Upregulation of juxtaglomerular NOS1 and COX-2 precedes glomerulosclerosis in fawn-hooded hypertensive rats

WILKO WEICHERT,1 ALEXANDER PALIEGE,1 ABRAHAM P. PROVOOST,2 AND SEBASTIAN BACHMANN1

1Anatomisches Institut, Charité, Humboldt Universität, 13353 Berlin, Germany; and 2Department of Pediatric Surgery, Erasmus University, 3000 DR Rotterdam, The Netherlands

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Weichert, Wilko, Alexander Paliege, Abraham P. Provoost, and Sebastian Bachmann. Upregulation of juxtaglomerular NOS1 and COX-2 precedes glomerulosclerosis in fawn-hooded hypertensive rats. Am J Physiol Renal Physiol 280: F706–F714, 2001.—This study describes elevated histochemical signals for nitric oxide synthase-1 (NOS1) and cyclooxygenase-2 (COX-2) in juxtaglomerular apparatus (JGA) and adjacent thick ascending limb of the kidney of fawn-hooded hypertensive rats (FHH). Two different age groups of FHH (8 and 16 wk; FHH8 and FHH16, respectively) were compared with genetically related fawn-hooded rats with normal blood pressure (FHL) that served as controls. Histopathological changes in FHH comprised focal segmental glomerulosclerosis (FSGS), focal matrix expression, and a moderate arteriolopathy with hypertrophy of the media, enhanced immunoreactivity for α-smooth muscle actin, and altered distribution of myofibrils. Macula densa NOS activity, as expressed by NADPH-diaphorase staining, and NOS1 mRNA abundance were significantly elevated in FHH8 (+153 and +88%; P < 0.05) and FHH16 (+93 and +98%; P < 0.05), respectively. Even higher elevations were registered for COX-2 immunoreactivity in FHH8 (+166%; P < 0.05) and FHH16 (+157%; P < 0.05). The intensity of renin immunoreactivity and renin mRNA expression in fferent arterioles was also elevated in FHH8 (+51 and +166%; P < 0.05) and FHH16 (+105 and +136%; P < 0.05), respectively. Thus we show that coordinate upregulation of tubular NOS1, COX-2, and renin expression precedes, and continues after, the manifestation of glomerulosclerotic damage in FHH. These observations may have implications in understanding the role of local paracrine mediators in glomerular disease.

Address for reprint requests and other correspondence: S. Bachmann, Anatomie der Charite, Elektronenmikroskopie, Campus Virchow Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany (E-mail: sbachm@charite.de).

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(COX-2) is constitutively expressed in cells of the thick ascending limb (TAL) adjacent to the MD and, to a lesser extent, in MD cells proper (19). Like NOS1, COX-2 appears to be involved in the regulation of glomerular arteriolar tone and renin synthesis (17, 18). Inhibition of COX-2 in progressive renal disease had a renoprotective effect (42). It has further been suggested that COX-2 function is under the control of NO and is negatively regulated by angiotensin II (9, 10). In FHH, increased urinary levels of various eicosanoids have previously been reported to coincide with hyperfiltration (14).

The present study addresses the question as to whether juxtагlomerular activity/expression levels of juxtагlomerular NOS1 and COX-2 are altered in 8- and 16-wk-old FHH compared with fawn-hooded rats with low blood pressure (FHL); FHL served as controls because they share most of the identified genetic loci associated with renal disease susceptibility in FHH but do not develop hypertension and early renal damage (29, 36, 39). Morphological parameters are documented to monitor age-related, specific vascular and glomerular damages and to evaluate their potential relationship to paracrine signals from the JGA.

MATERIALS AND METHODS

Animals. Experiments were performed in 8-wk-old male FHH rats (FHH8; n = 9), in 16-wk-old male FHH rats (FHH16; n = 10), and 16-wk-old male FHL rats (FHL16; n = 9). Rats were bred at the animal facilities of Erasmus University (Rotterdam, The Netherlands). Animals were housed and fed under standard conditions and received tap water ad libitum. Clinical parameters were not evaluated in details in the groups of the present study, because values (GFR, creatinine clearance, albuminuria, systolic blood pressure, etc.) had been solidly established by our group with rats of identical age that were kept under identical conditions (36, 39). Thus only blood pressure levels and urinary protein concentrations were measured before the death of the animals. Blood pressure was determined by indirect tail-cuff plethysmography in conscious animals. Proteinuria was measured before the death of the animals. Blood pressure was determined by indirect tail-cuff plethysmography in conscious animals. Proteinuria was measured before the death of the animals. Blood pressure was determined by indirect tail-cuff plethysmography in conscious animals. Proteinuria was measured before the death of the animals.

