Rats were anesthetized with a 50 mg/kg intraperitoneal injection of pentobarbital and sodium, and the left kidney was rapidly removed and placed in ice-cold (4°C), bicarbonate-buffered physiological salt solution (PSS) containing (in mM): 144 Na^+, 124 Cl^-, 2.5 Ca^{2+}, 4.7 K^+, 1.2 Mg^{2+}, 1.2 PO^{4-}, 15 HCO^{3-}, 11 glucose, 10 HEPES, and 0.026 EDTA at pH 7.4. The kidney was hemisected, and interlobular arteries (70-100 µm) were microdissected near the junction of the cortex and outer medulla by using a stereomicroscope (x60). One vessel was isolated from the kidney of each animal. Arterial segments 8-10 mm in length were placed in a perfusion chamber, cannulated at both ends with glass pipettes, and secured in place with 10.0 silk suture (Ethicon). Side branches, when present, were ligated with the same suture. The arterial segments were perfused and superperfused with PSS at 37°C. The inflow cannula was connected in series with a pressure reservoir and a transducer (Spectramed, Oxnard, CA). During the measurements, the outflow cannula was clamped off to maintain a given level of transmural pressure as previously described.[16,19]

The internal diameter of the vessel was monitored using a television camera (model KP130; Hitachi, Denshi, Tokyo, Japan) and a stereomicroscope (model DRC; Zeiss, Oberkochen, Germany). The image was displayed on a monitor (model CVM-1271; Sony, Tokyo, Japan), and vessel diameters were measured using a video dimension analyzer (VIA-100; Boeckeler Instruments, Tucson, AZ). Magnification on the screen was approximately x180, and the measurement system was calibrated with a micrometer to a diameter within ± 2.0 μm.

Cannulated rat renal interlobular arteries were maintained at 80 mmHg during a 30-min equilibration period. After an equilibration period of 30 min, the viability of each vessel was tested by constructing a cumulative dose-response curve to phenylephrine (PE) followed by acetylcholine (ACh). Next, a control myogenic response curve was constructed by measuring internal diameter of the vessels as transmural pressure was varied from 50 to 150 mmHg in steps of 10 mmHg. After this control relationship was determined, the procedure was repeated using Ca^{2+}-free PSS. After a 30-min equilibration period, the pressure-diameter curves were redetermined to obtain the passive properties of the vessel.

Glomerular Morphology

After completion of each *in vivo* study, coronal sections of both kidneys were immersed in 3% Formalin. After fixation, 2- to 3-mm slices of renal tissue were embedded in paraffin and prepared for light microscopy. The extent of glomerular damage was determined in 3-μm sections stained with periodic acid-Schiff reagent. In each animal, 50 glomeruli were scored for the presence of sclerotic lesions, mesangial matrix expansion, and adhesion formation between tuft and Bowman's capsule. The extent of glomerular damage was expressed as percentage of glomeruli exhibiting one or more of these features. The incidence of rats exhibiting the different types of renal damage was not assessed separately.

Calculations and statistics

Data are presented as mean values ± 1 SE. Whole kidney blood flow data were factored per gram of kidney weight. RBF autoregulatory indexes over the range of pressures from 100 to 140 (volume-replete) or 150 mmHg (volume-expanded) were calculated as the percentage change in the electromagnetic flow signal divided by the percentage change of RPP. The laser-Doppler flow data are presented as absolute RBF flux values in volts, and the autoregulatory indexes were calculated as described above.

Plasma protein concentrations were measured using a clinical refractometer (model N; Atago), and glomerular capillary oncotic pressure was assumed to equal the oncotic pressure of arterial blood. Plasma oncotic pressure was calculated from the plasma protein concentration using the Landis-Pappenheimer equation[20]

$$\pi = 0.0092\ C^3 + 0.16\ C^2 + 2.1\ C$$

where C is concentration in units of grams per dl (valid over the range of 4-10 g/dl) and π is plasma oncotic pressure in mmHg.

Active tension in the vascular wall was calculated from the measured active and passive vessel diameters using the following equation[22]

$$T_a\ (\text{dyne/cm}) = -\ 1{,}333\ (\text{dyne/cm}^2 \times \text{mmHg}) \times P \times (R_a - R_p) \times 10^{-4}\ (\text{cm}/\mu\text{m})$$

where T_a is the active wall tension, P is the transmural pressure in mmHg, and R_a and R_p are the radii of the vessel in micrometers measured in PSS and calcium-free PSS. Vascular diameters are presented as percentages of the control diameter measured in each vessel when it was perfused and bathed with PSS at a transmural pressure of 70 mmHg (pressure at which these vessels exhibited a maximal diameter). The level of relaxation in response to increasing doses of ACh was calculated as percent of PE (10^{-5} mol/L) preconstricted inner diameter (ID). The inhibitory effect of PE is expressed as the change in diameter reduction in active tension measured in the vessels during the control period at a pressure of 80 mmHg.

Significance of differences in measured values was evaluated using a two-way ANOVA for repeated measures followed by Duncan's multiple range test. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

RBF responses

Autoregulation of RBF was studied in volume-replete FHH and FHL rats, and after plasma volume was expanded, to minimize the contribution of TGF to the RBF response. The results in volume-replete rats are presented in Figure 1A. Control mean arterial pressures (MAP) averaged 149 ± 4 and 131 ± 4 mmHg in FHH (n=12) and FHL (n=9) rats. Baseline RBF measured at these pressures was significantly greater in FHH than in FHL rats, and averaged 9.3 ± 0.5, and 6.9 ± 0.3 ml/min/g kidney wt, respectively. RBF was autoregulated in both FHL and FHH rats over the range of pressures from 100 to 150 mmHg under these experimental conditions. However, autoregulation of RBF was less efficient in volume-replete FHH than in FHL rats. This is reflected in the autoregulatory indexes that were significantly different in volume-replete FHH and FHL rats and averaged 0.36 ± 0.12 *vs.* 0.19 ± 0.09, respectively. We also noted that the time course of the autoregulatory response differed in volume-replete FHH and FHL rats. Autoregulation of RBF was complete within 10 s after a fall in RPP in FHL rats, whereas the time course of the autoregulatory response was different in volume-replete FHH rats, and it generally took 3-4 min for RBF to return to control values after a fall in RPP.

A comparison of the relationships between RPP and whole kidney blood flow after plasma volume expansion in FHH and FHL rats is presented in Figure 1B. Baseline MAP in these rats measured before abdominal surgery averaged 147 ± 3 (n=12) and 132 ± 2 mmHg (n=9). Baseline RBF increased significantly more in FHH than in FHL rats after volume expansion and averaged 11.2 ± 0.5 and 6.7 ± 0.9 ml/min/g kidney wt, respectively. RBF was not well autoregulated after plasma volume expansion in FHH rats, and the RBF autoregulatory index averaged 0.96 ± 0.12 over a range of RPPs from 100 to 150 mmHg. In contrast, FHL rats retained some ability to autoregulate RBF after plasma volume expansion, and the autoregulatory index averaged 0.42 ± 0.04.

The relationship between RPP and cortical blood flow measured by laser-Doppler flowmetry in volume-replete FHH and FHL rats is presented in Figure 2A. Baseline MAP measured before abdominal surgery averaged 154 ± 3 and 129 ± 2 mmHg in volume-replete FHH (n=7) and FHL (n=7) rats, respectively. Baseline cortical blood flow under this condition was significantly higher in FHH than in FHL rats and averaged 3.20 ± 0.25 and 2.94 ± 0.05 V, respectively. Autoregulation of cortical blood flow was less efficient in volume-replete FHH compared with volume-replete FHL rats. Autoregulatory indexes over the

pressure range of 100-140 mmHg in FHH and FHL rats averaged 0.30 ± 0.17 and 0.19 ± 0.06, respectively.

The relationship between RPP and medullary blood flow measured by laser-Doppler flowmetry in volume-replete FHH and FHL rats is presented in Figure 2B. Autoregulation of blood flow was significantly less efficient in the medulla of FHH (n=7) compared with FHL rats (n=7), with indexes over the pressure range of 100-140 mmHg of 0.78 ± 0.29 and 0.37 ± 0.23, respectively.

The relationship between RPP and cortical blood flow in the volume-expanded state is shown in Figure 3A. Somewhat higher perfusion pressures could be obtained in the volume-expanded compared with the volume-replete state. Blood flow over the pressure range of 100-150 mmHg was not efficiently autoregulated under this condition in FHH rats (n=7), with an index of 0.86 ± 0.30. In contrast, blow flow in the cortex of volume-expanded FHL rats (n=5) was more efficiently autoregulated with an index of 0.37 ± 0.20.

Baseline medullary blood flow after acute volume expansion, shown in Figure 3B, was significantly higher in FHH than in FHL rats and averaged 1.71 ± 0.04 and 1.24 ± 0.32 V, respectively. Medullary blood flow was less efficiently autoregulated in volume-expanded FHH rats (n=7) than in FHL rats (n=5), with indexes over the pressure range of 100-150 mmHg of 1.06 ± 0.26, and 0.72 ± 0.20, respectively.

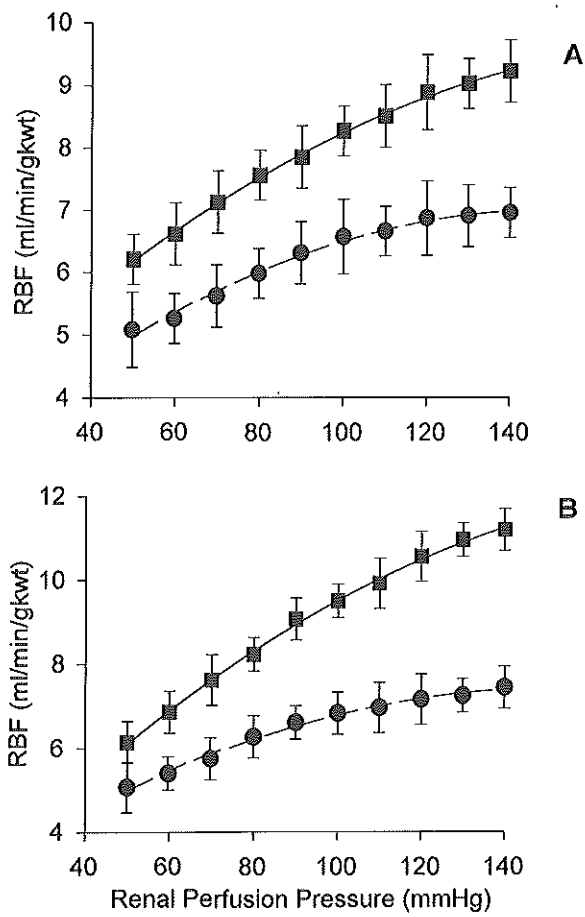

Figure 1A. Relationship between renal perfusion pressure (RPP) and whole kidney renal blood flow (RBF) in ml per minute per gram of kidney weight in volume-replete, and **B.** volume-expanded Fawn-Hooded Hypertensive (FHH, ■, n=12) rats and Fawn-Hooded Low blood pressure (FHL, ●, n=9) rats. Kidney weights averaged 2.50 ± 0.03, and 2.51 ± 0.06 g in FHH and FHL rats, respectively. Values are means ± SE.

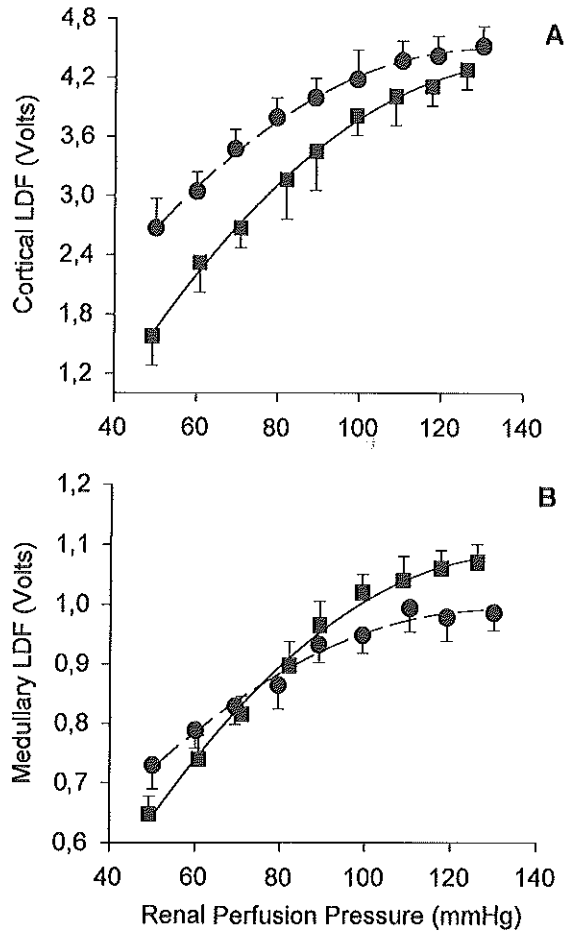

Figure 2A. Relationship between RPP and cortical and **B.** medullary laser-Doppler flux (LDF) in volume-replete FHH (■, n=7) rats and FHL (●, n=7) rats. Values are means ± SE.

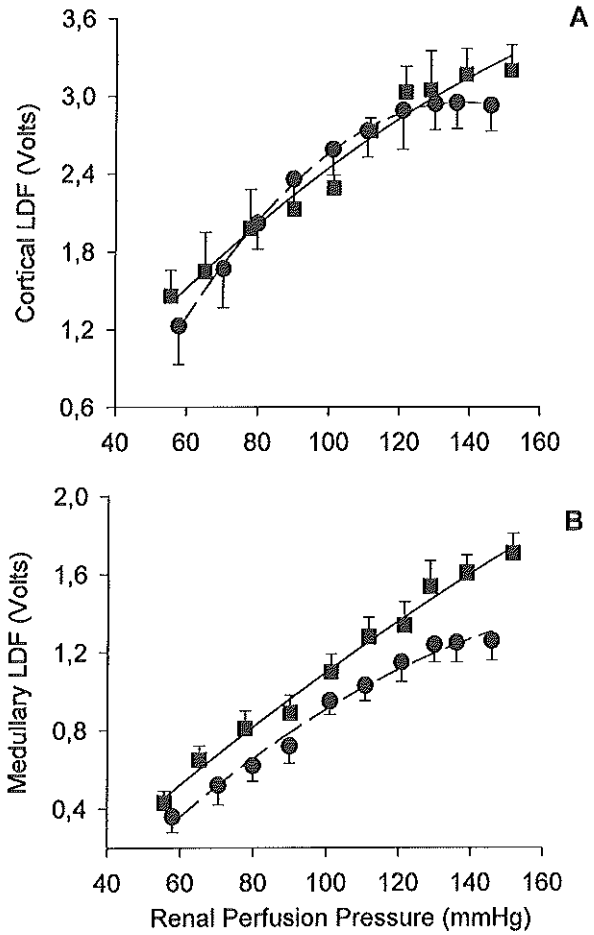

Figure 3A. Relationship between RPP, cortical and **B.** medullary LDF in volume-expanded FHH (■, n=7) rats and FHL (●, n=5) rats. Values are means ± SE.

