Glomerular and tubular damage in normotensive and hypertensive rats

Jarle Ofstad and Bjarne M. Iversen
Renal Research Group, Institute of Medicine, University of Bergen, and Haukeland University Hospital, Bergen, Norway

Submitted 18 June 2004; accepted in final form 8 November 2004

Ofstad, Jarle, and Bjarne M. Iversen. Glomerular and tubular damage in normotensive and hypertensive rats. Am J Physiol Renal Physiol 288: F665–F672, 2005. First published November 9, 2004; doi:10.1152/ajprenal.00226.2004.—Tubular cell damage is an important mediator of interstitial fibrosis in chronic renal diseases. Glomerular and tubular damage in genetic hypertension was therefore studied. Tubular and glomerular damage was investigated in 10-, 40-, and 70-wk-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) and compared with glomerular capillary pressure (PGC) and glomerulosclerosis in superficial (OC) and juxtamedullary (JMC). Tubular vimentin was used as criterion of tubular damage. Variation in tubular diameter was measured during change in perfusion pressure and, ureter ligation was used to demonstrate the relationship between tubular pressure and appearance of vimentin-positive cells. Tubular and glomerular damage was most pronounced in JMC and greater in SHR than in WKY. It was absent in 10-wk-old WKY and significantly higher in JMC of SHR compared with WKY at 70 wk of age. Numbers of vimentin-positive segments were 18 ± 9 vs. 38 ± 7% in JMC of 70-wk-old WKY and SHR (P < 0.02), and glomerulosclerosis was seen in 8 ± 3% vs. 19 ± 5% of glomeruli in JMC of 70-wk-old WKY and SHR, respectively (P < 0.01). PGC was 45 ± 3 mmHg in JMC of WKY and 57 ± 3 mmHg in JMC of 70-wk-old SHR (P < 0.001). Tubular diameter variation was greatest in SHR (P < 0.05) during pressure variation. Proteinuria was present only in 40- and 70-wk-old SHR and did not correlate with tissue damage. Tubular and glomerular damage in both strains develops in parallel and may be caused by a common mechanism, which may be glomerular capillary and tubular wall stretch during acute blood pressure variation which is greatest in JMC in SHR.

IN PROGRESSIVE HYPERTENSIVE disease, physical factors such as increased glomerular capillary pressure (PGC), filtration rate, and flow are considered to be important mediators of glomerulosclerosis (15, 21). Recent research has focused on other aspects, such as growth, tubular damage, accumulation of collagens, and appearance of immune cells in the peritubular interstitium. Altered tubular cell function seems to play a pivotal role in development of the tubulointerstitial pathologi

...glomerulosclerosis and tubular damage, as tubular degeneration was observed without proteinuria in old WKY. In old SHR, degeneration was accompanied by proteinuria but no further increase of the PGC. Based on these observations, and our previous work on RBF autoregulation (15, 18, 27), we suggest that pressure variation in glomerular and tubular during every day pressure variation of the systemic blood pressure might play a pathogenetic role in development of glomerular and tubular degeneration in SHR.

MATERIALS AND METHODS

Animals. Forty-six SHR and WKY rats (10, 40, and 70 wk old) were examined. The animals were obtained from Mollegaard, Skensved, Denmark and from the same batches as used in our previous study of PGC, glomerular sclerosis index (SI), and autoregulation (15).

The animals were kept, four in each cage, on a 12:12-h light-dark cycle, with free access to water and were fed ordinary rat chow (B&K Universal) containing 0.30% sodium, 0.70% potassium, 0.88% calcium, and 18% crude protein. All of the animal protocols were approved by the National Animal Research Authority of Norway.
Preparation of kidneys. The kidneys were removed from animals with pentobarbital sodium anesthesia, fixed in 4% formalin, and embedded in paraffin. Central longitudinal sections of the whole kidney including cortex, medulla, and papilla were prepared for examination in a Leica Orthoplan light microscope equipped with a video camera and connected to a Q500MC unit for picture analysis (Leica, Cambridge) and a Propaleta 8000 IT-digital visualization unit (Propaleta).

Staining and recording. Four-micrometer-thick dewaxed sections were incubated with unlabeled monoclonal mouse anti-vimentin primary antibody [clone V9, Isotype IgG1 kappa (DAKO)], followed by incubation with a biotinylated secondary antibody before incubation with a preformed avidin-biotinylated horseradish peroxidase macromolecular complex (ABC method). Negative control staining was carried out in all groups.