Perfusion fixation. Animals were anesthetized by using Nembutal. For morphological and histochemical analysis, animals were perfusion-fixed by cannulation of the abdominal aorta by using freshly prepared paraformaldehyde (3% in PBS) at a pressure of 220 mmHg initially, then 60 mmHg (32). To protect the tissues from freezing artifacts, subsequent perfusion was done with a sucrose-PBS solution adjusted to 800 mosmol/kgH2O. Kidneys were then removed and in part immediately shock-frozen in liquid nitrogen-cooled isopentane, in part processed for paraffin embedding, and in part postfixed in 1.5% glutaraldehyde for electron microscopy preparation.

Morphological analysis. For histostuctural study and for determination of the extent of glomerular damage, 5-μm-thick paraffin sections were stained with periodic acid-Schiff reagent (PAS). For fine structural morphology, semithin sections were stained with periodic acid-Schiff reagent and lead citrate. The extent of glomerular damage was determined on two 5-μm-thick PAS-stained paraffin sections/rat; a total of 400–600 glomeruli were evaluated per rat, and at least 4 rats/group were studied. Glomeruli showing significant features of segmental or global sclerosis, i.e., adhesion of the tuft to Bowman’s capsule, capillary collapse and/or balloononing, mesangial matrix expansion, and deposition of hyalin material, respectively, were scored. The percent values were taken as the sclerosis index.

Histochemical analysis. For demonstration of NOS tissue activity, NADPH diaphorase (NADPH-d) reaction was performed as described (1). Five-micrometer-thick cryostat sections were incubated in 0.1 M phosphate buffer containing nitro blue tetrazolium (NBT), NADPH, and Triton X-100. Tissues of all groups were processed simultaneously at an incubation temperature of 37°C. Reaction was stopped for all tissues after 30 min when the MD signal was clearly distinguishable and background staining had not yet appeared.

For visualization of mRNA expression, in situ hybridization was performed by using riboprobes transcribed from NOS1-, renin-, and α1-collagen IV-specific cDNAs as previously reported (1, 32). Briefly, riboprobes were generated from the respective vectors by in vitro transcription by using digoxigenin (DIG)-labeled UTP and T3 or T7 RNA polymerase for sense or antisense transcripts. DIG-UTP-labeled NOS1 and α1-collagen IV probes were subjected to time-controlled alkaline hydrolysis according to standard methodology. Probes were checked by formaldehyde-agarose gel electrophoresis and ethidium bromide staining. For in situ hybridization 7-μm-thick cryostat sections were cut, mounted on silane-coated slides, postfixed in 4% paraformaldehyde in PBS, acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, dehydrated in a graded ethanol series, prehybridized, and then hybridized with a riboprobe mix containing 5–10 ng/ml of the respective probe. Hybridization was performed at 40–47°C for 18 h. Slides were then washed sequentially with decreasing concentrations of sodium citrate (SSC) at 40°C, then in buffer 1 (0.1 M Tris-HCl, 0.15 M NaCl, pH 7.5) at room temperature, followed by an incubation with buffer 1 containing blocking medium (1% blocking reagent and 0.5% BSA) for 30 min. Sheep anti-DIG-alkaline phosphatase conjugate (diluted 1:500 in blocking medium) was administered for 60 min at room temperature and then overnight at 4°C. The slides were then washed twice with buffer 1 and rinsed in buffer 3 (0.1 M Tris-HCl, 0.1 M NaCl, 0.05 M MgCl2, pH 9.5). A solution of 4-NBT chloride (45 μl), 5-bromo-4-chloro-3-indolylphosphate (X-phosphate; 35 μl), and 2.5 mg levamisole in 10 ml of buffer 3 was applied for the color reaction. Reaction was stopped by washing twice with buffer 4 (0.1 M Tris-HCl, 1 mM EDTA, pH 8.0). For control, sense and antisense probes were applied in parallel. Slides were rinsed with PBS and coverslipped with PBS-glycerol.