Micropuncture experiments

A comparison of pressures measured in proximal tubules (P_T), star vessels corresponding with the pressure in efferent arterioles (P_E), and P_{GC} estimated from proximal tubular stop flow pressures (P_{SF}) in FHH and FHL rats is presented in Figures 4 and 5. Control MAP averaged 159 ± 4 mmHg (n=9) in FHH rats and 130 ± 2 mmHg (n=6) in FHL rats. Pressures measured in proximal tubules (Figure 4A) were similar and averaged 22 ± 2, 23 ± 2, and 24 ± 2 mmHg in FHH rats and 19 ± 2, 20 ± 2, and 21 ± 2 mmHg in FHL rats at an RPP of 100, 125, and 150 mmHg, respectively. P_E (Figure 4B) at an RPP of 100 and 125 mmHg averaged 23 ± 2 and 25 ± 2 mmHg in FHH rats and 22 ± 2 and 23 ± 2 mmHg in FHL rats, respectively. At a higher RPP of 150 mmHg, P_E was not autoregulated and rose to 34 ± 1 mmHg in FHH rats. This value was significantly higher than the corresponding value measured in FHL rats (24 ± 2 mmHg). In FHH rats, P_{GC} (Figure 5) increased dramatically as RPP was elevated and averaged 46 ± 1 mmHg at an RPP of 100 mmHg, 58 ± 2 mmHg at an RPP of 125 mmHg, and 71 ± 1 mmHg at an RPP of 150 mmHg. The rise in P_{GC} with an increase in RPP was less dramatic in FHL rats and averaged 39 ± 1, 47 ± 2, and 53 ± 1 mmHg at an RPP of 100, 125, and 150 mmHg, respectively. The relative change in P_{GC} as a function of the relative change in RPP (P_{GC} autoregulatory index) averaged 0.78 ± 0.11 in FHL *vs.* 1.06 ± 0.23 in FHH rats, and showed better preservation of P_{GC} in FHL than in FHH rats.

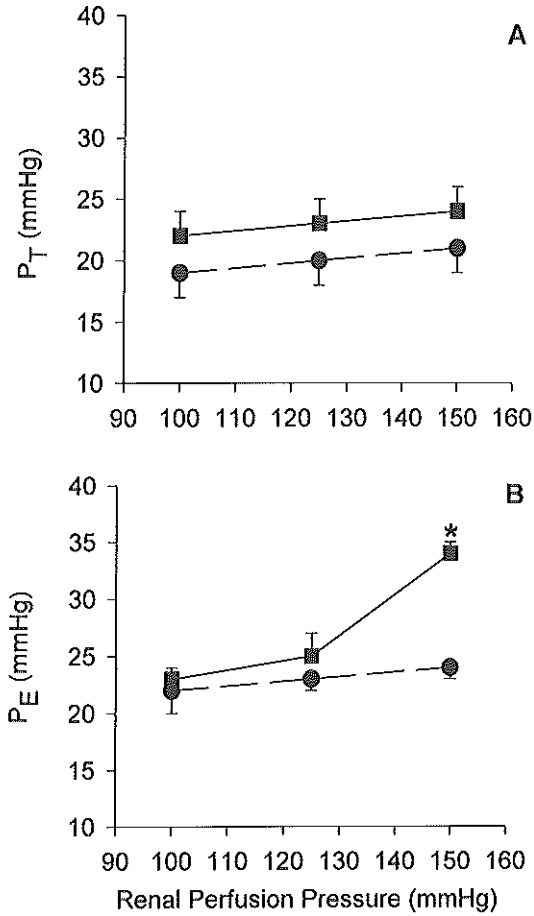

Figure 4A. Relationship between RPP, proximal tubular pressure (P_T) in mmHg, and **B.** efferent arteriolar pressure (P_E) in mmHg in FHH (■, n=9) and FHL (●, n=6) rats. Values are means ± SE. * $p < 0.05$, FHH rats compared with FHL rats.

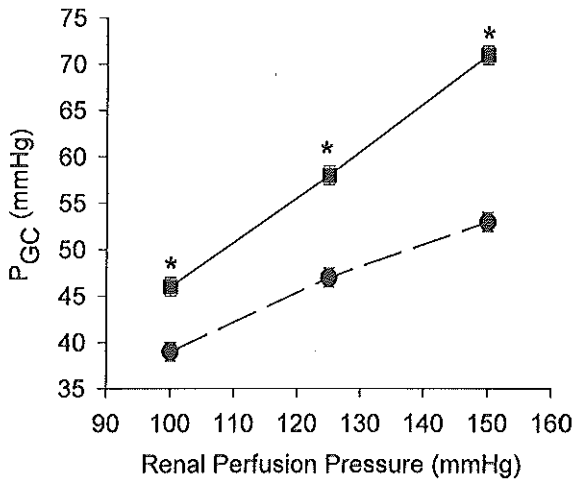

Figure 5. Relationship between RPP and glomerular capillary pressure (P_{GC}) in FHH (■, n=9) rats and FHL (●, n=6) rats. Values are means ± SE. * $p < 0.05$, FHH rats compared with FHL rats.

Myogenic response of renal interlobular arteries

Pressure-diameter relationships in renal interlobular arteries of FHH (n=5) and FHL (n=5) rats are presented in Figure 6A. Baseline diameters of interlobular arteries of FHH and FHL rats at a transmural pressure of 70 mmHg were not significantly different and averaged 99.6 ± 24.3 and 109.5 ± 13.6 µm, respectively. Vessels obtained from the kidneys of FHL rats exhibited a typical myogenic response, and the inner diameter of these vessels decreased to 92.2 ± 2.1% of control in response to an elevation in transmural pressure from 70 to 120 mmHg. After removal of calcium from the bath, the diameter of these vessels increased as the transmural pressure was varied over this same range. In contrast, renal interlobular arteries obtained from the kidneys of FHH rats did not constrict in response to an elevation in transmural pressure. More than that, the diameter of these vessels increased significantly, to 113.1 ± 1.7% of control when pressure was elevated from 70 to 120 mmHg.

The increase in vessel diameter with no calcium in the bath was similar in FHH and FHL vessels, although values were numerically higher in FHH compared with FHL. This might indicate that the inner diameter from vessels from FHH rats increased more in response to an increase in transmural pressure and therefore dilate more in response to increases in transmural pressure in the absence of calcium than vessels from FHL rats.

A comparison of the relationship between the percentage change in active wall tension and transmural pressure in vessels obtained from the kidneys of FHH and FHL rats is presented in Figure 6B. Active wall tension rose from 78 to 597% of active tension at 80 mmHg in response to an increase in transmural pressure from 70 to 120 mmHg in vessels obtained from the kidneys of FHL rats. In contrast, vessels obtained from the kidneys of FHH rats (n=5) failed to respond, and active tension did not increase significantly as pressure was increased over this same range.

To exclude the possibility that the failure of FHH vessels to respond to elevations in transmural pressure was due to nonspecific vascular injury, the vasoconstrictor responses of FHH and FHL rat vessels to phenylephrine were compared. The results of these experiments are presented in Figure 7A. Vessels from the kidneys of FHH and FHL rats constricted similarly to increasing doses of PE. The only significant difference in the dose-response curve was a slightly diminished response in vessels from FHH kidneys at a concentration of 10^{-7} mol/L.

The responses of FHH and FHL renal interlobular arteries to ACh were also compared because endothelial dysfunction has been reported to be common in many experimental and genetic models of hypertension. The results of these experiments are presented in Figure 7B. Acetylcholine increased the diameter of interlobular arteries obtained from the kidneys of FHL rats in a dose-dependent manner to 80% of control. In contrast, vessels obtained from the kidneys of FHH rats did not respond to even relatively high concentrations of ACh (10^{-6} mol/L).

Glomerular Injury

Total kidney weight was not significantly different in FHH and FHL rats, and averaged 2.50 ± 0.03 and 2.51 ± 0.06 g, respectively. The incidence of glomerulosclerosis in the kidneys of these 3-mo-old FHH and FHL rats was not significantly different. Only 1.6 ± 0.6% of the glomeruli exhibited signs of glomerulosclerosis in the kidneys of FHH rats *vs.* an incidence of 1.2 ± 0.4% observed in the kidneys of FHL rats.

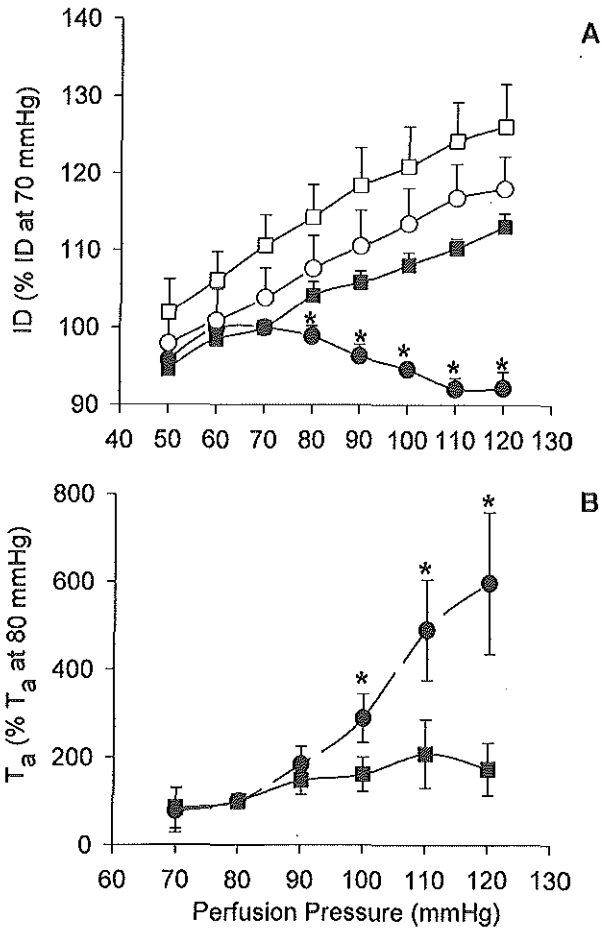

Figure 6A. Relationship between perfusion pressure and inner diameter (ID) of interlobular arteries in %ID at 70 mmHg in calcium containing (closed symbols) and calcium-free (open symbols) medium in interlobular arteries of FHH (■ and □, n=5) and FHL (● and ○, n=5) rats. **B.** Relationship between perfusion pressure and pressure-active tension (T_a) in % T_a at 80 mmHg in interlobular arteries of FHH (■, n=5) and FHL (●, n=5) rats. Values are means ± SE. * $p < 0.05$, FHL rats compared with FHH rats.

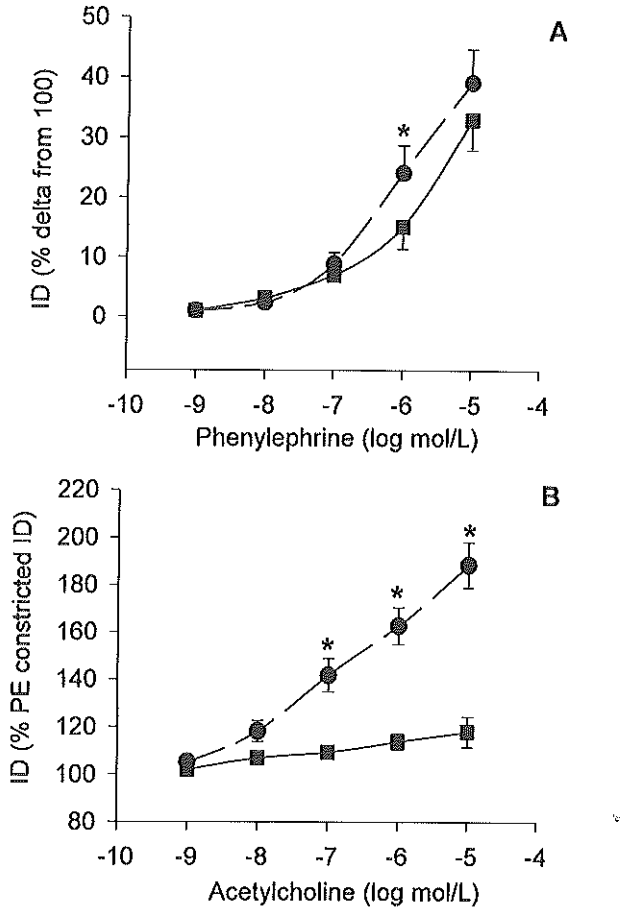

Figure 7A. Relationship between responses to increasing doses of phenylephrine (PE) and ID in % change (delta) from 100 in interlobular arteries of FHH (■, n=5) rats and FHL (●, n=5) rats. **B.** Relationship between responses to increasing doses of ACh in log mol/L and ID expressed as %PE (10^{-5} mol/L) constricted ID in interlobular arteries of FHH (■, n=5) and FHL (●, n=5) rats. Values are means ± SE. * $p < 0.05$, FHL rats compared with FHH rats.

DISCUSSION

The present study compared changes in RBF and cortical and medullary blood flow in response to alterations in RPP in the kidneys of FHL and FHH rats to determine whether an impairment in the myogenic component of renal autoregulation might contribute to the development of glomerular failure in FHH rats. We found that baseline RBF was significantly greater in volume-replete FHH than in FHL rats. Under this experimental condition, FHH and FHL rats both autoregulated RBF. However, the efficiency of autoregulation was slightly but significantly greater in FHL than in FHH rats. The most striking difference was seen in the time course of the response. Autoregulatory adjustments in renal vascular resistance were complete within seconds in FHL after a reduction in RPP, whereas it typically took more than 3-4 min for RBF to return to control in FHH rats. This finding suggests that the rapid myogenic component of autoregulation may be impaired in FHH rats but that the TGF component of renal autoregulation, which exhibits a much longer time constant, is still intact. This view is also consistent with the recent findings of Verseput *et. al.*,[28] who found and intact TGF response in FHH rats studied at about the same age as those used in the present study.

This hypothesis is further supported by the results of experiments performed in volume-expanded FHH and FHL rats to minimize the contribution of TGF to autoregulation of RBF.[15] Baseline whole kidney, cortical, and medullary blood flows were markedly elevated in volume-expanded FHH rats. Under these conditions, FHH rats did not autoregulate whole kidney, cortical or medullary blood flow as efficient as FHL rats. Overall, these results suggest that the myogenic response of the preglomerular vasculature to changes in transmural pressure is markedly impaired in FHH rats.

Micropuncture experiments were performed to further evaluate this hypothesis. P_{GC} was estimated from proximal tubule stop flow pressures and compared in volume-replete FHH and FHL rats at different levels of RPP. Under these conditions, flow to the macula densa is interrupted and the contribution of TGF to the autoregulation of P_{GC} is eliminated. Our results indicate that volume-expanded FHL rats retain some ability to autoregulate P_{GC} via activation of myogenic mechanisms but FHH rats do not. Indeed, the percentage change in P_{GC} as a function of RPP (P_{GC} autoregulatory index) averaged 0.78 in FHL rats versus 1.06 in FHH rats. Another interesting observation is that, although P_{GC} was not autoregulated and markedly elevated in FHH rats, there was little difference in the relationship between pressures in the peritubular capillaries and RPP in FHH and FHL rats. This implies that the resistance of the efferent arteriole must have increased in FHH rats because RPP was elevated. This may be related to some

capacity of the efferent arteriole to respond actively to elevations in P_{GC}, or to some unknown mechanism that remains to be explored.

The hypothesis that the myogenic response of the preglomerular vasculature is altered in FHH rats was directly tested using isolated perfused renal interlobular arteries. The results of these studies indicate that vessels obtained from FHL rats constricted and active wall tension increased in response to an elevation in transmural pressure, whereas vessels obtained from FHH rats failed to exhibit a myogenic response. Indeed, the diameter of these vessels increased in response to an elevation in transmural pressure, and there was no significant difference in the pressure-diameter curve in these vessels studied in the presence and absence of calcium in the bath. These isolated vessel data further support the micropuncture data and indicate that an impairment in the myogenic response of the preglomerular vasculature of FHH rats contributes to the lack of autoregulation of RBF and P_{GC} in these animals, especially after acute volume expansion. However, impairment of the myogenic responses in interlobular arteries alone should not have that much impact on autoregulation of RBF or P_{GC} because in the rat, most of the preglomerular pressure drops occur along the afferent arteriole. For this reason, we believe that myogenic response must be impaired throughout the preglomerular renal vasculature and particularly in the afferent arteriole in the kidney of the FHH rat.