Because vimentin normally is present in the glomerular visceral epithelium and in the smooth muscle cells of arterioles and arteries, automatic recording of the vimentin-positive tubular area was unpractical. Tubular vimentin appeared in all or most cells in clusters of convoluted tubular segments (Fig. 1A). Clusters represent cross sections of proximal tubuli lying closely together, all or nearly all tubular cells being vimentin positive. The number of vimentin-positive segment clusters was counted in a 450-μm zone in the outer cortex and in a corresponding 450-μm deep juxtamedullar cortical zone in the whole cortical section. The area was analyzed by a PC program and calculated as percentage of total cortical area but adjusted for numbers of glomeruli in the region. Considering the difference of kidney size between the groups and the possible difference in glomerular numbers between the species, number of vimentin-positive segment clusters was related to the number of glomeruli in the corresponding OC and JMC areas.

Tubular casts, glomerular focal sclerosis, and adhesions between the glomerular tuft and Bowman’s capsule were recorded in similar examinations in a Leica Orthoplan light microscope equipped with a video camera and connected to a Q500MC unit for picture analysis (Leica, Cambridge) and a Propaleta 8000 IT-digital visualization unit (Propaleta).

Measurements of PGC. As supplement to our earlier study of glomerular SI and capillary pressure in OC and JMC glomeruli in 10- and 70-wk-old animals, similar measurements in 40-wk-old animals were done as described before (2, 23). Details of these methods are given in a previous study (15). In short, the left kidney was placed in a Lucite cup with the dorsal aspect facing upwards and immobilized by cotton moistened in saline, and micropuncture was done with glass micropipettes with sharpened tips of 3–5 μm, filled with 0.5 M NaCl colored with Evans blue under microscopic control (Wild M5 stereomicroscope with a magnification of ×60) as previously described (2). The micropipettes were connected to a servo-controlled counter pressure system. Pump pressure and aortic pressure were recorded continuously and illumination was provided by a two-armed fiber-optic lamp. The localization of the micropipette within the glomerular capillary cannot be defined visually; indirect criteria were employed for acceptance of pressure measurements as described by Aukland et al. (2).

SI. SI was measured in 3- to 4-μm-thick sections, stained with eosin and hematoxylin or PAS. It was calculated by a semiquantitative technique scoring each glomerulus from 0–4 as described before (15, 23). SI was calculated in both superficial and juxtamedullary glomeruli.

Measurements of tubular diameters. Diameters of proximal tubule were measured in 7 WKY rats and 7 SHR rats, 40 wk of age, after the kidney was placed in a Lucite cup with the dorsal aspect facing upwards and immobilized by cotton moistened in saline.

The perfusion pressure to the kidney was measured by a PE-50 catheter in the femoral artery connected to a transducer. A sling of thread was placed around the aorta above the renal arteries that were used for reduction of the renal perfusion pressure. The objective of a microscope was placed close to the surface of the kidney, and an ocular with optic scale was used with an enlargement of 20 × 16. An arbitrary tubule was punctured and free flow pressure was measured. A small amount of Evans blue was injected to identify the tubule as proximal, i.e., when it was possible to see three or more loops filled with blue color (18). The maximal tubular diameter from basement to basement membrane was measured in arbitrary units; diameter changes were calculated as percent of control diameter. After measurements of the control diameter, the renal perfusion pressure was reduced to the lower limit of autoregulation [mean blood pressure (MAP) ≈85–90 mmHg in WKY and MAP ≈105–110 mmHg in SHR] and the diameter was measured at the low perfusion pressure in the same tubulus. Thereafter, the perfusion pressure was increased abruptly by release of the thread around the aorta and the diameter of the same tubulus was measured shortly after the perfusion pressure increase. This diameter was also calculated as percentage of the control diameter.

Relationship between tubular pressure and vimentin formation. In five WKY rats, 10 wk of age, the left ureter was ligated and the kidneys were examined for appearance of vimentin after 1, 4, 6, and 11 days. The right kidney was used as control. Tubular hydrostatic
pressures ("free flow") were studied after 1 and 4 days using micro-puncture technique.