For immunohistochemical detection of COX-2, goat antibody directed against a rat COX-2 COOH-terminal peptide was used on 5-μm-thick paraffin sections. To improve antigen retrieval, the slides after deparaffinization, were placed in 0.1 M sodium citrate buffer, pH 6.0, and boiled for 20 min in a microwave oven at 600 W. After several rinses in PBS and pretreatment with 10% native swine serum (NSS) for 30 min, slides were incubated with specific antibody (dilution 1:250 in PBS containing 1% NSS) for 2 h at room temperature and then at 4°C overnight. Slides were washed in PBS, and Cy3-conjugated donkey anti-goat IgG, diluted 1:500 in PBS, was applied for 1 h. After several washes in PBS, slides were coverslipped by using PBS-glycerol. For detection of immunoreactive renin, rabbit polyclonal antibody directed against purified rat renin was used on paraffin sections as

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described (6); signal was detected by the peroxidase-antiperoxidase method. For detection of α-smooth muscle actin, monoclonal mouse anti-human antibody was used on paraffin sections. After deparaffinization, slides were boiled for better antigen retrieval. Slides were then incubated with 4% BSA in PBS, followed by specific antisera (dilution 1:100 in PBS containing 1% NSS). After washing in PBS, bound antibody was visualized by using a silver-enhanced gold-labeling method. Slides were incubated with goat anti-mouse IgG conjugated with 4-nm colloidal gold particles (diluted 1:200) in PBS for 90 min at room temperature. After postfixation with 2% glutaraldehyde in PBS for 15 min and several rinses in PBS, slides were treated with freshly prepared silver enhancement reagent for 15 min. Reaction was stopped in PBS, and sections were coverslipped in PBS-glycerol. For immunolabeling of α1α2-collagen IV, rabbit polyclonal antibody directed against mouse α1α2-collagen IV was used on cryostat sections. After pretreatment with 2% BSA in PBS for 30 min, slides were incubated with primary antibody (1:500 in PBS), rinsed in PBS, and bound antibody was detected with Cy3-conjugated goat anti-rabbit IgG serum (diluted 1:250 in PBS) for 1 h. Slides were washed and coverslipped in PBS-glycerol. Control experiments were made by omitting first antibody and using nonimmune serum instead. Basic controls for antibody specificity had been proven in preceding studies.

Quantification of histochemical NOS and renin signal. NOS1, COX-2, and renin histochemical signals were semiquantitatively evaluated as described (6). To establish changes in MD, NOS1 enzyme activity, and transcription rate, the mean number of NOS-positive cells per single glomerulus was determined by evaluating a total of 400–600 glomeruli/animal. NOS enzyme activity at the MD was detected by the NADPH-d reaction. Colocalization of NADPH-d and NOS in the same cells has been demonstrated earlier (1). It was assumed that the intensity of the NADPH-d reaction on sections from perfusion-fixed kidneys was proportional to the activity of NOS1 and to the amount of NO released. Using standardized histochemical conditions with constant exposure time and temperature for each experiment, we performed the semiquantitative evaluation under the premise that, under control conditions, not all MD cells are histochemically positive for NADPH-d; instead, a proportion of cells regularly fall below the detection level of a signal. Thus under stimulatory conditions, previously absent NADPH-d signal in some MD cells would shift from below to above the detection limit of the method; conversely, under suppressive conditions a reduction of the number of positive cells below control level could be established. By analogy, NOS1 transcription rate and immunoreactive COX-2 abundance in the MD were estimated. NOS1 mRNA abundance was estimated by counting MD cells with a positive hybridization signal; it was assumed that changes in the number of reactive MD cells were proportional to changes in MD-NOS1 mRNA abundance. To this end, the color reaction after in situ hybridization had to be stopped simultaneously in the experimental groups to be compared. All COX-2-immunoreactive cells of TAL segments in the vicinity of the JGA and of MD were counted to determine changes in the COX-2 protein abundance in FHH compared with FHL. The evaluation of renin expression was based on the well-established fact that, with varying stimuli, a metaplastic transformation occurs between renin-synthesizing cells and typical smooth muscle cells of the afferent arteriolar wall, resulting in a length shift of and a shift of intensity in the immunoreactive portion of a vessel in an up- or downstream direction of the bloodstream. The changes correspond to the levels of renal renin synthesis and to plasma renin levels under various conditions (6, 31). Both renin mRNA and protein levels were evaluated. The values are presented as means ± SD. For statistics of all experiments, the Mann-Whitney U-test was applied. P < 0.05 was considered significant.