The inability of renal interlobular arteries from FHH rats to respond to elevations in transmural pressure was not due to nonspecific vascular damage, because they exhibited a normal vasoconstrictor response to PE. We did, however, find that the vasodilator response to acetylcholine was blunted in FHH rats. The importance of this observation remains to be determined, but it is consistent with a large body of emerging evidence indicating that hypertension is often associated with endothelial dysfunction.[5,7,8,19,24]

Perspectives.

The results of the present study indicate that the myogenic response of preglomerular renal arteries is impaired in FHH rats, and that they exhibit an impaired ability to buffer changes in intraglomerular pressure, especially in response to rapid fluctuations in arterial pressure. This defect in the myogenic response of the preglomerular vasculature, in combination with the previously reported increased efferent vascular resistance that elevated baseline P_{GC},[25,26] and the tendency of these animals to develop systolic hypertension, promotes the transmission of elevated pressures to the glomerulus. Because elevations in P_{GC} have been previously linked to the development of glomerulosclerosis in many different experimental models of renal disease,[2,14,25] it is likely that these also contribute to the development of proteinuria and renal failure in FHH rats as well, and probably greatly depend on the genetic susceptibility of this rat strain to develop renal failure.[4,12,13]

Acknowledgments

Studies were performed with financial support from the National Heart, Lung, and Blood Institute (Grant R01-HL-36279 to R.J Roman and Grant R01-HL-56284-01 to H.J Jacob and A.P Provoost). The work of R.P.E. van Dokkum at the Medical College of Wisconsin was supported by grants from the Royal Dutch Academy of Sciences (VWF97/SCW/42), the Trustfund of Erasmus University Rotterdam (97030.35/96.0311/evt), and by foundation 'The Three Lights' (96/54 AH/rvv) of The Netherlands.

Parts of this study were presented in May 1998 at the *13th Scientific Meeting of the American Society of Hypertension*, New York, NY, in June 1998 at the *17th Scientific Meeting of the International Society of Hypertension*, Amsterdam, The Netherlands, and in September 1998 at the *52nd Annual Fall Conference and Scientific Sessions of the Council for High Blood Pressure*, Philadelphia, PA and have been published in abstract form (*Am J Hypertens.* 1998;11:1A, *J Hypertens.* 1998;16:S189, and *Hypertension* 1998;32:602).

REFERENCES

1. Arendshorst WJ, Finn WF, Gottschalk CW. Autoregulation in the rat kidney. *Am J Physiol.* 1975;228:127-133.
2. Azar S, Johnson MA, Scheinman J. Regulation of glomerular capillary pressure and filtration rate in young Kyoto hypertensive rats. *Clin Sci.* 1979;56:203-209.
3. Bidani AL, Schwartz MM, Lewis EJ. Renal autoregulation and vulnerability to hypertensive injury in remnant kidney. *Am J Physiol.* 1987;252:F1003-F1010.
4. 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 Gen.* 1996;12:44-51.
5. Carmines PK, Inscho EW, Gensure RC. Arterial pressure effects on preglomerular microvasculature of juxtamedullary nephrons. *Am J Physiol.* 1990;258:F94-F102.
6. Casellas D, Moore LC. Autoregulation of intravascular pressure in preglomerular juxtamedullary vessels. *Am J Physiol.* 1993;264:F315-F321.
7. Chen Y-M, Holstein-Rathlou N-H. Differences in dynamic autoregulation of renal blood flow between SHR and WKY rats. *Am J Physiol.* 1993;264:F166-F174.
8. Clausen G, Øien AH, Aukland K. Myogenic vasoconstriction in the rat kidney elicited by reducing perirenal pressure. *Acta Physiol Scand.* 1992;144:277-290.
9. Cowley AW, Roman RJ, Fenoy FJ, Mattson DL. Effect of renal medullary circulation on arterial pressure. *J Hypertens.* 1992;10:S187-S193.
10. De Keijzer MH, Provoost AP, Molenaar JC. Glomerular hyperfiltration in hypertensive fawn-hooded rats. *Renal Physiol Biochem.* 1988;11:103-108.
11. Dokkum RPE van, 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(1):in press.
12. Dokkum RPE van, Jacob HJ, Provoost AP. Genetic differences define severity of renal damage after L-NAME-induced hypertension in rats. *J Am Soc Nephrol.* 1998;9:363-371.
13. Dokkum RPE van, Jacob HJ, Provoost AP. Difference in susceptibility to develop renal damage in normotensive Fawn-Hooded (FHL) and ACI rats after L-NAME-induced hypertension. *Am J Hypertens.* 1997;10:1109-1116.
14. Dworkin LD, Feiner HD. Glomerular injury in uninephrectomized spontaneously hypertensive rats. A consequence of glomerular capillary hypertension. *J Clin Invest.* 1986;77:797-809.
15. Feldberg R, Colding-Jørgensen M, Holstein-Rathlou N-H. Analysis of interaction between TGF and the myogenic response in renal blood flow autoregulation. *Am J Physiol.* 1995;269:F581-F593.
16. Harder DR. Pressure-induced myogenic activation of rat cerebral arteries is dependent on intact endothelium. *Circ Res.* 1989;60:102-107.
17. Harrison-Bernard LM, Navar LG. Renal cortical and medullary microvascular blood flow autoregulation in rat. *Kidney Int.* 1996;50:S23-S29.

18. Holm L, Morsing P, Casellas D, Persson AEG. Resetting of the pressure range for blood flow autoregulation in the rat kidney. *Acta Physiol Scand.* 1990;138:395-401.
19. Kauser K, Stekiel WJ, Rubanji G, Harder DR. Mechanism of action of EDRF on pressurized arteries: Effect on K^+ conductance. *Circ Res.* 1989;65:199-204.
20. Kauser K, Clark JE, Masters BS, Ortiz de Montellano PR, Ma YH, Harder DR, Roman RJ. Inhibitors of cytochrome P-450 attenuate the myogenic response of dog renal arcuate arteries. *Circ Res.* 1991;68:1154-1163.
21. Mattson DL, Lu S, Roman RJ, Cowley AW. Relationship between renal perfusion pressure and blood flow in different regions of the kidney. *Am J Physiol.* 1993;264:R578-R583.
22. Monos E, Contney SJ, Cowley AW, Stekiel WJ. Electrical and mechanical responses of rat saphenous vein wall exposed to short-term pressure loads. *Am J Physiol.* 1989;256:H47-H55.
23. Provoost AP. Spontaneous glomerulosclerosis: Insights from the fawn-hooded rat. *Kidney Int.* 1994;45:S2-S5.
24. Sanchez-Ferrer CF, Roman RJ, Harder DR. Pressure-dependent contraction of rat juxtamedullary afferent arterioles. *Circ Res.* 1989;64:790-798.
25. Simons JL, Provoost AP, Anderson S, Troy JL, Rennke HG, Sandstrom DJ, Brenner BM. pathogenesis of glomerular injury in the fawn-hooded rat: early glomerular capillary hypertension predicts glomerular sclerosis. *J Am Soc Nephrol.* 1993;3:1775-1782.
26. 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.
27. Takenaka T, Harrison-Bernard LM, Inscho EW, Carmines PK, Navar LG. Autoregulation of afferent arteriolar blood flow in juxtamedullary nephrons. *Am J Physiol.* 1994;267:F879-F887.
28. Verseput GH, Braam BB, Provoost AP, Koomans HA. Tubuloglomerular feedback and prolonged ACE-inhibitor treatment in the hypertensive fawn-hooded rat. *Nephrol Dial Transplant.* 1998;13:893-899.
29. Wang X, Aukland K, Ofstad J, Iversen BM. Autoregulation of zonal glomerular filtration rate and renal blood flow in spontaneously hypertensive rats. *Am J Physiol.* 1995;269:F515-F521.

CHAPTER 7

GENERAL DISCUSSION

CONCLUSIONS

PERSPECTIVES

7.1 GENERAL DISCUSSION

Evidence in humans and experimental and genetic animal models of hypertension and renal failure suggest that the development of hypertension-associated renal failure depends on the genetic background.[29,31,32,52,63,99] The Fawn-Hooded Hypertensive (FHH) rat is a genetic model for hypertension-associated renal failure and develops systolic and intraglomerular hypertension, leading to proteinuria (UpV) and focal glomerulosclerosis (FGS), whereas the Fawn-Hooded Low blood pressure (FHL) rat does not.[72] The FHH rat has several genes for renal failure (at least five) and hypertension (at least two).[17,82] Experiments described in this thesis examined the susceptibility for renal damage after L-NAME-induced hypertension in rat strains with different genetic backgrounds. Physiological experiments were described studying the impact of increases in renal perfusion pressure on renal failure in FHH and FHL rats.

7.1.1 Physiology, genetics, and the susceptibility to renal damage

The FHH and FHL rat carry two genes involved in the development of renal failure, *Rf-1* and *Rf-2*, but have a different susceptibility to develop renal damage after the induction of hypertension. This might be caused by a different mutation in the *Rf*-genes in the FHL rat or by the interaction of these genes with other genes that protect the kidney of the FHL rat from developing intraglomerular hypertension. The difference might also be due to the lower blood pressure level in FHL rats. Heterozygous F_1 rats from a cross of the FHH rat and the renal damage resistant ACI rat, develop less renal damage upon blood pressure elevation than FHL rats, although we found a significant increase in renal damage. This increase was observed when systemic blood pressure exceeds a certain threshold level, which might be related to baroreceptor function. Surmising that the gene for this receptor is mutated in FHH rats, this means that being heterozygous for the gene as in F_1 rats needs a certain pressure level for the mutation to become apparent.

In rats, only one study, by Churchill *et al.*, has directly investigated the genetic susceptibility to develop renal damage. Transplantation of a kidney from a congenic SHR, carrying a region from chromosome one of the Brown-Norway rat, indicated the presence of one or more genes modifying the susceptibility to hypertension-related renal failure.[20] At least one of the chromosomal regions identified by Brown *et al.*[17] overlaps with a segment of chromosome one that has recently been reported to contain a gene influencing susceptibility to stroke in the stroke-prone SHR.[77] Interestingly, this area on chromosome 1 contains the *Rf-2*

region. This might indicate that chromosome one contains genes increasing the risk for hypertension-induced vascular injury, not only in the kidney, but also in the brain.[77] This points towards common mechanisms in the development of end-organ damage, and the vascular abnormalities observed in the FHH rat might very well be present in other organ systems. Therefore, it might be useful to evaluate other organ systems for their regulatory abilities, for instance, to measure autoregulation of cerebral blood flow. Finally, studies in transgenic and gene knock-out mice revealed increased susceptibility to develop renal damage.[65,86]

7.1.2 L-NAME, hemodynamics, and the susceptibility to renal damage

The different responses to L-NAME-induced hypertension in the rat strains with two kidneys and after unilateral nephrectomy (UNX) observed in this thesis are summarized in Table 1. Most previous studies do not directly assess the relation between blood pressure and renal damage and are difficult to compare with our studies, also because blood pressure measurements and glomerular injury scores were different. The doses used were much higher than the doses used in our studies, yet the animals developed less hypertension and renal damage.[5,85,91]

Table 1. Comparison of several parameters in different strains used in this thesis after L-NAME-induced hypertension.

Strain	time	dose	n	SBP	UaV	FGS	Ch.
FHH-2K	7	9	15	209 ± 20	211 ± 89	31 ± 7	3
FHL-2K	7	23	6	199 ± 11	191 ± 72	25 ± 5	2
F_1-2K	7	13	10	201 ± 16	30 ± 25	20 ± 4	3
ACI-2K	11	18	6	206 ± 18	13 ± 9	7 ± 2	2, 3
F_1-UNX	12	13	10	212 ± 12	109 ± 48	24 ± 4	4
ACI-UNX	12	8	10	165 ± 15	22 ± 9	10 ± 6	4

FHH, Fawn-Hooded Hypertensive rat; FHL, Fawn-Hooded Low blood pressure rat; F_1, (FHH x ACI) rat; ACI, August x Copenhagen Irish rat; 2K, two kidneys; UNX, unilateral nephrectomy; time, weeks after start of treatment; n, number of rats; SBP, systolic blood pressure measured by tail-cuff; UaV, urinary albumin excretion in mg/24h; FGS, focal glomerulosclerosis (% incidence); Ch., chapter of this thesis.

Table 1 shows equal functional and structural renal damage in two-kidney FHH and FHL rats at similar blood pressure levels. However, in FHL rats, higher L-NAME doses induced similar blood pressure levels as in FHH rats. Differences in basal SBP level between FHH rat and FHL rats most likely account for this phenomenon. A similar level of renal damage at similar SBP in both Fawn-Hooded strains might indicate that these strains share most of their genome with regard to the *Rf*-genes. The functional renal damage in both Fawn-Hooded strains is much larger than that of F_1 (FHH x ACI) rats at similar blood pressure levels. However, the structural renal damage in FHL and F_1 rats is similar. The F_1 rats in turn develop more renal damage than the ACI rats. This was even more pronounced after UNX, although ACI rats did not receive the same amount of L-NAME and did not develop the same blood pressure level as the F_1 rats. Thus, with regard to the susceptibility to renal damage after similar levels of L-NAME-induced hypertension, we can give the following equation:

$$\mathbf{FHH = FHL >> F_1 > ACI.}$$

Simons *et al.* showed that FHH rats treated with L-NAME exhibit high systemic blood pressure, heavy UpV, further elevation of the ultrafiltration pressure with a decrease in the ultrafiltration coefficient, and high incidence of FGS after only eight weeks of treatment.[85] Glomerular filtration and perfusion were diminished and single nephron filtration fraction increased.[83] Total renal vascular resistance was increased threefold, and proportional increases in afferent and efferent arteriolar resistance were observed. These observations are in agreement with previous findings in two-kidney[5,33] and remnant Munich-Wistar rats.[33] Rats responding to renal mass reduction by an increase in the ultrafiltration coefficient, while maintaining normal P_{GC}, are protected from progressive glomerular injury. The Wistar-Kyoto (WKY) rat is exemplary as well: UNX[25] and remnant WKY rats[7] show a compensatory increase in single nephron glomerular filtration rate (GFR), which is mainly due to an increase in the ultrafiltration coefficient, whereas P_{GC} remains at a normal level. Both models of renal ablation in WKY rats are remarkably resistant to the development of FGS.[7,25] Ono *et al.*, studying L-NAME treated SHR rats, found that single nephron plasma flow and the ultrafiltration coefficient were reduced despite an unchanged single nephron GFR and P_{GC}.[67,68] This may be explained by the severely increased afferent resistance and a lesser increase in efferent resistance associated with the reduced single nephron plasma flow. In support of these findings, Herrera-Acosta *et al.* also reported a sharper increase in afferent resistance and a greater decrease of single nephron glomerular filtration rate with L-NAME in Goldblatt hypertensive rats compared with normotensive rats.[43] In

contrast, FHH rats treated with L-NAME do not show increased afferent resistance that prevent the elevated levels of systemic blood pressure from entering their glomerular (micro)circulation.