For measurement of tubular hydrostatic pressure, a micro-puncture technique was used that we described before (18). In short, the left kidney was immobilized in a Lucite cup with its dorsal aspect facing upward and immobilized by cotton moistened in saline. The urethra was annulated with a PE-50 catheter and the open abdomen was covered with mineral oil and the kidney was continuously irrigated with warmed Ringer acetate. Micro-puncture was performed with glass pipettes with tips of 3–6 μm in diameter, mounted in micromanipulators, and the punctures were performed under a stereomicroscope at ×50 magnification and a fiberoptic light source. The micropipettes used to measure intratubular pressure were filled with 0.5 M NaCl with Evans blue and neutralized with NaHCO3 to prevent precipitation. The micropipettes were connected to a servo-controlled counter pressure pump system. An arbitrary tubule was punctured, and free flow pressure was measured. Small amounts of Evans blue were injected to identify the tubule as proximal, i.e., when it was possible to see three or more loops filled with blue color. Measurements were performed only in proximal tubuli.

Urinary protein excretion. The 24-h urine excretions were collected in metabolic cages and urinary protein was measured with the pyrogallol red-molybdate method of Watanabe et al. (31).

Measurements of immunohistochemical staining. In both WKY and SHR, vimentin appeared in all or most cells in clusters of proximal convoluted segments (Fig. 1A). The distal tubule including macula densa and the easily identifiable ascending part of the loop of Henle, which in the rat surrounds the vascular bundles in the inner part of outer medulla, were vimentin negative.

In both species, the vimentin-positive proximal segment clusters appeared first and were most numerous in the deep juxtamedullary cortex, but increasing numbers of vimentin-positive segment clusters were found in more superficial layers with increasing age. The clusters presented an irregular, patchy pattern. The staining of these segments was substantially stronger in SHR than in WKY, indicating greater tubular derangement in SHR (Fig. 1B).

In all SHR groups and in 40- and 70-wk-old WKY, interstitial fibrosis with infiltration of lymphocytes and macrophages were present, starting in the deepest cortex and substantially enlarged with age.

The quantitative data for measurement of vimentin are shown in Fig. 2. In both outer and juxtamedullary cortex, the numbers of vimentin-positive segment clusters as percentage of the number of glomeruli in the same area increased with age in both species. At 10 wk, vimentin-positive segment clusters appeared only in the juxtamedullary layer of SHR and increased significantly in 40- and 70-wk-old SHR (P < 0.02). The percentage of positive segment clusters was always greater in SHR than in WKY; in JMC of 70-wk-old animals, this difference was significant (P < 0.02). The percentage of vimentin-positive segment clusters was greater in JMC than in OC in both species (P < 0.01).

Glomerular adhesions, glomerular focal sclerosis, and tubular casts. The presence of glomerular focal sclerosis and adhesions between the capillary tuft and Bowman capsular was examined in 250 glomeruli in each group, i.e., a total of 1,500 glomeruli, and presented as percent of observed glomeruli (Fig. 3). In OC adhesions were absent in 10-wk-old rats and also in JMC of 10-wk-old WKY. In JMC of SHR the percentage of glomeruli with adhesions increased substantially from 10 to 70 wk of age (P < 0.05); in JMR in 70-wk-old SHR, the percentage with glomerular adhesions was significantly greater than in OC (P < 0.01) and also greater than in JMC of 70-wk-old WKY (P < 0.01). A corresponding difference between cortical layers was not observed in WKY.

The percentage of glomeruli with focal sclerosis presented a similar pattern (Fig. 3, bottom). The percentage of glomerular focal sclerosis increased in the juxtamedullary cortex from 10 to 70 wk of age in SHR, but the difference between JMC in SHR and WKY at 70 wk of age was, however, not significant.
In 40- and 70-wk-old SHR, the percentage of focal sclerosis was greater in JMC than in OC (P < 0.05). Longitudinal, central cortical sections, five per group, were examined for tubular casts. Protein and granular casts were absent in 10-wk-old animals and in 40-wk-old WKY; two of five 40-wk-old SHR presented protein casts, less than two per section. The 70-wk-old animals presented multiple, mostly protein casts, for the greater part localized to the outer medulla and in substantially dilated collecting ducts in the deep cortex (data not shown).