RESULTS

Clinical parameters. FHH8 and FHH16 rats had moderate but significant hypertension, with mean systolic blood pressure levels of 156 ± 12 (P < 0.05) and 160 ± 17 mmHg (P < 0.05), respectively, compared with 138 ± 18 in the FHL16 group. Proteinuria was elevated only in the older hypertensive group (24 mg/day in FHH16, 19 mg/day in FHH8, and 217 mg/day in FHH16; P < 0.05), indicating advanced nephron damage.

Renal histopathology. Renal damage was absent from the FHL16 group, occasionally present to a moderate extent in FHH8, and very prominent in FHH16. Both alterations of the vasculature and the nephron were encountered. Pathomorphology of cortical arteries and arterioles in FHH16 included mild-to-significant hypertrophy of the media, enhanced levels of immunoreactive α-smooth muscle actin, and the presence of intramural PAS-positive deposits (Fig. 1). Ultrastructural analysis also revealed that vascular remodeling also included degenerative changes of the smooth muscle cells with loss of myofilaments; degeneration apparently took its origin from the innermost layer of the media. Marked increases in the quantity of interstitial matrix compounds and thickening of the basement membrane were seen in the arteriolar wall, preferentially in subendothelial localization. Intima swelling was obvious, and subendothelial spaces were often widened (Fig. 1).

Glomeruli were substantially damaged in FHH16. Occasionally, collapsed glomeruli were observed (Fig. 2). Typically, however, they showed focal segmental glomerulosclerosis (FSGS), as evidenced by incipient stages with ballooning of the capillaries in the vascular pole region, where primary branches of the afferent arteriole were affected, and by more advanced stages with detachment of podocytes from the glomerular basement membrane, adherence of the glomerular tuft to the capsule (Fig. 2), and eventual opening of a
filtration/exsudation route toward the cortical interstitium. Hyalinization and collapse of capillaries were characterizing final stages, with global sclerosis. FSGS was occurring at a mean rate of 23.5 ± 6.6% (p < 0.05) whereas, in FHL16 and FHH8, glomeruli were largely intact. Areas of focal synechiae in FHH16 showed manifest overexpression of α₁-collagen IV mRNA in mesangial cells and parietal epithelial cells and in nascent synechiae of the glomerular stalk, as revealed by in situ hybridization. An enhanced collagen IV immunoreactivity was found in analogous positions (results not shown). In advanced stages, collagen IV expression in the glomerular periphery was located in fibroblasts lining the newly formed paraglomerular spaces. Tubular damage was also accompanied by fibroblast accumulations that showed an enhanced collagen IV syn-

Fig. 1. Arteriolopathy at the glomerular vascular pole in 16-wk-old fawn-hooded hypertensive rats (FHH16). A: profile of the preglomerular afferent arteriole (++) with subendothelial vacuolation and signs of incipient necrosis next to a macula densa (arrows). Glomerulus with segmental sclerosis; semithin section is shown. B: electron micrograph of a preglomerular portion of afferent arteriole with markedly increased subendothelial hyalin deposits (++); a renin-containing cell is obvious by specific granules (arrowhead). C and D: media hypertrophy of afferent and efferent arteriolar wall of 16-wk-old fawn-hooded rats with normal blood pressure (FHL16; C) compared with FHH16 (D). Note the thickening of media (black signal in D) in pre- and postglomerular vessels of FHH16 as revealed by silver-enhanced gold labeling of immunoreactive α-smooth muscle actin. Light microscopy with interference contrast optics was used. Approximate magnifications: ×1,000 (A), ×4,500 (B), ×500 (C and D).

Fig. 2. Glomerular pathomorphology in FHH (periodic acid-Schiff reagent; PAS). A: intact glomerulus from an 8-wk-old FHH (FHH8). B: glomerulus showing incipient collapse with precipitate of plasma proteins in Bowman’s space and in the tubular lumen (FHH16). C: glomerulus typically showing advanced segmental glomerulosclerosis and broad tuft synechia to Bowman’s capsule of the lower lobule; the upper lobule is intact (FHH16). Approximate magnification: ×250 (A–C)
thesis. Tubular portions in the vicinity of sclerotic glomeruli showed epithelial degeneration and proteinaceous content of the lumen and were enclosed by a thickened layer of matrix (Fig. 2). Matrix overexpression was largely absent from cortical parenchyma in FHH8.