The findings by Simons *et al.* in UNX-FHH rats treated with L-NAME may indicate that the mechanism maintaining a stable P_{GC} is less effective.[55,83] It remains to be determined, however, whether the observed P_{GC} differences are to some extent due to suppression of the tubuloglomerular feedback (TGF) response during stop-flow conditions or due to altered autoregulation as a consequence of UNX.[70] The L-NAME studies described in chapters 2, 3, and 4 of this thesis are well in accordance with these observations. Although P_{GC} was not assessed in the rats used in these studies, the susceptibility to develop renal damage might be directly related to the P_{GC} level. The homozygous FHH rat in our studies appeared to be extremely susceptible to renal damage due to high P_{GC}. Susceptibility to renal damage in the FHL rat is probably also related to elevated P_{GC} levels after L-NAME-induced hypertension. An important observation in this regard is that FHL rats have slightly elevated P_{GC} levels under normal conditions.[84] The heterozygous F_1 rat seems to develop renal damage above a certain pressure level (chapter 3). Beyond this level, P_{GC} could probably not be maintained at low levels, initiating the development of renal damage. The renal damage resistant ACI rat is able to maintain low GFR levels (chapter 5 of this thesis), which might indicate that these rats are also capable to maintain low P_{GC} levels, even after considerable blood pressure elevation. The data indicate that the development of renal failure is related to genetic susceptibility to elevated P_{GC}, which is possibly related to the ability of the afferent arteriole to respond adequately to changes in systemic blood pressure. Moreover, an inherited weakness in the structure of the capillary wall may be another factor.[54,55] The studies described in chapter 6 of this thesis seem to confirm this hypothesis.

7.1.3 Hemodynamic studies

Data from animal models indicate that preservation of normal glomerular hemodynamics is fundamental to minimize the progression of glomerular damage in hypertension,[13] diabetes,[45] and after reductions in renal mass.[1,2,46] As stated before in this thesis, glomerular damage develops differently in different strains of hypertensive rats. For example, SHR rats have a filtration area similar to that in control WKY rats, but show a reduced filtration rate before the development of hypertension, possibly due to a reduced ultrafiltration coefficient. SHR rats present with early hypertrophy of the afferent arteriole, which increases its resistance and protects the glomerulus from elevations in P_{GC} and glomerular

blood flow when hypertension develops. In contrast, the FHH rat develops only mild systemic hypertension (chapter 5), but shows increased efferent arteriolar resistance in combination with the inability to increase afferent vascular tone, resulting in glomerular hypertension.[95] The data from the FHH rat so far indicate that abnormalities in the control of renal vascular resistance may play a greater role in the development of renal failure than the presence of hypertension *per se*.

Data generated by Verseput *et al.*[96] and by Simons *et al.*[84] together with the results described in chapter 6 of this thesis, indicate that operating P_{GC} in the young FHH rat is elevated during normal flow to the macula densa, whereas operating P_{GC} in SHR and MHS rats is normal.[8,18,23,71] However, in the MNS rat the development of FGS is thought to be caused by impaired TGF,[8] which also leads to increases in P_{GC} and histologic damage, similar to that in the FHH rat.[54] A relatively high TGF responsiveness is thought to contribute to the development of hypertension on account of an associated depression of single nephron GFR.[9] The diminished resistance of the afferent arterioles in FHH rats allows P_{GC} and GFR to be elevated at lower systemic pressures than in other hypertensive strains. The glomerular hemodynamics in the FHH rat may thus limit the degree of systemic hypertension, though at the cost of increasing the risk to develop FGS.[95] The fact that the efferent arteriolar pressure in FHH rats significantly increased when renal perfusion pressure was raised from 125 to 150 mmHg (chapter 6) is in accordance with a threshold level for the systemic pressure to be transmitted into the glomeruli, as observed in F_1 (FHH rat x ACI) rats (chapter 3).

It remains to be determined why in the FHH rat, afferent arteriolar resistance is low and control by the TGF system relatively inefficient. These phenomena may be related to a generalized abnormality in mechanisms controlling myogenic tone in the preglomerular vasculature as indicated by our isolated vessel data in chapter 6 of this thesis. In this respect, the behavioral characteristics of the renal vasculature in the FHH rat resemble that of diabetic and galactose-fed rats.[30,40] Altered glomerular hemodynamics in the FHH rat, such as impaired myogenic response and impaired autoregulation of renal blood flow (RBF), P_{GC}, and GFR (chapter 6) are also observed in spontaneous and induced rat models of diabetes and rats with glomerulonephritis.[27,38,50,69,81] However, these abnormalities might be of different origin. The hypothesis presented here, *i.e.*, impaired autoregulation leads to renal failure, is a novel approach compared to that reported in most papers, supporting the hypothesis that glomerular failure leads to impaired renal autoregulation.

Hypertension in the FHH rat is very sensitive to blockers of the renin-angiotensin system,[85,98] indicating important interaction in the (micro)vasculature, between the abnormality in vascular reactivity and the prevailing levels of angiotensin II (Ang II). Since high Ang II activity is associated with enhanced

TGF activity in hypertensive models such as the two-kidney, one-clip model[9] and the transgenic mRen-2 rat,[65] one could surmise that, in the FHH rat, the endogenous defense against the afferent arteriolar constriction by Ang II is characterized by a paracrine disturbance. In this respect, the bleeding diathesis and platelet dysfunction due to defective serotonin and adenosine di-phosphate (ADP) release,[92,93] and the increased urinary excretion of eicosanoids[21] might be important.[42,95]

The P_{GC} level in FHL rats was found to be higher than in other normotensive strains.[84] Moreover, autoregulation of RBF and P_{GC} was less efficient in FHL rats compared with other normotensive strains.[25,84] The data indicate that the FHL rat also has some degree of abnormal renal hemodynamics similar to the FHH rat, but might be protected because of low systemic blood pressure or different mutations in the *Rf*-areas. Indeed, levels for GFR, P_{GC}, and RBF in the FHL rat in response to changes in renal perfusion pressure were in between those for ACI and FHH rats.

The studies in interlobular arteries of FHH rats showed numerically higher increases in vessel diameter compared with FHL rats in the absence of calcium. This might indicate an increased calcium-independent dilation in vessels from FHH rats in response to increases in transmural pressure. This might also explain the decreased efficacy of calcium antagonists in the prevention and treatment of renal disease in FHH rats compared with ACEi.[98] Therefore, an impairment might be present in the function of the calcium-dependent potassium channels (K_{Ca}), which are thought to buffer vasoconstrictor responsiveness to a variety of agonists.[16,24,78-80] A large body of evidence shows increased activity and upregulation of these channels in different models of hypertension.[26,28,56,57] Patch-clamp studies need to be performed in order to compare single-channel K_{Ca} channel currents between isolated membrane patches of renal vascular smooth muscle cells from FHH and normotensive control rats. Recent studies describing the involvement of NO and cytochrome P-450 metabolites of arachidonic acid (AA) in the activation of the K_{Ca} channels and the regulation of vascular tone[36,37,47] might provide other avenues for investigating the abnormalities in control of renal vascular tone in the Fawn-Hooded rat.[15,39-41]

Recent genetic analysis by Stelzner *et al.* showed the presence of an unknown genetic locus on chromosome one, called *PH1*, which was linked to pulmonary hypertension in the FH/IR rat.[88,89] However, genetic linkage with the earlier found increased ET-1 production by FH/IR rat lung endothelium could not be established.[88,89]

The pulmonary artery of the FH/IR rat used by Ashmore *et al.*[4] also exhibited increased constrictor sensitivity to serotonin (5-HT), which may also contribute to the paradoxical responses to aggregating platelets in arteries of this rat.[53,94]

Similar to ADP, 5-HT may also stimulate release of an endothelium-derived constrictor.[58-60] Alternatively, although the overall response to 5-HT in rat vessels is constriction, the endothelium may modulate these responses by the release of endothelium-derived relaxing factor (EDRF).[58-60] Therefore, 5-HT-induced release of EDRF may be impaired in the FHH rat, as in humans.[3,14] Preliminary data on the responses of isolated renal arteries of FHH rats to various pharmacological agents showed no profound differences in response to constrictor or dilator agents compared with FHL and Wistar control rats. This suggests that there is no specific pharmacological pathway involved in the impairment of the myogenic response in the FHH rat, favoring an impairment in pressure-induced mechanosensor mechanisms. Also, the impaired dilatory response of FHH rat interlobular arteries to acetylcholine (chapter 6) deserves to be explored further.

Findings by Roman *et al.* suggest that inhibition of 20-HETE, a potent vasoconstrictor formed from AA by the cytochrome P-450 enzyme, contributes to the cyclic GMP independent actions of NO and mediates myogenic responses.[48,76,101] Thus, in the FHH rat, vasodilatory epoxy-eicosatrienoic acids (EETs) may be elevated and 20-HETE production reduced in FHH vessels. Preliminary data from Roman *et al.* in kidneys of FHH rats show that this might actually be true.

In general, the release of NO is impaired in animal models of hypertension.[49,58,64] Therefore, an impairment in the production or release of EDRFs could explain the effect seen in the renal vessels of the FHH rat (chapter 6). Because studies in this rat showed increased resistance of the efferent arteriole, this impairment might be due to the inability of producing relaxing factors or to a decreased vascular responsiveness to nitric oxide in this arteriole. In addition, the increased susceptibility to the effects of NOi in the FHH rat (chapter 3) might be due to the inability of the afferent arteriole to constrict in response to NOi because of increased levels of EDRF.[85] Indeed, Resch *et al.* reported increased NO synthase activity in the macula densa of the FHH rat.[75] On the other hand, the reduced responses to acetylcholine in renal arterioles of the FHH kidney (chapter 6) are in favor of a decreased NO release, as observed in the Dahl salt-sensitive rat and in the hypertension-prone Sabra rat,[39,58,74] dependent on which NO synthase gene is expressed. The expression of this gene might also be important in the FHH rat. The SHR, however, is thought to have an increased production of endothelium-derived hyperpolarizing factor, but not a deceased NO production. The DOCA-salt model of hypertension, which shares altered renal hemodynamic responses such as impaired autoregulation of RBF and impairments in the myogenic response[40] with the FHH rat, showed an

enhanced expression of endothelin-1 in blood vessels and glomeruli, especially in mesangial cells.[22]

7.1.4 Impact of gene discovery on renal failure in rat and man

The finding that the genetic background controls the development of renal failure in a hypertensive animal model, raises the possibility that the same may be true for humans with hypertension who develop renal failure. Clinical and epidemiological data support this hypothesis, because improved blood pressure control has reduced the incidence of heart failure and stroke, but not ESRF. Moreover, renal failure only occurs in a subset of patients with hypertension. Familial clustering of hypertension-associated complications has not been extensively studied, although a few reports suggest familial correlation.[29,52]

Hypertension results in renal damage in a limited percentage of patients only. Apparently other - genetic - factors are important in determining the susceptibility to develop renal failure. The *Rf*-loci revealed in the genetic studies with the FHH rat may be important contributors to the development of renal failure. However, the question remains whether this renal failure is hypertension-associated. The strongest evidence provided so far, is that renal failure in the FHH rat is prevented by antihypertensive treatment with ACEi, although this protection could be independent of its effects on blood pressure.[97] However, this does not exclude the possibility that *Rf*-genes may cause renal impairment in both hypertensive and normotensive humans and animals when the *Rf*-loci are general loci affecting renal function. As all forms of renal disease are exacerbated by increases in blood pressure,[11] it is possible that the effect of *Rf*-genes on renal physiology only becomes readily apparent later in life in the presence of high blood pressure.

The *Rf-2* locus is interesting because its differential effect on plasma albumin levels is substantially larger than for *Rf-1*, suggesting that this index of severe renal impairment may be independent of UpV. Although the effect of *Rf-2* on plasma albumin alone could be explained by a gene affecting albumin synthesis rather than renal impairment, the fact that *Rf-2* also affected the macroscopic renal index score suggests that it acts at the level of the kidney. Because *Rf-2* maps near another factor affecting blood pressure, it was difficult to assess the effect of this gene on blood pressure. Therefore, *Rf-2* might act through a mechanism other than a direct effect on blood pressure. *Rf-2* maps to the same region as the platelet storage-pool bleeding disorder in the fawn-hooded rat and could be identical with the gene causing this disorder and may have an impact on renal hemodynamics. There are several reports of patients with renal failure and

various platelet disorders including a platelet storage pool disease.[34,35] However, the FH/IR rat also has prolonged bleeding times but does not develop renal failure. Furthermore, the region containing *Rf-2* is homologous to a region on mouse chromosome 7 that contains loci involved in renal failure associated with systemic lupus erythematosus.[66]

Genetic susceptibility for end-organ damage independent from blood pressure is not restricted to the kidney. In recent years, others have reported on QTLs affecting cardiac hypertrophy and stroke.[77] End-organ damage in susceptible individuals may develop with normal blood pressure, but is usually accelerated when hypertension is present. In complex situations, animal models can be useful to reduce the complexity and help to elucidate mechanisms that may be important in the progression of human renal disease.

Cloning of the *Rf*-genes will allow isolation of the mouse and human homologues and allow direct examination of variation in the genes in patients and families. In fact, studies are ongoing in which the genetic material of a large population of patients on hemodialysis with no underlying primary renal disease is tested for markers of the synthenic *Rf*-regions of the FHH rat. Several physiological parameters can subsequently be tested in these patients, so that the relation between phenotype and genotype may be determined.

7.1.5 Independent control of renal impairment

The identification of genes that determine the development of renal failure in animal models[62] with or without hypertension may help to understand why some hypertensive patients develop renal disease.[6,90] Many investigators support the theory that hypertension *causes* renal failure. Establishing the causative element, both clinically and experimentally, is problematic because hypertension is known to accelerate renal failure.[19,29]

Brown *et al.* showed that hypertension alone is not enough to cause renal impairment.[17] The fact that the major loci explaining renal impairment in the FHH x ACI backcross are not the same as those causing high blood pressure indicates that the majority of animals in the backcross have blood pressure levels that simply accelerate the development of renal damage. In such a setting, the development of renal impairment appears to depend on the inheritance of downstream susceptibility factors. The fact that systemic blood pressure alone does not determine subsequent renal damage is consistent with previous observations done in other rat crosses. For example, MNS rats develop renal sclerosis, whereas MHS rats do not, despite higher blood pressure levels.[10,87]

Studies in the *Os/+* mouse, a model of reduced nephron number,[100] revealed that the susceptibility to develop FGS is controlled by multiple recessive loci independent of blood pressure, glomerular size, and number of glomeruli. Multiple recessive traits (at least 8-10 loci) were hypothesized to be involved, which illustrates that in a severe complication of diseases such as diabetes mellitus, FGS affects only a minority of patients. In this regard, a locus on chromosome 19q13 for focal segmental glomerulosclerosis with autosomal dominant inheritance was found in a family with no history of hypertension.[61]

Another QTL for UpV was reported in a cross of Buffalo/Mna and WKY rats.[62] This QTL was detected on chromosome 13 near the renin locus and is different from the *Rf-1* to *Rf-5* QTLs found in the FHH rat so far. The studies in rats and mice point towards extreme complex patterns of inherited factors determining the increased susceptibility to develop renal damage. As a consequence, the quest for genetic factors influencing the progression of renal failure in humans and experimental models has not yet resulted in any definitive conclusion. Our studies in the FHH model are at the forefront of the current experimental knowledge in this field. These studies show, however, that even a relatively simple homogeneous model such as the FHH rat, appears to be extremely elaborate. No less than five genes appear to affect the initiation and progression of renal failure. It is to be expected that the genes will show complex gene-gene and gene-environmental interactions. Thus, not only the function of each individual gene, but all gene-gene and gene-environmental interactions need to be studied to get the full picture of progressive chronic renal failure. Congenic rats are ideally suited for the further studies into the role of the individual genes as well as their interactions. We fully anticipate being able to use the animals that are currently constructed for many future critical studies.

A complete understanding of the renal failure genes will clearly require their isolation. The rat genetic map that was available when the study by Brown *et al.* was published, included no obvious candidate genes in the region.[17,51] At present, however, we can combine our knowledge on the renal abnormalities in the FHR with the genetic analysis by Brown *et al.*[17] and Shiozawa *et al.*[82] Using the currently available databases, we can combine physiology and genetics and compare *Rf*-regions to homologous areas in the mouse and in humans. Because the impairment in the myogenic response is most likely the major player in the development of renal damage in the FHH rat, we can screen for genes that are involved in vascular tone regulation. Although there are various genes in the homologous *Rf*-regions, the most likely candidates involve several potassium channels, adrenergic receptors, and members of the cytochrome P-450 family (internet: http://www.ncbi.nlm.nih.gov/Omim/Homology).