Urinary protein excretion and glomerular filtration rate. Urinary protein excretion increased linearly from 10 to 70 wk of age in SHR, whereas it was constant in WKY and did not exceed 20 mg/24 h (Fig. 4). Glomerular filtration rate (GFR) was significantly lower in SHR compared with WKY and declined continuously with age being significantly lower in 10-, 40-, and 70-wk-old SHR compared with aged-matched WKY. In WKY, GFR did not change significantly from 10 to 70 wk of age (Fig. 4).

MAP, RBF, PGC, and SI. MAP was highly significantly different in WKY and SHR at 10, 40, and 50 wk of age, whereas RBF was not different (Fig. 4). The new data in 40-wk-old SHR and WKY are presented together with similar data for 10- and 70-wk-old animals (PGC and SI), which have been published earlier (Fig. 4) (15).

PGC in JMC was greater than in OC in all SHR groups and significantly greater than JMC PGC in the corresponding WKY groups. In 40-wk-old SHR, PGC was 59 ± 3 mmHg in JMC, substantially higher than in 10-wk-old SHR (P < 0.01) but not significantly different from PGC in 70-wk-old SHR. In OC, PGC was 47 ± 5 mmHg, which is not significantly different from that in 10- and 70-wk-old SHR. In 40-wk-old WKY OC, PGC was greater than in 70-wk-old WKY OC (P < 0.05).

SI was greater in JMC than in OC in all SHR groups. In 40-wk-old SHR, JMC SI was 0.73 ± 0.12, significantly greater than in OC, where it was 0.55 ± 0.16 (P < 0.01), but substantially less than in JMC of 70-wk-old SHR (P < 0.01). An increase of SI from 40 to 70 wk of age was also observed in OC of SHR (P < 0.01), but SI in JMC was significantly greater in SHR than in WKY in all groups. In WKY, SI was also significantly (P < 0.05) greater in JMC than in OC in all groups. In WKY, SI increased both in superficial and deep cortex significantly (P < 0.01) from 40 to 70 wk (Fig. 4).

Relationship between proximal tubular diameters and renal perfusion pressure. The proximal tubular diameters in WKY were slightly smaller than in SHR (Fig. 5). During reduction in renal perfusion pressure to 85–90 mmHg in WKY and 105–110 mmHg in SHR, the tubular diameter decreased 25%. After increase of the renal perfusion pressure to normal, the diameter of the proximal tubuli expanded and reached the control diameter in WKY, whereas in SHR a slightly but significantly increased diameter compared with control values was found. The measurements of tubular diameters were only performed in outer cortex of both strains.

Relationship between appearance of tubular vimentin-positive cells and tubular pressure. To estimate the relationship between tubular pressure and development of vimentin, tubular pressure was measured 1 and 4 days after ureter legation. These pressures were not different, and the data were pooled. The tubular pressures were 23.3 ± 1.2 mmHg in the kidneys with ligated ureter and 13.1 ± 0.9 mmHg in the kidneys with free ureteral flow (P < 0.001). Proximal tubular vimentin was not seen in kidneys with free ureteral flow. Vimentin appeared already after 1 day of ureteral ligation; it increased after 4 days and was very pronounced after 6 and 11 days (data not shown).

DISCUSSION

The main finding in the present study was the parallel development of glomerulosclerosis and tubular damage in
normotensive and hypertensive rats. The glomerular and tubular changes were both greater in JMC than in OC and also greater in hypertensive than in normotensive animals.

The present study provides new information about the development of cortical damage in normotensive and hypertensive animals. In both stains, nearly normal glomerular as well as tubular structures and PGC were found in the outer cortex of the kidney, although a small increase in vimentin-positive clusters was seen in outer cortex of 70-wk-old SHR. In juxtamedullary cortex in SHR, the glomerular and tubular damage were pronounced and the PGC was increased substantially. There is therefore a good correlation between PGC and cortical damage in outer cortex of both strains and in the juxtamedullary region of SHR. Development of tubular and

Fig. 4. Urinary protein excretion, glomerular filtration rate (GFR), mean blood pressure, total renal blood flow (RBF), glomerular capillary pressure, and glomerulosclerosis index from OC and JMC in 10-, 40-, and 70-wk-old WKY and SHR. Data from 10- and 70-wk-old animals are from Iversen et al. (15) and Wang et al. (30). Data from 40-wk-old animals are new. *P < 0.05, **P < 0.01 SHR different from WKY.