Histochemistry of juxtaglomerular vasoactive parameters. Compared with the normotensive FHL16 group, FHH groups presented significant augmentation of NOS1 activity and mRNA abundance (Fig. 3) and COX-2 immunoreactive signal in MD and adjacent TAL cells (Fig. 4). The preglomerular portion of the afferent arteriole showed marked enhancements of immunoreactive renin and renin mRNA expression levels, respectively (Fig. 5). NOS activity, as expressed by the number of NADPH-d-stained MD cells, was significantly increased in FHH8 (+153%; \( P < 0.05 \)) and in FHH16 (+93%; \( P < 0.05 \)); accordingly, MD cells stained for NOS1 mRNA transcripts were numerically increased as well in FHH8 (+88%; \( P < 0.05 \)) and in FHH16 (+98%; \( P < 0.05 \)). Thus increased NOS signals in FHH were evident by significant augmentation of histochemically labeled MD cells (Fig. 3).

In probing for COX-2 immunoreactivity, even more pronounced increases were encountered in MD and adjacent TAL portions of FHH (Fig. 4). In FHL like in other control rat strains, COX-2 immunoreactive cells were located mostly next to, but rarely within, the MD. Compared with FHL16, COX-2 immunoreactivity, as expressed by the number of fluorescent cells, was significantly increased in FHH8 (+166%; \( P < 0.05 \)) and in FHH16 (+157%; \( P < 0.05 \)). Renin immunohistochemical signal, as expressed by the number of renin-positive juxtaglomerular sites per defined section area, was
increased in FHH8 (+51%; \( P < 0.05 \)) and FHH16 (+105%; \( P < 0.05 \)), and, similarly, renin mRNA expression was elevated in FHH8 (+166%; \( P < 0.05 \)) and FHH16 (+136%; \( P < 0.05 \)), respectively, compared with FHL16 (Fig. 5).

DISCUSSION

The present study revealed a coordinate enhancement of mRNA and activity levels of NOS1 and protein levels of COX-2 in the MD and juxtaglomerular portion of the TAL and of renin mRNA and protein levels in the afferent arteriole in FHH rats. This phenomenon occurs before the manifestation of glomerular disease and continues at least up to the age of 16 wk.

The observed patterns of FSGS largely corresponded to what has been reported earlier on renal pathogenesis in FHH (24, 26) and were thus in agreement with the rapid progression of the damage leading to chronic renal failure in FHH after \( \sim 1 \) yr (13, 27). The older FHH group also showed a modest-to-medium-strength arteriolopathy of the smaller resistance arterioles. In these vessels, a pronounced media hypertrophy coincided with myocyte degeneration of the innermost media layers, and these changes had also affected the preglomerular portion of the afferent arteriole, a functionally prominent site for the regulation of glomerular perfusion (31).

The animals of the FHH groups in our study were moderately hypertensive. Among the possible causes leading to the progression of renal failure in this rat strain, systemic hypertension may play a significant, although not the central, role in FHH, and blood pressure correlated closely with capillary hypertension and hyperfiltration (36). Accordingly, pharmacological treatment that decreases blood pressure reduced or prevented glomerular and renal vascular damage in FHH (35). However, other hypertensive strains such as spontaneously hypertensive rats (SHR) have a much higher blood pressure but are quite resistant to renal damage unless an additional impact such as uninephrectomy is imposed (15). Because variation in blood pressure in FHH correlates with renal damage only at a rate of 42%, additional adverse factors must be effective (8); in fact, insufficient afferent arteriolar tone in conjunction with a relatively high efferent arteriolar resistance seem to be the key factors leading to glomerular hypertension and consequent damage (36).

We found markedly increased NOS1 enzyme activity and mRNA abundance in the MD of FHH. As established for other models, activation of NOS1 may result in a constitutively increased release of NO from this site (3, 5, 6, 43). Under the given condition of an established hyperfiltration and the probably resulting elevation in distal tubular NaCl load in FHH, an enhanced expression of NOS1, however, is an unexpected finding because this condition should rather reduce than enhance NOS1 activity and synthesis (3, 6, 37). A secondary, reactive upregulation of NOS1 in FHH would therefore seem unlikely, whereas a primary, as yet unidentified, stimulation of NOS1 would appear
more likely. As a consequence, enhanced levels of NO from MD may diffuse into the JGA (5), where binding to its receptor, soluble guanylyl cyclase (sGC), could induce a relaxation of intra- and extraglomerular mesangial cells and nearby located vascular cells (2). As a result, basal afferent tone could be inadequately reduced, which would agree with an impaired myogenic autoregulation in FHH compared with FHL (40) and abnormal operating levels of TGF despite an intact responsiveness of this mechanism (41). The resulting elevated PGC may in fact be related to impaired local NO abundance, which is known to affect juxtaglomerular vasoconstrictor mediators (31, 43, 40).