7.2 CONCLUSIONS

Combining physiology and genetics in the Fawn-Hooded rat from present and previous studies results in the following conclusions:

(I) The genetic background together with the blood pressure level determine the course and severity of the development of renal failure.

(a) The inheritance of the susceptibility genes in homozygous form as in the FHH rat means rapid development of severe renal damage upon blood pressure elevation.

(b) The inheritance of the susceptibility genes in heterozygous form as in the F_1 (FHH x ACI) rat means the development of renal damage beyond a threshold level of systemic blood pressure.

(c) The absence of susceptibility genes as in the ACI rat means that little or no renal damage will develop upon blood pressure elevation.

(d) The FHL rat, which shares a substantial part of its genome with that of the FHH rat, develops severe renal damage upon blood pressure elevation.

(e) Rats with one kidney, being heterozygous for the susceptibility genes, develop more renal damage upon blood pressure elevation compared with rats that have none of these genes.

(II) The normotensive FHL rat is susceptible to the development of renal damage upon blood pressure elevation.

(III) De presence of a genetic background for renal failure in heterozygous form does not imply complete protection from the development of renal damage.

(IV) Blood pressure rather than the specific NO-synthase inhibitor used determine the development of renal damage after L-NAME-induced hypertension.

(V) A defect in the ability to regulate afferent arteriolar tone causes a severely impaired renal autoregulation in the FHH rat.

(VI) Genes that are important in the regulation of renal hemodynamics, are in mutated form responsible for the phenotype of the FHH rat.

Although it is difficult to extrapolate results from animal models to the development of renal failure in humans, similar mechanism may apply to hypertensive patients. This might explain why only a limited number of hypertensives eventually develop progressive renal failure.

7.3 PERSPECTIVES

7.3.1. From linkage to locus for polygenic traits

Although there are indications about possible candidate genes for renal impairment in the FHH rat, it is important to know how to proceed from the initial detection of linkage to the definitive identification of a trait determined by multiple genes. The reader is therefore provided with a brief description of the approach to clone a QTL based on position.[51] First, the linkage data from the (FHH x ACI) FHH backcross generated by Brown *et al.*[17] were confirmed in a larger F_2 cross.[82] Secondly, a small region containing the QTL (*Rf-1*) allele was moved from the FHH to the ACI rat by successive backcrossing. Thus congenic strains are now being constructed differing only in the region of the QTL. To ensure the introgression of the QTL allele from FHH, progeny was selected that inherited alleles of genetic markers within and flanking the QTL. To accelerate the removal of FHH alleles elsewhere in the genome, genetic markers in other regions were used to identify and select animals that have lost more than the expected 50% proportion ACI alleles per generation (marker-assisted selection). The number of backcross generations required for introgression can thus be limited. Comparing congenics with parental strains, we can make detailed physiological studies, and examine the separate and interacting effects of the QTLs, and their mode of inheritance. Thirdly, using the congenic strains and a denser map of genetic markers we want to narrow down the *Rf*-regions. Fourth, a physical map will be constructed and genes in the region will be identified by selecting and testing plausible candidate genes in the region, using the results of the physiological studies. This is the critical point for the implementation of physiological and genetic data: physiological genomics. Fifth, to prove that a candidate gene corresponds to a QTL, we have to use homologous recombination to construct gene knockouts in strains carrying a dominant allele (which can than be tested for loss of dominance at the QTL) or to substitute the FHH allele into ACI and demonstrate that the QTL effect has been transferred as well.[51]

7.3.2 Congenics of the renal failure genes

Further studies are needed to identify the precise genes at each of the *Rf*-loci, their function, the interaction between the various loci and between genetic and environmental factors, such as blood pressure and dietary intake. For this purpose, congenic strains will be developed carrying each of the *Rf*-loci homozygous from the FHH rat on the renal damage resistant background of the ACI rat. To study the interaction with hypertension, similar FHH congenic rats could be constructed on an SHR background. Finally, FHH rats will be rescued from developing renal failure by moving the various *Rf*-loci from the ACI rats into the FHH genome, thus by making reverse congenic strains. Currently, we are generating congenic strains carrying *Rf-1* and *Rf-4* regions separately and a double congenic strain carrying both loci. Detailed physiological studies ahead of gene identification, as well as their separate and interacting effects on the development of renal damage can thus be performed. Chronic studies will be done under three conditions: (a) during aging, (b) after UNX, and (c) by increasing blood pressure with chronic L-NAME treatment. Acute *in vivo* studies into the autoregulation of RBF and P_{GC} will be done, as well as *in vivo* and *in vitro* studies on renal vascular activity.

Although functional defects within the FHH kidney play an important role in the development of renal damage, it is not yet clear whether the genetic susceptibility of the FHH rat resides completely within the kidney or is partially related to genetic defects in the rest of the body. This may be unraveled by studying kidney transplantation, as a means of organ-specific genome transfer.[20] Congenic rats are highly suitable for such studies as they share the genes of the major histocompatibility complex.

7.3.3 Further characterization of the Fawn-Hooded rat

Because vascular abnormalities seem to have a common ground in the pathologic findings observed in the FHH rat, studies into the exact mechanism of mainly the vascular smooth muscle cell in this model, such as patch-clamp studies, have to be carried out in order to elucidate alterations in both calcium ion regulation and in voltage-dependent calcium channels of vascular smooth muscle cells in FHH and congenic rats. Furthermore, several *in vitro* models to evaluate renal function, such as juxtamedullary nephron preparations, isolated perfused hydronephrotic kidneys, and isolated afferent arterioles, have to be evaluated in FHH and *Rf*-congenic rats. The vascular abnormalities found in FHH kidneys might very well be present in other organ systems, and it might be useful to

evaluate these systems for their function. For instance, measurement of cerebral blood flow autoregulation may provide useful information on the properties of other vascular beds in FHH rats.

Furthermore, the mechanism underlying the development of hypertension in the FHH rat needs to be elucidated. For instance, measurement of cardiac output at different ages and disease states might reveal how changes in the peripheral resistance contribute to the development of hypertension in the FHH rat. Furthermore, more chronic blood pressure measurements in FHH and FHL rats must be performed because all studies so far were based on tail-cuff measurements. It would be wise to characterize renal function in the FHH and FHL rat compared with additional control strains. For instance, the FH/IR rat, which does not develop any hypertension or renal damage, could prove to be a useful control animal. FHL rats should be studied as well, both genetically and physiologically, as they are less susceptible to renal damage than FHH rat rats, though more susceptible to the development of renal failure than other rat strains. Hence, additional genetic studies will have to be carried out to find the origin of the differences between both Fawn-Hooded strains.

REFERENCES

1. Anderson HS, Brenner BM. The critical role of nephron mass and of intraglomerular pressure for initiation and progression of experimental hypertensive-renal disorder. In: *Hypertension: Pathophysiology, Diagnosis and Management.* (Laragh JH, Brenner BM, eds.) New York, NY, Raven, 1990, p 1163-1176.
2. 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.
3. Anh-Tuan DX, Higenbottan TW, Pepke-Zaba J, Clelland CA, Wallwork J. Pulmonary artery endothelium-dependent relaxation is impaired in human secondary pulmonary hypertension. *Circulation* 1989;80:II-435 (Abstract).
4. Ashmore RC, Rodman DM, Sato K, Webb SA, O'Brien RF, McMurtry IF, Stelzner TJ. Paradoxical constriction to platelets by arteries from rats with pulmonary hypertension. *Am J Physiol.* 1991;260:H1929-H1934.
5. Baylis C, Mitruka B, Deng A. Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest.* 1992;90:278-281.
6. Bianchi G, Cusi D, Barlassina B, Lupi GP, Ferrari P, Picotti GB, Gatti M, Polli E. Renal dysfunction as a possible cause of essential hypertension in predisposed subjects. *Kidney Int.* 1983;23:870-875.
7. Bidani AK, Griffin KA, Plott W, Schwartz WW. Renal ablation acutely transforms 'benign' hypertension to 'malignant' nephrosclerosis in hypertensive rats. *Hypertension* 1994;24:309-316.
8. Boberg U, Persson AEG. Increased tubuloglomerular feedback activity in Milan hypertensive rats. *Am J Physiol.* 1986;250:F967-F974.
9. Braam BB, Navar LG, Mitchell KD. Modulation of tubuloglomerular feedback by angiotensin type II type 1 receptors during the development of Goldblatt hypertension. *Hypertension* 1995;25:1232-1237.
10. Brandis A, Bianchi G, Reale E, Helmchen U, Kühn KW. 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.
11. Brazy PC, Stead WW, Fitzwilliam JF. Progression to renal insufficiency: role of blood pressure. *Kidney Int.* 1989;35:670-674.
12. Brenner BM. Hemodynamically mediated glomerular injury and the progressive nature of kidney disease. *Kidney Int.* 1983;23:647-655.
13. Brenner BM, Garcia DL, Anderson S. The renal abnormality in hypertension: A proposed defect in glomerular filtration surface area. *Am J Hypertens.* 1988;6:335-347.
14. Brink C, Cerrina J, Labat C, Verley J, Benveniste J. The effects of contractile agonists on isolated pulmonary arterial and smooth muscle preparations derived

from patients with primary pulmonary hypertension. *Am Rev Resp Dis.* 1988;137:106 (Abstract).
15. Bolotina VM, Najibi S, Palacino JJ, Pagano PJ, Cohen RA. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature* 1994;368:850-853.
16. Brayden JE, Nelson MT. Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science* 1992;256:532-535.
17. 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. *Nat Genet.* 1996;12:44-51.
18. Chen Y, Yip K, Marsh DJ, Holstein-Rathlou NH. Magnitude of TGF-initiated nephron-nephron interactions is increased in SHR. *Am J Physiol.* 1995;269:F198-F204.
19. Chung O, Rohmeiss P, Sponer G, Strauch M, Gretz N. Renal involvement in spontaneously hypertensive rats. In: *Rat Models of Chronic Renal Failure* (Gretz N, Strauch M, eds) Karger, Basel 1993;357-370.
20. Churchill P, Churchill MC, Bidani AK, Griffin KA, Picken M, Pravenec M, Kren V, St Lezin E, Wang JM, Wang N, Kurtz TW: Genetic susceptibility to hypertension-induced renal damage in the rat. Evidence based on kidney-specific genome transfer. *J Clin Invest.* 1997;100:1373-1382.
21. De Keijzer MH, Provoost AP, Zijlstra FJ. Enhanced urinary excretion of eicosanoids in Fawn-Hooded rats. *Nephron* 1992;62:454-458.
22. Deng LY, Day R, Schiffrin EL. Localization of sites of enhanced expression of endothelin-1 in the kidney of deoxycorticosterone acetate-salt hypertensive rats. *J Am Soc Nephrol.* 1997:in press.
23. Dilley JR, Arendshorst WJ. Enhanced tubuloglomerular feedback activity in rats developing spontaneous hypertension. *Am J Physiol.* 1984;247:F672-F679.
24. Dopico AM, Kirber MT, Singer JJ, Walsh JV. Membrane stretch directly activates large conductance Ca^{2+}-activated K^+ channels in mesenteric artery smooth muscle cells. *Am J Hypertens.* 1994;7:82-89.
25. Dworkin LD, Feiner HD. Glomerular injury in uninephrectomized spontaneously hypertensive rats. A consequence of glomerular capillary hypertension. *J Clin Invest.* 1986;77:797-809.
26. England SK, Woolridge TA, Stekiel WJ, Rusch NJ. Enhanced single-channel K^+ current in arterial membranes from genetically hypertensive rats. *Am J Physiol.* 1993;264:H1337-H1345.
27. Epstein M, Hayashi K, Forster H. Impaired myogenic afferent arteriolar (AA) vasoconstriction in genetically diabetic rats. *J Am Soc Nephrol.* 1990;1:627 (Abstract).
28. Falcone JC, Granger HJ, Meininger GA. Enhanced myogenic activation in skeletal muscle arterioles from spontaneously hypertensive rats. *Am J Physiol.* 1993;265:H1847-H1855.

29. Ferguson R, Grim CE, Opgenorth TJ. A familial risk of chronic renal failure among blacks on dialysis ? *J Clin Epidemiol.* 1988;41:1189-1196.
30. Forster HG, Ter Wee PM, Hohman TC, Epstein M. Impairment of afferent arteriolar myogenic responsiveness in the galactose-fed rat. *P.S.E.B.M.* 1994;206:365-374.
31. Freedman BI, Spray BJ, Tuttle AB, Buckalew VM. The familial risk of end-stage renal disease in African Americans. *Am J Kidney Dis.* 1993;21:387-393.
32. Freedman BI, Iskandar SS, Appel RG. The link between hypertension and nephrosclerosis. *Am J Kidney Dis.* 1995;25:207-221.
33. Fujihara CK, Michellazo SM, De Nucci G, Zatz R. Sodium excess aggravates hypertension and renal parenchymal injury in rats with chronic NO inhibition. *Am J Physiol.* 1994;266:F697-F705.
34. Gawaz MP, Bogner C, Gurland HJ. Flow-cytometric analysis of mepacrine-labelled platelets in patients with end-stage renal failure. *Hemostasis* 1993;23:284-292.
35. Gordge MP, Faint RW, Rylance PB, Neild GH. Platelet function and the bleeding time in progressive renal failure. *Thromb Haemostasis* 1989;60:83-87.
36. Harder DR, Campbell WB, Roman RJ. Role of cytochrome P-450 enzymes and metabolites of arachidonic acid in the control of vascular tone. *J Vasc Res.* 1995;32:79-92.
37. Harder DR, Lange AR, Gebremedhin D, Birks EK, Roman RJ. Cytochrome P-450 metabolites of arachidonic acid as intracellular signalling molecules in vascular tissue. *J Vasc Res.* 1997;34:237-243.
38. Hashimoto Y, Ideura T, Yoshimura A, Koshikawa S. Autoregulation of renal blood flow in streptozotocin-induced diabetic rats. *Diabetes* 1989;38:1109-1113.
39. Hayakawa H, Hirata Y, Suzuki E, Sugimoto T, Matsuoka H, Kikuchi K, Nagano T, Hirobe M, Sugimoto T. Mechanisms for altered endothelium dependent vasorelaxation in isolated kidneys from experimental hypertensive rats. *Am J Physiol.* 1993;264:H1535-H1541.
40. Hayashi K, Epstein M, Saruta T. Altered myogenic responsiveness of the renal microvasculature in experimental hypertension. *J Hypertens.* 1996;14:1387-1401.
41. Hayashi K, Loutzenhiser R, Epstein M, Suzuki H, Saruta T. Multiple factors contribute to acetylcholine-induced afferent arteriolar vasodilation during myogenic, norepinephrine- and KCl-induced vasoconstriction: studies in the isolated perfused hydronephrotic kidney. *Circ Res.* 1994;75:821-828.
42. Hayashi K, Epstein M, Loutzenhiser R, Forster H. Impaired myogenic responsiveness of the afferent arteriole in streptozotocin-induced diabetic rats: role of eicosanoid derangements. *J Am Soc Nephrol.* 1992;2:1578-1586.
43. Herrera-Acosta J. The role of systemic and glomerular hypertension in progressive glomerular injury. *Kidney Int.* 1994;45:S6-S10.
44. Hostetter TH, Rennke HG, Brenner BM. The case of intrarenal hypertension and progression of diabetic and other glomerulopathies. *Am J Med.* 1982;72:375-380.