Fig. 5. Tubular diameter in subcapsular cortex after pressure reduction and increase within the pressure range of RBF autoregulation. *P < 0.05, different from control diameter at control pressure. **P < 0.01, from diameter at control pressure and diameter after release of reduced pressure. A: WKY. B: SHR.
glomerular damage was closely related to the increase in glomerular pressure in juxtamedullary cortex of SHR from 10 to 40 wk of age, although the SI, glomerular adherence, focal sclerosis, as well as tubular damage continued to develop without further increase in P\textsubscript{GC} after the age of 40 wk in SHR. In the present study, degenerative changes at glomerular and tubular level were also found in the juxtamedullary cortex of 70-wk-old WKY with a normal glomerular pressure. In this situation, other mechanisms may be involved.

Although the high P\textsubscript{GC} may be of major importance in the development of renal deterioration, other mechanisms may also play a role. Proteinuria is recognized as an important promoter of renal damage and was found only in 40- and 70-wk-old SHR (25). Recent studies from our laboratory showed that the percentage of glomeruli with adsorption droplets in podocytes was increased in the inner cortex of 70-wk-old SHR and the numbers of electrical charges are reduced in this area (7). Previous investigations also indicate that proteinuria in hypertensive animals comes from glomeruli localized to the inner cortex, and the content of protein in tubular fluid per nephron in the present study was probably comparable to that assumed to cause tubular damage in global affection (11, 25). The finding of a substantial number of protein casts, some blocking the tubule, has also been reported as typical for kidneys with tubular damage due to proteinuria. Thus the accelerating tubular and interstitial damage appearing pari-passu with the heavy proteinuria supports the hypothesis that tubular protein was an important cause of tubular cell degeneration and interstitial inflammation in SHR.

The tubular and glomerular damage in juxtamedullary cortex of 70-wk-old WKY was neither associated with high P\textsubscript{GC} nor protein leakage into the tubular lumen. The tubular damage was substantial, both proteinuria and casts were absent; increased tubular fluid protein reabsorption without proteinuria does not seem to have toxic effects (25). In the juxtamedullary cortex of 70-wk-old WKY, one may suggest that a harmful direct effect of systemic pressure on both glomeruli and tubuli might constitute a possible explanation for the findings in deep cortex of normotensive animals and represent a pathogenic mechanism in addition to the generally accepted toxic effect of tubular fluid protein and increased P\textsubscript{GC}. The parallel development of glomerular and tubular damage in our study suggests a common pathogenic mechanism; this obviously can be neither the P\textsubscript{GC} nor proteinuria.

An acute increase of the renal perfusion pressure is followed by a transient overshoot of P\textsubscript{GC}, RBF, and GFR (6, 16, 27) and a reduction of reabsorption in proximal tubules. Our study shows that this reaction also includes an increase in proximal tubular diameter. The RBF overshoot induced by an acute perfusion increase has been shown to be greater in SHR than in WKY and greater in the inner than in the outer cortex of both species; this is probably also the case for the P\textsubscript{GC} and GFR (27). The present finding of a change in proximal tubular diameter during increase in perfusion pressure indicates that acute systemic pressure variation is transmitted to the tubuli and this is more pronounced in SHR than in WKY and probably more in juxtamedullary cortex compared with more superficial areas of the cortex. Intratubular pressure variations have been seen using micropuncture technique, but the observation of change in tubular diameter measured at the tubular basement membrane level is new. Due to technical problems, it cannot be performed in deep cortex. This pattern of vascular reaction on acute perfusion pressure increase is thus similar to that of glomerular and tubular damage. Monitoring the systemic pressure in conscious SHR and WKY shows that the pressure variations necessary for this vascular reaction to take place are a normal and frequently occurring phenomenon (3).