Previous studies have identified the podocyte as the cell type centrally involved in the glomerular structural and functional deterioration in FSGS (25, 26); however, an increased physical impact on the glomerular capillaries in FHH may also require the countering forces from the mesangial side (12, 25). Altered NO release from the JGA may therefore alter this parameter as well in pathogenetic respect.

Concomitantly enhanced immunoreactivity for COX-2, the rate-limiting enzyme in prostaglandin and thromboxane synthesis, in MD and adjacent TAL cells is likely to reflect an augmented release of one or several of the biologically active metabolites such as PGE₂, PGI₂, thromboxane B₂, or others (for review, see Refs. 7, 31, and 34). Release of these substances would be in line with previous findings in FHH on enhanced excretion of all eicosanoids, including PGE₂, coincident with the onset of hyperfiltration (14), whereas subsequent progressive proteinuria in these rats was associated with an increase in thromboxane B₂ and a decrease in PGE₂ excretion. Although the array of prostanooids produced by the MD has not been characterized, the primary product synthesized by the TAL is PGE₂ (4),
which in the setting of volume depletion appears to maintain GFR by dilating the afferent arteriole (20). A recent study has suggested a pathogenetic role for COX-2 in progressive renal failure induced by the reduction of renal mass (42); inhibition of COX-2 significantly reduced the damage, which may have implications for the role of prostaglandins in FHH.

The reason COX-2 is upregulated in FHH is as unclear as that for NOS1, because an augmented filtrate should suppress rather than enhance expression of the enzyme, and an increased NaCl load at the MD substantially decreased expression of the enzyme (44). On the other hand, COX-2 may be activated by NO that probably interacts with its heme domain by means of the intermediate coupling product peroxynitrite (28). Accordingly, renal cortical COX-2 is likely to be under the stimulatory control of NO, as has been suggested from studies in rabbit (30), rat (9, 33), and mice with targeted deletion of NOS1 (Bachmann S and Theilig F, unpublished observations). Therefore, enhanced NO levels generated in MD may be related to the enhanced juxtaglomerular COX-2 expression seen in FHH.

We also observed an enhanced expression of renin in the afferent arteriole of FHH. This result agrees with previous findings on coordinate changes in the juxtaglomerular levels of NOS1 and renin (6, 31, 37). In fact, NO may exert a direct, cGMP-mediated effect on the granular renin-producing cells because these were shown to contain significant amounts of soluble guanylyl cyclase (2). In addition, there are several lines of evidence that COX-2 may also be directly involved in the transmission of MD-stimulated renin release (17, 38).

Although data on plasma renin activity in FHH are somewhat controversial (16, 21, 27, 29), it is evident that elevated renin levels and elevated blood pressure correlated with variations in renal damage (27). We also found that FHH were protected from renal damage by an inhibitor of the angiotensin-converting enzyme, especially when it was administered before the development of FSGS (35, 41, 43). Local upregulation of the type 1 angiotensin II receptor may also be involved in the regulation of glomerular perfusion because elevated levels of the receptor were found in FHH (22).

Altered renal gene expression in FHH is likely to be responsible for the susceptibility to FSGS because transplantation of FHH kidney into a normotensive, renal damage-resistant strain has proven that the susceptibility for renal damage depended on the graft kidney when the recipient was exposed to high blood pressure (23). Genetic analysis of this strain has identified two genes that are responsible for a proportion of the genetic variation related to renal damage, and one locus on chromosome 1, Rf-1, was reported to explain 37% of the total variance in proteinuria, but, in addition, a number of other loci were detected as well, thus underscoring the complexity of the genesis of renal deficits in FHH (8). A clear relationship to the aberrant juxtaglomerular gene expression observed in this study thus awaits further clarification.

In conclusion, we have observed enhanced expression of epithelial NOS1 and COX-2 at the JGA, along with increased renin expression in the glomerular afferent arteriole. In other models, it has been shown that locally enhanced NOS1 and COX-2 activity may affect local vascular tone. These changes may thus have implications for the experimentally established hyperfiltration in FHH. The enhanced renin expression may be related as well to the pathogenesis in this rat model. An application of selective pharmacological inhibitors will be necessary to clarify these issues. A potential link between the genetic variations in FHH and the alterations of juxtaglomerular gene expression remains to be discovered.

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REFERENCES