45. Hostetter TH, Troy JL, Brenner BM. Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int.* 1981;19:410-415.
46. 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-F93.
47. Hu S, Kim HS. Activation of K^+ channel in vascular smooth muscles by cytochrome P-450 metabolites of arachidonic acid. *Eur J Pharmacol.* 1993;230:215-221.
48. Imig JD, Zou A-P, Stec DE, Harder DR, Falck JR, Roman RJ. Formation and actions of 20-hydroxyeicosatetraenoic acid in rat renal arterioles. *Am J Physiol.* 1996;270:R217-R227.
49. Ito S, Carretero OA. Impaired response to acetylcholine despite intact endothelium-derived relaxing factor/nitric oxide in isolated microperfused afferent arterioles of spontaneously hypertensive rats. J *Cardiovasc Pharmacol.* 1992;20:S187-S189.
50. Iversen BM, Ofstad J. Loss of renal blood flow autoregulation in chronic glomerulonephritic rats. *Am J Physiol.* 1988;254:F284-F290.
51. Jacob HJ, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao Y-P, Ganten D, Dzau VJ, Lander ES. Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. *Cell* 1991;67:213-224.
52. Jacob HJ, Shiozawa M, Broeckel U, Brown DM, Naff B, Majewski RR, Gauthier S, Kissebah A, Provoost AP. Genetics of end stage renal disease (ESRD): Of Rat and Man ? *Am J Hypertens.* 1997;10:216A (Abstract).
53. Kentera D, Susic D, Veljkovic V, Tucakovic G, Koko V. Pulmonary artery pressure in rats with hereditary platelet function defect. *Respiration* 1988;54:110-114.
54. Kriz W, Hosser H, Hähnel B, Gretz N, Provoost AP. From segmental glomerulosclerosis to total nephron degeneration and interstitial fibrosis: a histopathological study in rat models and human glomerulopathies. *Nephrol Dial Transplant.* 1998;13:2781-2798.
55. Kriz W, Hosser H, Hähnel B, Simons JL, Provoost AP. Development of vascular pole associated glomerulosclerosis in the fawn-hooded rat. *J Am Soc Nephrol.* 1998;9:381-396.
56. Liu Y, Knaus H-G, Rusch NJ. Enhanced Ca^{2+}-activated K^+ channel expression in aortic smooth muscle membranes from spontaneously hypertensive rats. *Hypertension* 1996;28:P34 (Abstract).
57. Liu Y, Jones AW, Sturek M. Ca^{2+}-dependent K^+ current in arterial smooth muscle cells from aldosterone-salt hypertensive rats. *Am J Physiol.* 1995;269:H1246-H1257.
58. Luscher TF, Raij L, Vanhoutte PM. Endothelium-dependent vascular responses to nitric oxide in normotensive and hypertensive Dahl rats. *Hypertension* 1987;9:157-163.

59. Luscher TF, Vanhoutte PM. Endothelium-dependent relaxation to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension* 1986;8:344-348.
60. Luscher TF, Vanhoutte PM. Endothelium-dependent responses to platelets and serotonin in spontaneously hypertensive rats. *Hypertension* 1986;8:55-60.
61. Mathis BJ, Kim SH, Calabrese K, Haas M, Seidman JG, Seidman CE, Pollak M. A locus for inherited focal segmental glomerulosclerosis maps to chromosome 19q13. *Kidney Int.* 1998;53:282-286.
62. Murayama S, Yagyu S, Higo K, Ye C, Mizuno T, Oyabu A, Ito M, Morita H, Maeda K, Serikawa T, Matsuyama M. A genetic locus susceptible to the overt proteinuria in BUF/Mna rats. *Mamm Genome* 1998;9:886-888.
63. McClellan W, Tuttle E, Issa A. Racial differences in the incidence of hypertensive end-stage renal disease (ESRD) are not entirely explained by differences in the prevalence of hypertension. *Am J Kidney Dis.* 1988;4:285-290.
64. Miller JS, Pinto A, Mullane KM. Impaired endothelium-dependent relaxations in rabbits subjected to aortic coarctation hypertension. *Hypertension* 1987;10:164-170.
65. Mitchell KD, Mullins JJ. ANG II dependence of tubuloglomerular feedback responsiveness in hypertensive ren-2 transgenic rats. *Am J Physiol.* 1995;268:F821-F828.
66. Morel L, Rudofski UH, Longmate JA, Schiffenbauer J, Wakeland EK. Polygenic control of susceptibility to murine systemic lupus erythematosis. *Immunity* 1994;1:219-229.
67. Ono H, Ono Y, Frohlich ED. ACE inhibition prevents and reverses L-NAME-exacerbated nephrosclerosis in spontaneously hypertensive rats. *Hypertension* 1996;27:176-183.
68. Ono H, Ono Y, Frohlich ED. Nitric oxide synthase inhibition in spontaneously hypertensive rats. *Hypertension* 1995;26:249-255.
69. Parving H-H, Kastrup H, Smidt UM, Anderson AR, Feldt-Rasmussen B, Christiansen JS. Impaired autoregulation of glomerular filtration rate in type I (insulin-dependent) diabetic patients with nephropathy. *Diabetologica* 1984;27:547-552.
70. Pelayo JC, Westcott JY. Impaired autoregulation of glomerular capillary hydrostatic pressure in the rat remnant nephron. *J Clin Invest.* 1991;88:101-105
71. Persson AEG, Bianchi G, Boberg U. Tubuloglomerular feedback in hypertensive rats of the Milan strain. *Acta Physiol Scand.* 1985;123:139 (Abstract).
72. Provoost AP. Spontaneous glomerulosclerosis: insights from the Fawn-Hooded rat. *Kidney Int.* 1994;45:S2-S5.
73. Provoost AP, Kouwenhoven EA, Van Dokkum RPE, De Bruin RWF, Marquet RL. Donor and recipient factors in the development of albuminuria after kidney transplantation (KTx) in rats. *J Am Soc Nephrol.* 1997;8:3096A (Abstract).
74. Rees D, Ben-Ishay D, Moncada S. Nitric oxide and the regulation of blood pressure in the hypertension-prone and hypertension-resistant Sabra rat. *Hypertension* 1996;28:367-371.

75. Resch S, Provoost AP, Bachmann S. Morphology of the renin-angiotensin system (RAS) and the L-arginine nitric oxide (NO) pathway in Fawn-Hooded (FH) rats. *J Am Soc Nephrol.* 1995;5:590 (Abstract).
76. Roman RJ, Harder DR. Cellular and ionic signal transduction mechanisms for the mechanical activation of renal arterial vascular smooth muscle. *J Am Soc Nephrol.* 1993;4:986-996.
77. Rubattu S, Volpe M, Kreutz R, Ganten U, Ganten D, Lindpaintner K. Chromosomal mapping of quantitative trait loci contributing to stroke in a rat model of complex human disease. *Nature Gen.* 1996;13:429-434.
78. Rusch NJ, Liu Y. Potassium channels in hypertension: Homeostatic pathways to buffer arterial contraction. *J Lab Clin Med.* 1997;130:245-251.
79. Rusch NJ, Liu Y. Mechanisms for regulation of arterial tone by Ca^{2+}-dependent K^+ channels in hypertension. *Clin Exp Pharmacol Physiol.* 1996;23:1077-1082.
80. Rusch NJ, De Lucena RG, Woolridge TA, England SK, Cowley AW Jr. A Ca^{2+}-dependent K^+ current is enhanced in arterial membranes of hypertensive rats. *Hypertension* 1992;19:301-307.
81. Scholey JW, Meyer TW. Control of glomerular hypertension by insulin administration in diabetic rats. *J Clin Invest.* 1989;83:1384-1389.
82. Shiozawa M, Provoost AP, Majewski RR, Van Dokkum RPE, Jacob HJ. Interacting genes determine the suceptibility to proteinuria after uninephrectomy in rats. *J Am Soc Nephrol.* 1998;9:394A (Abstract).
83. Simons JL. Pathogenesis of glomerular sclerosis in the Fawn-Hooded rat. Thesis, Rotterdam, December 1994.
84. Simons JL, Provoost AP, Anderson S, Troy JL, Rennke HG, Sandstrom DJ; Brenner BM. Pathogenesis of glomerular injury in the fawn-hooded rat: early glomerular capillary hypertension predicts glomerular sclerosis. *J Am Soc Nephrol.* 1993;3:1175-1782.
85. 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.
86. Morel L, Rudofski UH, Longmate JA, Schiffenbauer J, Wakeland EK. Polygenic control of susceptibility to murine systemic lupus erythematosis. *Immunity* 1994;1:219-229.
87. Stella P, Cusi D, Duzzi L, Bianchi G. Relations between hypertension and glomerulosclerosis in first-generation hybrid rats of the Milan strains. *Child Nephrol Urol.* 1991;11:6-9.
88. Stelzner T, Hofmann TA, Brown DM, Deng A, Jacob HJ. Genetic determinants of pulmonary hypertension in fawn-hooded rats. *Chest* 1997;111:96S (Abstract).
89. Stelzner TJ, O'Brien RF, Yanagisawa M, Sakurai T, Sato K, Webb S, Zamora M, McMurtry IF, Fisher JH. Increased lung endothelin-1 production in rats with idiopathic pulmonary hypertension. *Am J Physiol.* 1992;262:L614-L620.
90. Summerson JH, Bell RA, Konen JC. Racial differences in the prevalence of microalbuminuria in hypertension. *Am J Kidney Dis.* 1995;26:577-579.

91. Tolins JP, Shultz PJ. Endogenous nitric oxide synthesis determines sensitivity to the pressor effect of salt. *Kidney Int.* 1994;46:230-236.
92. Tschopp TB, Weiss HJ. Decreased ATP, ADP and serotonin in young platelets of fawn-hooded rats with storage pool disease. *Thrombos Diathes Haemorrh.* 1974;32:670-677.
93. Tschopp TB, Zucker MB. Hereditary defect in platelet function in rats. *Blood* 1972;40:217-226.
94. Vanhoutte PM, Houston DS. Platelets, endothelium, and vasospasm. *Circulation* 1985;72:728-734.
95. Verseput GH. Renoprotective actions of ACE inhibition in the hypertensive Fawn-Hooded rat developing glomerulosclerosis. *Thesis, Utrecht*, November 1998, ISBN 90-9011986-8.
96. Verseput GH, Braam BB, Provoost AP, Koomans HA. Tubuloglomerular feedback and prolonged ACE-inhibitor treatment in the hypertensive fawn-hooded rat. *Nephrol Dial Transplant.* 1998;13:893-899.
97. 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.
98. Verseput GH, Braam BB, Van Dokkum RPE, Weening JJ, Koomans HA, Provoost AP. Renoprotection with ACE-inhibition is only partially dependent on blood pressure reduction in hypertensive Fawn-Hooded rats. *Thesis G.H. Verseput, Utrecht*, November 1998, ISBN 90-9011986-8.
99. Walker WG. Hypertension-related renal injury: a major contributor to end-stage renal disease. *Am J Kidney Dis.* 1993; 22: 164-173.
100. Zalups RK. The Os/+ mouse: a genetic animal model of reduced renal mass. *Am J Physiol.* 1993;264:F53-F60.
101. Zou A-P, Fleming JT, Falck JR, Jacobs ER, Gebremedhin D, Harder DR, Roman RJ. 20-HETE is an endogenous inhibitor of the large-conductance Ca^{2+}-activated K^+ channel in renal arterioles. *Am J Physiol.* 1996; 270: R228-R237.

CHAPTER 8

SUMMARY

SAMENVATTING

SUMMARY

Chapter 1 introduces the reader to the thesis and the subjects that are related to hypertension-associated renal failure. It gives an overview of the current literature on the Fawn-Hooded rat and contains the scope and outline of the thesis.

Chapter 2 describes experiments in which the susceptibility to hypertension and renal failure in the normotensive FHL and ACI rat strains was investigated. These strains were treated with the nitric oxide synthase inhibitor, N^{ω}-L-arginine methyl ester (L-NAME), to induce increases in systemic blood pressure. The FHL strain, which is genetically identical to the FHH strain, does not develop hypertension or renal damage with age under normal conditions. In this experiment, under the influence of L-NAME-induced hypertension, the FHL rat developed significantly more functional and structural renal damage than the ACI rat at similar blood pressure levels. It was concluded that there is an interaction between blood pressure and the genetic susceptibility to renal failure in the FHL rat and that the susceptibility to renal damage is genetically determined in rats.

Chapter 3 describes experiments under similar conditions, in which the susceptibility to develop renal damage was studied in F_1 (FHH x ACI) rats and in both parental strains. At similar blood pressure levels FHH rats developed severe renal damage and showed high mortality, in contrast to ACI rats, which did not develop renal damage. Values for blood pressure and renal damage in the F_1 rats were intermediate, being closer to values in ACI than in FHH rats, and increased over an SBP level of 180 mmHg. The F_1 rats, being heterozygous for the renal failure genes, were largely, but not completely, protected from the development of L-NAME-induced renal damage. From these findings it was concluded that susceptibility to hypertension-associated renal damage in rats primarily depends on the presence of predisposing genes for renal failure.

Chapter 4 describes the relationship between blood pressure level and the susceptibility to develop renal damage after unilateral nephrectomy in F_1 and ACI rats. In this study, NO synthase was chronically blocked with L-NAME, while blood pressure was normalized using the angiotensin-converting enzyme inhibitor, lisinopril. Results of the study were similar to results obtained in two kidney F_1 and ACI rats, *i.e.*, the F_1 rats developed more renal damage compared with ACI rats. As in the previous studies, a positive correlation between blood pressure and functional and structural renal damage was found, which increased

with time and was more severe in F_1 than in ACI rats. The conclusion of this study was that the development of renal damage is directly related to the level of blood pressure or indirectly dependent on actions of the renin-angiotensin system.

Chapter 5 describes experiments performed to elucidate the mechanisms underlying the renal abnormalities in the Fawn-Hooded rat. The responses to changes in renal perfusion pressure (RPP) were measured in FHH, FHL, and ACI rats to determine whether an abnormality in autoregulation of renal blood flow (RBF) may contribute to the development of glomerulosclerosis in the FHH rat. Moreover, the first long-term blood pressure measurements in chronically instrumented FHH and FHL rats were presented. Apart from the fact that RBF was significantly greater in FHH rats compared with FHL and ACI rats, RBF was not autoregulated in FHH rats. Glomerular filtration rate was also significantly higher by 20-30% in FHH rats than in FHL and ACI rats at every RPP studied. Urinary protein excretion correlated with RPP in FHH rats, but not in FHL and ACI rats. However, no differences were observed in the pressure-natriuresis studies that were also performed. This could explain the mild elevation of SBP in the FHH rat measured in the long-term blood pressure recordings. The conclusion of the study was that autoregulation of RBF is impaired in FHH rats, and that the abnormality may contribute to the development of hyperfiltration and proteinuria, before any glomerulosclerosis develops in these animals. The data provide evidence for the development of intrinsic hemodynamic abnormalities in the kidney of the FHH rat and for the phenomenon that the abnormalities in renal hemodynamics precede the development of severe hypertension and renal failure in the FHH rat.

Chapter 6 describes additional studies performed to examine whether an abnormality in the myogenic response of preglomerular renal arterioles impairing autoregulation of RBF and glomerular capillary pressure (P_{GC}), contributes to the development of glomerulosclerosis in the FH rat. Autoregulation of RBF, cortical and medullary blood flow and P_{GC} were compared in volume-replete and volume-expanded FHH and FHL rats to minimize the influence of tubuloglomerular feedback on these responses. In volume-expanded rats, baseline RBF, cortical and medullary blood flow were all significantly greater in FHH than in FHL rats. Autoregulation of whole kidney, cortical, and medullary blood flow was significantly impaired in FHH compared with FHL rats. Myogenically-mediated autoregulation of P_{GC} was also impaired in FHH compared with FHL rats. Furthermore, studies in isolated perfused interlobular arteries showed that vessels of FHH rats were unable to constrict in response to increases in transmural pressure. Vessels of these rats even dilated in response to the same increase in

perfusion pressure. The normal responses of arterioles from FHH rats to the vasoconstrictor compound phenylephrine showed that the vascular abnormalities were not due to nonspecific vascular injury. However, the vasodilator responses to acetylcholine in these vessels were severely impaired compared with arterioles of FHL rats, pointing towards endothelial dysfunction in FHH rats.