Because the interstitial pressure in the kidney is very low, a wave of transtubular increase is induced by the transient increase of GFR that follows acute increase of systemic pressure and creates a corresponding increase of the tubular diameter and wall stretch. When exposed to acute ureter obstruction or to cyclic mechanical stretch in culture, tubular cells increase their expression of osteopontin mRNA and protein, possibly induced by production of ANG II (26). Osteopontin is a cell adhesion and chemoattractant molecule and is assumed to be an important mediator of interstitial infiltration of macrophages and thus a mediator of interstitial inflammatory derangement. The deleterious effect of pressure on the glomerular capillaries has similarly been referred to increased wall stretch activating constitutive molecules in the vessel wall and interference with the integrin-extracellular matrix interaction (29); recently, stretch applied to podocytes has been shown to induce increased osteopontin synthesis also in these cells (9). Thus parallel variations of wall stretch in glomerular capillaries and in proximal tubuli, a direct effect of frequently repeated acute pressure variations, may represent a unifying explanation of the parallel development of glomerular and tubular damage as well as the difference between the damage in outer and inner cortex (Fig. 6). It also explains the appearance of similar patterns of derangement in normotensive and hypertensive animals as well as the lack of close correlation between both glomerulosclerosis and tubular damage and the P\textsubscript{GC} in anesthetized animals in a steady state of systemic pressure (5, 33). According to Lemley et al. (19), the primary cause of glomerulosclerosis is increased P\textsubscript{GC}. The presence of glomerular and
Early tubular damage and glomerulosclerosis may be induced parallel with glomerulosclerosis, starting in the deep cortex. The acceleration of tubular damage in the phase of profuse proteinuria in SHR, however, indicates that the toxic effect of tubular fluid protein may be the main pathogenic mechanism in progressive renal damage in hypertension. Our hypothesis does not contradict the hypothesis of Remuzzi and Bertani (25) and Kriz et al. (19) but might be considered as a supplement to both. Possibly the glomerulotubular damage in 70-wk-old SHR was caused by high P_GC, proteinuria, and the glomerulotubular pressure wave induced by acute changes in perfusion pressure.

The RBF and GFR overshoot caused by acute systemic pressure increase endures until autoregulation has brought RBF, GFR, and the intrarenal pressure back to control value. This takes about a half minute or more, longer in the inner than in the outer cortex (27). We suggested that this difference and also the difference in overshoot between cortical layers occur because the slow component of autoregulation, the macula densa feedback mechanism, dominates over the rapid myogenic component in the inner cortex (10, 12).

We want to underline that our hypothesis needs further confirmation, especially by direct measurement of intratubular pressure during everyday activities and also measurements of osteopontin and other signal substances in the renal cortex as a function of these tubular pressure variations. We have not developed the technique necessary to perform such experiments. We feel, however, that the substantial direct and indirect evidence supporting our hypothesis makes it of interest.

Vimentin has been used by others as a marker of tubular cell damage and also quantitated by counting of vimentin-positive proximal tubuli but not in SHR (32). Vimentin positivity in cells with only little damage, such as partial loss of brush border and minor blebbing of cytoplasm, signifies that the method is quite sensitive. The findings in ureter-obstructed kidneys indicate that wall stretch induces vimentin-positive tubular cell damage. However, some reservations should be added; we measured vimentin induction in tubular cells after ureter obstruction, which creates a constant pressure increase, while our hypothesis suggests that vimentin must be induced due to pressure variations. During pressure variation, vimentin will probably develop more slowly than during ureter obstruction.

The use of SHR as a model for human essential hypertension is also still debated: in our study, the significantly reduced GFR in 70-wk-old SHR and the morphological picture with severe glomerulosclerosis and tubular degeneration, widespread interstitial fibrosis, and infiltration of lymphocytes and macrophages, sclerotic arteries, proteinuria, and abundant focal sclerosis, intratubular cylinders, and glomerular adherences were quite similar to the findings in advanced human essential hypertension.

In conclusion, the development of tubular damage is similar but more severe in SHR than in WKY, is localized to the proximal tubule, has a patchy distribution, and develops in parallel with glomerulosclerosis, starting in the deep cortex. Early tubular damage and glomerulosclerosis may be induced by variations of capillary and tubular wall stretch caused by everyday variations of the systemic blood pressure. Increasing proteinuria, tubular casts, and permanently increased P_GC have an additional severe accelerating effect on tubular damage in the later course of hypertensive proximal tubular derangement.

ACKNOWLEDGMENTS
We thank D. A. Sandnes and A. Drange for excellent technical assistance.

GRANTS
The present work was supported with a grant from the Norwegian Council of Cardiovascular Diseases.

REFERENCES