Chapter 7 contains the general discussion and conclusion of this thesis, followed by the perspectives. Combining physiology and genetics with regard to blood pressure and renal failure in the Fawn-Hooded rat, results in the following conclusions:
(I) The genetic background and the blood pressure level determine the initiation and progression of renal damage.
(II) The normotensive FHL rat is susceptible to renal damage upon blood pressure elevation.
(III) The presence of a genetic background in heterozygous form does not imply complete protection from the development of renal damage.
(IV) The level of blood pressure and not the specific NO-synthase inhibitor used determine the level of renal damage after L-NAME-induced hypertension.
(V) A defect in the ability to regulate afferent arteriolar tone causes a severely impaired renal autoregulation in the FHH rat.
(VI) Genes that are important in the regulation of renal hemodynamics, are in mutated form responsible for the phenotype of the FHH rat.

SAMENVATTING

De Fawn-Hooded Hypertensieve (FHH) rat is een genetisch model voor nierschade die geassocieerd is met hoge bloeddruk (hypertensie). Deze rat ontwikkelt reeds op jonge leeftijd systolische en glomerulaire hypertensie, proteinurie (eiwit in de urine; UpV) en focale glomerulosclerose (FGS). De FHH rat bezit tenminste vijf genen voor nierschade en tenminste twee genen voor hypertensie. In dit proefschrift worden een aantal experimenten beschreven die tot doel hadden de gevoeligheid voor het ontwikkelen van nierschade te onderzoeken. Verder worden een aantal experimenten beschreven die tot doel hadden te onderzoeken welke mechanismen ten grondslag liggen aan het ontstaan van de nierschade in de FHH rat.
De genetische en pathofysiologische aspecten van hypertensie en nierschade, alsmede andere eerder gevonden afwijkingen in dit diermodel, worden beschreven in **Hoofdstuk 1** en gerelateerd aan de beschikbare literatuur.

Om verschillen in gevoeligheid voor het ontwikkelen van nierschade te onderzoeken, werd als eerste de Fawn-Hooded Lage bloeddruk (FHL) rat onderzocht, die genetisch identiek is aan de FHH rat, maar geen hypertensie en nierschade ontwikkelt met de leeftijd. De FHL rat werd vergeleken met de normotensieve August x Copenhagen Irish (ACI) rat. Dit experiment wordt beschreven in **Hoofdstuk 2**. Daartoe werd in beide stammen door toediening van een stof die de aanmaak van stikstofoxide remt, N^{ω}-L-arginine methyl ester (L-NAME), de bloeddruk verhoogd. De FHL rat ontwikkelde meer functionele en structurele nierschade dan de ACI rat bij vergelijkbare bloeddrukniveaus. De conclusie van dit experiment was dat er in de FHL rat een interactie is tussen bloeddruk en de genetische gevoeligheid voor het ontwikkelen van nierafwijkingen, en dat deze rat gevoeliger is voor het ontwikkelen ervan dan de ACI rat.

In een vergelijkbare proefopzet beschreven in **Hoofdstuk 3**, werd de gevoeligheid voor het ontwikkelen van nierschade in de FHH en de ACI rat, en in een F_1 (FHH x ACI) kruising van deze beide stammen onderzocht. Bij vergelijkbare bloeddrukniveaus ontwikkelde de FHH rat de meeste en de ACI rat de minste nierschade, terwijl de waarden van de F_1 (FHH x ACI) ratten tussen de waarden van de beide ouderstammen inlagen, maar dichter bij die van de ACI dan die van de FHH ratten. Boven een bloeddrukniveau van 180 millimeter kwik werd een toename van de nierschade gevonden. Het bleek dat F_1 ratten, die heterozygoot zijn voor de genen verantwoordelijk voor nierschade, slechts

gedeeltelijk beschermd zijn tegen de ontwikkeling van hypertensie-geïnduceerde nierschade. De conclusie was dat volledige gevoeligheid voor hypertensie-geassocieerde nierschade in ratten primair afhankelijk is van de aanwezigheid van genen die het risico op het ontwikkelen van nierschade verhogen, zelfs na een significante stijging van de bloeddruk.

In **Hoofdstuk 4** worden experimenten beschreven waarbij de rol van een toegenomen hemodynamische last op de glomeruli op het ontstaan van nierschade door de combinatie van éénzijdige nierverwijdering (unilaterale nefrectomie, UNX) en L-NAME-geïnduceerde hypertensie in F_1 (FHH x ACI) en ACI ratten. Verder werd onderzocht of een algemeen toxisch effect van L-NAME, onafhankelijk van de bloeddruk, nierschade veroorzaakte in F1 ratten. Daartoe werden deze ratten tegelijkertijd behandeld met L-NAME en lisinopril, een stof die de aanmaak van angiotensine-converting enzym remt. In overeenstemming met de resultaten van de studies in ratten met twee nieren, ontwikkelden de F_1 (FHH x ACI) meer nierschade dan de ACI ratten. Er werd een positieve relatie gevonden tussen het bloeddrukniveau en de hoeveelheid functionele en structurele nierschade. In de F_1 ratten die met lisinopril waren behandeld, werd geen significante nierschade gevonden bij een laag bloeddrukniveau, hetgeen aangeeft dat nierschade in hypertensieve F_1 ratten niet een direct effect is van de L-NAME behandeling, maar het resultaat is van de hoge bloeddruk of een andere werking afhankelijk van de werking van het renine-angiotensine systeem. De conclusie van dit experiment luidde dat in ratten die heterozygoot zijn voor de genen die de ontwikkeling van nierschade beïnvloeden in de FHH rat, de gevoeligheid van de nier voor het ontwikkelen van schade na UNX en systemische hypertensie is toegenomen.

De effectiviteit van de autoregulatie van de nierdoorbloeding en het niveau van de glomerulaire filtratiesnelheid in FHH, FHL, en ACI ratten werd onderzocht door de responsen van de nier op veranderingen in renale perfusiedruk te meten, en worden beschreven in **Hoofdstuk 5**. Het basale niveau van de nierdoorbloeding in de FHH rat bleek significant hoger te liggen dan in de FHL en ACI rat, hetgeen mogelijk veroorzaakt wordt door een permanente verwijding van de nierarterie in de FHH rat. Verder werd gevonden dat de nier van de FHH rat niet in staat is de bloed flow naar de nier acceptabel laag te houden, hetgeen duidt op een defect in de autoregulatie van de nier. De FHL en de ACI rat waren beter in staat de nierdoorbloeding te reguleren. De glomerulaire filtratiesnelheid in beide fawn-hooded stammen nam toe met een toename in de renale perfusiedruk, en was 20-30% hoger in de FHH ratten vergeleken met FHL en de ACI ratten bij iedere gemeten renale perfusiedruk. De hoeveelheid eiwit in

de urine was ook lineair gecorreleerd met de renale perfusiedruk in de FHH ratten, maar niet in de FHL en ACI ratten. De incidentie van de glomerulosclerose was niet verschillend tussen de beide stammen en geeft aan dat de gemeten verschillen al aanwezig waren voordat de nieren structurele schade ontwikkelden. De conclusie was dat autoregulatie van de nierdoorbloeding in de FHH rat afwijkend is, en dat dit deze afwijking bijdraagt tot de ontwikkeling van hyperfiltratie van de glomeruli en proteinurie, voordat er structurele nierschade aanwezig is.

De waargenomen afwijkingen aan de nieren van de FHH rat werden verder geïmplementeerd in **Hoofdstuk 6**, waar werd onderzocht of een afwijking in de myogene respons in nierarteriolen, die de autoregulatie van de nierdoorbloeding en de intraglomerulaire druk (P_{GC}) aantast, bijdraagt tot het ontstaan van glomerulosclerose in de FHH rat. Autoregulatie van de nierdoorbloeding werd op drie verschillende niveaus onderzocht. Afgezien van het feit dat de nierdoorbloeding in de FHH rat significant hoger was vergeleken met de FHL rat, vertoonde de autoregulatie in de FHH rat op elk niveau afwijkingen vergeleken met de FHL rat. In micropunctie experimenten werd de directe relatie tussen de renale perfusiedruk en de intraglomerulaire druk onderzocht. Deze bleek een significante correlatie te vertonen: een stijging in de renale perfusiedruk veroorzaakte een stijging in de P_{GC}, en dit leidde tot de conclusie dat er geen adequate autoregulatie van de P_{GC} in de FHH rat aanwezig is. Tenslotte werd onderzocht of de gevonden afwijkingen verklaard zouden kunnen worden uit een afwijking in de myogene respons van nierarteriolen van de FHH rat. Hiertoe werden arteriolen van beide FHH rat en FHL ratten geïsoleerd en *in vitro* aan een toenemende perfusiedruk blootgesteld. Het bleek dat de arteriolen van de FHH rat, in tegenstelling tot die van de FHL rat, niet in staat waren adequaat te contraheren bij een toename in de perfusiedruk. Deze contractie is noodzakelijk voor het in stand houden van een constant niveau van nierdoorbloeding *in vivo*. Het berekende vermogen van de vaatwand om weerstand te bieden aan een toename in perfusiedruk bleek sterk verminderd in vaten van de FHH rat, waar zelfs enige vaat*verwijding* gemeten werd. Niervaten van de FHL rat daarentegen, waren in staat weerstand te bieden aan een toename in perfusiedruk. De gevonden afwijkingen in de vaten van de FHH rat zijn karakteristiek voor een defect in de myogene respons. Dat dit effect niet werd veroorzaakt door non-specifieke vaatschade bleek uit het feit dat de vaten normaal reageerden op de constrictoire stof fenylefrine. Opmerkelijk was de bevinding dat de relaxatie van FHH vaten na toediening van acetylcholine geheel afwezig was, terwijl arteriolen van de FHL rat toenemende relaxatie vertoonden bij een toenemende dosis van deze stof, mogelijk veroorzaakt door een reeds aanwezige relaxatie. Uit de beschreven

experimenten werd geconcludeerd dat de myogene respons van nierarteriolen in de FHH verminderd is. De arteriolen zijn daardoor niet in staat de arteriële druk te bufferen en de P_{GC} laag te houden. Dit defect in de myogene respons van de preglomerulaire vasculatuur, in combinatie met de eerder beschreven verhoogde weerstand van de efferente arteriole, leidt tot een verhoogde basale P_{GC}. Hierdoor wordt de aanwezige systolische hypertensie doorgelaten naar de glomeruli, hetgeen leidt tot de ontwikkeling van proteinurie en glomerulosclerose.

In **Hoofdstuk 7** werden de beschreven experimenten samengevat en werden de bevindingen in een algemene discussie aan de reeds bestaande literatuur gerelateerd. Daarbij werden de fysiologie en de genetica van de Fawn-Hooded rat gecombineerd, wat resulteerde in de volgende conclusies:
(I) De genetische achtergrond en het bloeddrukniveau bepalen het ontstaan en beloop van nierschade.
(II) De normotensieve FHL rat is gevoelig voor het ontstaan van nierschade wanneer de bloeddruk wordt verhoogd.
(III) De bezit van een genetische aanleg voor nierschade in heterozygote vorm betekent nog geen volledige bescherming tegen de ontwikkeling van nierschade.
(IV) De mate van nierschade door L-NAME-geïnduceerde hypertensie wordt bepaald door het bloeddrukniveau en niet de gebruikte NO-synthase inhibitor zelf.
(V) Een defect in het vermogen om de tonus van de afferente arteriole te reguleren is de oorzaak van een ernstig gestoorde renale autoregulatie in de FHH rat.
(VI) Genen die waarschijnlijk van belang zijn bij regulatie van de renale hemodynamica, zijn in gemuteerde vorm verantwoordelijk voor het fenotype van de FHH rat.

DANKWOORD

Het boekje dat voor u ligt, beschrijft een stukje van de puzzel die bestaat uit de ontwikkeling van hypertensie-geassocieerde nierschade in de Fawn-Hooded rat. Hoewel het aantal stukjes van deze puzzel nog niet eens bekend is, hebben veel mensen aan het tot stand komen van dit deel meegewerkt. Enkele 'denksporters' wil ik graag met name noemen.

Prof.dr. J.C. Molenaar. Dank u voor het in mij gestelde vertrouwen, de rustpunten en de waardering die u gaf wanneer we het onderzoek bespraken. Vooral uw intensieve betrokkenheid bij de definitieve versie van het manuscript heb ik zeer gewaardeerd. Ik beschouw het als een eer dat u mijn promotor bent.

Dr. A.P. Provoost. Bram, dank voor de vrijheid die je hebt gegeven me te ontplooien. Je verstaat als geen ander de kunst te sturen zonder dat ik dat als een last ervaar. Ik denk dat de zelfstandigheid die binnen jouw groep essentieel is te overleven de ideale voedingsbodem vormt voor de wetenschapsbeoefening in het algemeen. Dank voor je vriendschap en steun, ook tijdens mijn verblijf in de V.S. We hebben samen meerdere reizen gemaakt en een betere gids had ik me niet kunnen wensen.

Prof.dr. M.A.D.H. Schalekamp. Dank u voor de beoordeling van het manuscript, voor uw bereidheid zitting te nemen in de commissie en hiervoor als secretaris te fungeren.

Prof.dr. P.R. Saxena. Dank u voor de beoordeling van het manuscript en voor uw bereidheid zitting te nemen in de commissie. Dank ook voor de gastvrijheid op uw afdeling te komen experimenteren. Van uw afdeling wil ik graag Antoinette Maassen-van den Brink en René de Vries bedanken voor hun hulp bij de 'natte' Farmacologie.

Prof.dr. B.A. Oostra. Dank u voor de beoordeling van het manuscript en voor uw bereidheid zitting te nemen in de commissie.

A.W. Cowley Jr., MD. Thank you for your support and the opportunity to work in 'your' department. From your lab, I would like to thank Meredith Skelton and Mary Kaldunski for their tremendous support.

R.J. Roman, PhD. Rich, I'm very grateful that you gave me the opportunity to come to the Medical College and work in your 'Roman Empire'. Thank you for your friendship, inspiration, and excellent teaching. The boat races we had with your 'Perpetuum Motion' on Lake Michigan are characteristic for our interaction. From your lab, I would like to thank Magdalena Alonso-Galicia, PhD, for her enormous support and help, and for the chronic blood pressure measurements, Cheng-Wen Sun for performing the vessel studies, and Lisa Geiger for her help preparing the slides for my seminar before the Department of Physiology in the winter of 1997, and for doing some studies after my departure. I would also like to thank Kathleen Hopp from the Department of Pediatric Nephrology for her friendship and support while working at the Roman lab.

H.J. Jacob, PhD. Howard, thank you for your invitation to visit your lab as well. Your inspiring discussion gave more meaning to the term 'Functional Genomics'. You were not at all disappointed when it became clear that my time in Milwaukee was going to be spent in Physiology. From your lab, I would like to thank everybody for their support, especially Michelle Brown for providing me with 'our' Fawn-Hooded rats.

M. Nobrega, MD. Marcello, the time we spent as roommates when I was in Milwaukee can be described by the word you so frequently use: excellent. You always managed to put things into perspective whenever I lost sight. Thank you for your friendship and support you gave my wife and me.

M. Shiozawa, MD, PhD. Masahide, although my participation in the genotyping was limited to harvesting tissue and sending the samples to Milwaukee, I would like to thank you for the unlimited energy you put into the project over the past years. Somehow, your data sheets were always printed out at night and during the weekends and Christmas. To us, you are really the hole-in-one of the Jacob lab.

Dr. J.H.C. Meijers. Carel, dank voor de prettige gesprekken die we hadden. Je kritische houding heeft mij vaak aan het denken gezet en zal dat in de toekomst ook blijven doen. De korte periode die ik op je lab heb doorgebracht heb ik als leerzaam en interactief ervaren.

Dr. G.H. Verseput. Gaico, dank dat je mijn paranimf wil zijn en voor de steun, hulp en gedeelde smart in hetzelfde onderzoek. Ik heb de intensieve interactie die we door onze beider promoties de laatste tijd hebben gehad en je hulp bij het tot stand komen van het uiteindelijke manuscript zeer gewaardeerd.

Dr. H.J. Duckers. Eric, dank dat je mijn paranimf wil zijn en voor je introductie in de wetenschap toen ik mijn afstudeeronderzoek bij je deed op het Rudolf Magnus Instituut. Jij gaf de eerste aanzet die uiteindelijk tot dit boekje heeft geleid.

Drs. R.J.E. van Staveren. Rob, dank voor de gesprekken die we hadden over het reilen en zeilen van het Instituut en je soepele opstelling wanneer ik weer eens wat nodig had. Helaas voor ons besloot je die kwaliteiten elders toe te passen.

Drs. J. Hagoort. Ko, dank voor je hulp bij het redigeren van het proefschrift. Je nauwgezetheid en redactionele vaardigheden, alsmede je grote kennis van de Engelse taal kwamen zeer goed van pas.

C. Luhrman-Schlomski. Cordula, dank voor je enorme inzet, nauwkeurigheid en betrokkenheid bij het onderzoek van onze groep. Zonder je steun waren en zijn we nergens. Over de bloeddruk- en metaboolmetingen en de verzorging en fok van 'onze' ratten zullen we ons nooit zorgen hoeven te maken.

J. Mahabier. John, dank voor je expertise en inzet bij de fok van onze ratten. De uitdrukking 'zonder dieren geen experiment' heeft door jou een extra dimensie gekregen.

Ing. I. Hekking-Weijma. Ineke, dank voor je inzet en doorzettingsvermogen bij onze experimenten en je grote analytische vermogen. Je nuchterheid, vaardigheden en kritische werkinstelling heb ik zeer gewaardeerd.

Ing. A.L. van den Bosch, Drs. M.R. Korte, en Ing. D.R. Tulling. Angela, Mario en Debby, dank voor jullie hulp en inzet bij de experimenten. Studenten begeleiden is toch een vak apart, en jullie hebben mij op dat gebied heel wat bijgebracht.

Ing. W.P. van Schalkwijk. Pim, dank voor je hulp op velerlei gebied. Je staat altijd voor iedereen klaar: de termen dienstverlening en klantvriendelijkheid zijn bij jou in goede handen.

Ing. T. Boijmans. Ton, dank voor het eindeloos analyseren van onze plasma's en zoals jij dat noemt 'vieze piezen'. Ook je hulp als officiële en officieuze ACP'er (weinig mensen weten wat het betekent) heeft een bijdrage geleverd aan de verdere automatisering van onze groep.

C. Peekstok. Coby, dank voor het eindeloos inbedden, snijden en kleuren van ruim 3.000 coupes. Ons contact was van het begin af aan prettig en ik ben blij dat je na veel jaren van hard werken ongetwijfeld intensief van je vrije tijd gaat genieten.

J. Lagerman. Jan, dank voor het redden en opnieuw configureren van mijn eerste PC. Het was een echte Windows-machine: uit het raam ermee.

Alle medewerkers van het E.D.C. Dank voor jullie inzet, hulp en het beantwoorden van al mijn lastige vragen: Thijs van Aken, Ron Briegoos, Amelie Byma, Enno Colly, Henk Dronk, Lydia van Gastel-Quist, Frans Greeven, Reier Hoogendoorn, Jaap Kasbergen, Wil Kort, Marijke Lagerman, Ed Lansbergen, Rob Meijer, Pim van Schalkwijk, en Roy Spruijt.

Op de laatste alinea van het dankwoord een ereplaats voor Elnetha, Eline en Laurens. Dankbaar ben ik voor hun steun en liefde die de drijfveer vormde om door te gaan. Zonder thuisfront geen promotie !

CURRICULUM VITAE

Richard Pierre Edwin van Dokkum werd geboren op 1 augustus 1969 te Hoek (Zeeuws-Vlaanderen). Hij volgde het voortgezet onderwijs aan het Christelijk College Nassau-Veluwe te Harderwijk, waar hij eindexamen deed in 1987. De Universitaire opleiding werd na de propaedeuse van het Hoger Laboratorium Onderwijs begonnen aan de Landbouw Universiteit te Wageningen. In het tweede jaar van de studie Zoötechniek werd van studie veranderd en zette hij de biologisch ingeslagen weg voort aan de Hogeschool van Utrecht, differentiatie Medische Biologie. Tijdens het laatste jaar van de studie en het jaar daarop deed hij wetenschappelijk onderzoek op het Rudolf Magnus Instituut voor Neurowetenschappen van de Vakgroep Medische Farmacologie van de Universiteit Utrecht, Afdeling Zenuwregeneratie (hoofd: Prof.dr. W.H. Gispen). Daar onderzocht hij met Dr. H.J. Duckers de neurotrofe effecten van een ACTH-analogon in een diermodel voor Multiple Sclerose, Experimentele Allergische Encephalomyelitis. Tegelijkertijd werkte hij op het Psychologisch Laboratorium van de Vakgroep Vergelijkende en Fysiologische Psychologie van de Faculteit Psychologie van de Katholieke Universiteit te Nijmegen (hoofd: Prof.dr. J.M.M. Vossen). In 1994 werkte hij als onderzoeker op de Pharma Divisie van Organon International te Oss, waar hij op de Afdeling Neurofarmacologie een onderzoek opzette naar de invloed van intraveneuze anaesthetica op het electroencephalogram bij de rat onder leiding van Dr. G.S.F. Ruigt. In januari 1995 werd hij aangesteld als Assistent In Opleiding bij het Instituut Kinderheelkunde (toenmalig hoofd: Prof.dr. J.C. Molenaar) van de Erasmus Universiteit Rotterdam onder leiding van Dr. A.P. Provoost. Op het voormalige Laboratorium voor Chirurgie werd het in dit proefschrift beschreven onderzoek uitgevoerd. Tijdens zijn AIO-schap volgde hij in 1996 de Erasmus Summerschool. Van mei tot en met november 1997 was hij werkzaam als research-fellow op het Department of Physiology (hoofd: Prof.dr. A.W. Cowley Jr.) van het Medical College of Wisconsin te Milwaukee onder leiding van Prof.dr. R.J. Roman, waar hij door middel van onder meer *in vivo* micropunctie en fysiologisch onderzoek, en *in vitro* vasculair onderzoek deed naar de oorzaken van het ontstaan van nierschade in de Fawn-Hooded rat. Tevens bezocht hij in Milwaukee het Laboratorium voor Genetisch Onderzoek van Dr. H.J. Jacob. Het door hem uitgevoerde onderzoek werd op diverse internationale congressen gepresenteerd. In het laatste jaar van zijn promotieonderzoek was hij nog werkzaam op de afdeling Farmacologie van Prof.dr. P.R. Saxena, waar hij onderzoek deed naar de responsen van nierarteriën van de Fawn-Hooded rat op diverse farmaca. De schrijver is sinds 1995 getrouwd met Elnetha Hoving, zij hebben een dochter en een zoon, Eline (1996) en Laurens (1998).

PAPERS

Duckers HJ, Van Dokkum RPE, Verhaagen J, Lopes da Silva FH, Gispen WH. Functional and neurophysiological evidence of the neuroprotective efficacy of trophic pharmacotherapy using an adrenocorticotrophic hormone$_{4-9}$ analog in experimental allergic encephalomyelitis, an animal model of multiple sclerosis. *Neuroscience* 1996;71:507-521.

Van Dokkum RPE, Jacob HJ, Provoost AP. Difference in susceptibility of developing renal damage in normotensive Fawn-Hooded (FHL) and August x Copenhagen Irish (ACI) rats after N^{ω}-Nitro-L-arginine methyl ester induced hypertension. *Am J Hypertens.* 1997;10:1109-1116.

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.

Duckers HJ, Van Dokkum RPE, Verhaagen J, Van Luijtelaar ELJM, Coenen AM, Lopes da Silva FM, Gispen WH. Neurotrophic Neurotrophic ACTH$_{4-9}$ analogue therapy normalizes electroencephalographic alterations in chronic experimental allergic encephalomyelitis. *Eur J Neuroscience* 1998;10:3709-3720.

Brown DM, Van Dokkum RPE, Korte MR, McLaughlin MG, Shiozawa M, Jacob HJ, Provoost AP. Genetic control of susceptibility for renal damage in hypertensive fawn-hooded rats. *Ren Fail.* 1998;20:407-411.

Van Dokkum RPE, Provoost AP, Jacob HJ, Roman RJ. Impaired autoregulation of renal blood flow in the Fawn-Hooded rat. *Am J Physiol.* 1999;276:R189-R196.

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:R855-R863.

Kouwenhoven EA, Van Dokkum RPE, Marquet RL, De Bruin RWF, IJzermans JNM, Provoost AP. Genetic susceptibility of the donor kidney contributes to the development of chronic transplant dysfunction. *Am J Hypertens.* 1999:in press.

Van Dokkum RPE, Jacob HJ, Provoost AP. Blood pressure and the susceptibility to renal damage after unilateral nephrectomy (UNX) and L-NAME-induced hypertension in rats. *Submitted for publication.*

Verseput GH, Braam BB, Van Dokkum RPE, Weening JJ, Koomans HA, Provoost AP. Renoprotection with ACE-inhibition is only partially dependent on blood pressure reduction in hypertensive Fawn-Hooded rats. *Submitted for publication.*

ABSTRACTS

Duckers HJ, Van Dokkum RPE, Verhaagen J, Gispen WH. A neurotrophic $ACTH_{4-9}$ analogue prevents the formation of central demyelinating lesions in CEAE and normalizes central conduction slowing. *J Neuroimmunol.* 1994;54:164.

Van Dokkum RPE, Jacob HJ, Provoost AP. Normotensive Fawn-Hooded (FHL) rats are susceptible to develop renal damage after L-NAME induced hypertension. *Am J Hypertens.* 1996;9:20A.

Provoost AP, Verseput GH, Van Dokkum RPE, Koomans HA. The efficacy of clonidine in the prevention and treatment of renal damage in the Fawn-Hooded (FHH) rat. *Am J Hypertens.* 1996;9:160A.

Van Dokkum RPE, Jacob HJ, Provoost AP. Differences in renal damage susceptibility after L-NAME-induced hypertension in inbred rat-strains. *Nephrol Dial Transplant.* 1996;11:A54.

Van Dokkum RPE, Jacob HJ, Provoost AP. In rats the susceptibility to develop renal damage after L-NAME-induced hypertension is genetically determined. *Paediatr Nephrol.* 1996;10:C112.

Van Dokkum RPE, Jacob HJ, Provoost AP. Genetic control of renal damage in rats with L-NAME-induced hypertension. *J Am Soc Nephrol.* 1996;7:1866.

Van Dokkum RPE, Jacob HJ, Provoost AP. Genetic control of susceptibility to glomerular damage in rats with L-NAME induced hypertension. *Kidney Int.* 1997;52:566.

Van Dokkum RPE, Jacob HJ, Provoost AP. L-NAME-induced hypertension reveals genetic differences in susceptibility to renal damage. *Am J Hypertens.* 1997;10:13A.

Provoost AP, Van Dokkum RPE, Jacob HJ. Genetic factors rather than blood pressure determine the development of proteinuria after unilateral nephrectomy (UNX) in rats. *Am J Hypertens.* 1997;10:32A.

Provoost AP, Kouwenhoven EA, Van Dokkum RPE, De Bruin RWF, Marquet RL. Donor and recipient factors in the development of albuminuria after kidney transplantation (KTx) in rats. *Am J Hypertens.* 1997;10:29A.

Provoost AP, Van Dokkum RPE, Jacob HJ. Genetic susceptibility is more important than systemic blood pressure in determining the development of renal damage after unilateral nephrectomy (UNX) in rats. *Kidney Int.* 1997;52:575.

Van Dokkum RPE, Jacob HJ, Provoost AP. Genetic susceptibility to renal damage after uninephrectomy and L-NAME-induced hypertension in rats. *J Am Soc Nephrol.* 1997;8:632A.

Provoost AP, Kouwenhoven EA, Van Dokkum RPE, De Bruin RWF, Marquet RL. Donor and recipient factors in the development of renal damage after kidney transplantation (KTx) in rats. *J Am Soc Nephrol.* 1997;8:664A.

Provoost AP, Shiozawa M, Majewski RR, Van Dokkum RPE, Jacob HJ. Genetic susceptibility to renal damage after unilateral nephrectomy in rats. *J Hypertens.* 1998;16:S46.

Provoost AP, Kouwenhoven EA, Van Dokkum RPE, De Bruin RWF, Marquet RL. Donor and recipient factors in the development of renal damage after kidney transplantation in rats. *J Hypertens.* 1998;16:S184.

Van Dokkum RPE, Jacob HJ, Provoost AP, Roman RJ. Increased intraglomerular pressure in Fawn-Hooded Hypertensive (FHH) rats due to impaired autoregulation of renal blood flow. *J Hypertens.* 1998;16:S189.

Provoost AP, Van Dokkum RPE, Jacob HJ. Differences in susceptibility to renal damage after unilateral nephrectomy (UNX) in rats. *Clin Exp Pharmacol Physiol.* 1998;25:A5.

Van Dokkum RPE, Jacob HJ, Provoost AP. Genetic susceptibility to renal damage after L-NAME-induced hypertension in uninephrectomized rats. *Clin Exp Pharmacol Physiol.* 1998;25:A20.

Provoost AP, Shiozawa M, Van Dokkum RPE, Jacob HJ. Phenoype and genotype variability in 4 strains of fawn-hooded (FH) rats. *Clin Exp Pharmacol Physiol.* 1998;25:A33.

Van Dokkum RPE, Cheng-Wen S, Provoost AP, Jacob HJ, Roman RJ. Impaired autoregulation of renal blood flow and myogenic response in the Fawn-Hooded rat. *Hypertension* 1998;32:602.

Van Dokkum RPE, Provoost AP, Jacob HJ, Roman RJ. Intraglomerular hypertension and hyperfiltration due to impaired renal autoregulation in the fawn-hooded hypertensive (FHH) rat. *Am J Hypertens.* 1998;11:1A.

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.

Provoost AP, Korte MR, Van Dokkum RPE. Development of renal damage in hypertensive fawn-hooded rats is not affected by gonadectomy. *Am J Hypertens.* 1998;11:89A.

Van Dokkum RPE, Cheng-Wen S, Provoost AP, Roman RJ. Impaired renal vascular responses in the Fawn-Hooded Hypertensive (FHH) rat. *J Am Soc Nephrol.* 1998;9:317A.

Provoost AP, Shiozawa M, Van Dokkum RPE, Jacob HJ. Differences in phenotype and genetic make-up between four strains of Fawn-Hooded rats. *J Am Soc Nephrol.* 1998;9:393A.

Shiozawa M, Provoost AP, Majewski RR, Van Dokkum RPE, Jacob HJ. Interacting genes determine susceptibility to proteinuria after uninephrectomy in rats. *J Am Soc Nephrol.* 1998;9:394A.