Transgenic rats carrying the mouse renin gene—Morphological characterization of a low-renin hypertension model

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Transgenic rats carrying the mouse renin gene—Morphological characterization of a low-renin hypertension model. Transgenic rats (TGR; strain name TGR(mRen2)27) harboring the mouse Ren-2 renin gene have been recently generated as a model for the study of primary hypertension that offers the advantage of a clearly-defined genetic alteration. Expression of the mouse Ren-2 gene causes severe hypertension (200 to 260 mm Hg) which is responsive to converting enzyme inhibitors. Compared to control transgene-negative littermates, plasma renin and angiotensin II values are lowered in TGR, whereas plasma prorenin values are strongly elevated. The adrenal gland in TGR shows marked overexpression of mouse renin messenger RNA; in situ hybridization using a 35S-labelled mouse-renin RNA probe reveals that enhanced renin expression is mainly localized to cells of the zona glomerulosa and outer zona fasciculata. Immunohistochemically, renin protein in the TGR adrenal gland is stored in larger quantities than in controls. Adrenal transgene expression probably accounts for most of the elevated plasma prorenin level in TGR, since bilateral adrenalectomy (ADX) causes a significant decrease in prorenin level (318±79 ng angiotensin I/ml/hr before ADX to 70±43 ng 4 days after ADX, P<0.0005). In the kidney, renin synthesis is almost completely suppressed in TGR. In situ hybridization demonstrates that few juxtaglomerular afferent arterioles express renin. Immunohistochemically, the TGR kidney shows significantly reduced renin and angiotensin II immunoreactivity at the afferent arteriole. Ultrastructural analysis of the afferent arteriolar wall frequently shows the complete absence of renin secretary granules since the granular cells are mostly converted into smooth muscle cells. Beginning at an age of approximately four to six months, TGR develop hypertension-related alterations and pathological lesions in various tissues. In the kidney, the wall thickness of arterioles and arteries is strongly increased, and glomerular lesions including different stages of sclerosis are observed. The thoracic aorta displays a considerable increase in tunica media thickness due to both myocyte hypertrophy and interstitial fibrosis. Coronary arteries and arterioles of the heart are thickened and perivascular fibrosis is observed. The data show that TGR(mRen2)27 transgenic rats display all typical characteristics of hypertensive pathology, making them an interesting model for therapeutic interventions. The fact that these changes occur in animals with a single gene difference to normotensive rats makes them a particularly suitable model for studies on gene-related hypertensive processes.

Disorders of the circulating renin-angiotensin system (RAS) play a central role in the genesis and development of hyperten-

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mouse and rat renin gene transcripts, Mullins, Peters and Ganten [20] confirmed that renin gene transcripts in the adrenal gland were of Ren-2 origin. To a lesser extent, mouse transcripts are expressed also in other tissues, such as the brain, the thymus and parts of the intestinal tract. Thus, it is anticipated that these rats will provide a valuable tool for the investigation of low plasma-renin hypertension and of local tissue RAS function.

The present study describes the local tissue renin expression by in situ hybridization histochemistry, establishes the tissue renin-protein localization by immunocytochemistry and presents a detailed analysis of the microanatomical characteristics in TGR of different age at the renal and cardiovascular levels. In addition, bilateral adrenalectomy was performed in order to investigate the role of the adrenal gland in elevated prorenin levels in TGR.

Methods

Animals

Transgenic rats of the hypertensive line TGR(mRen2)27 were used in this study. These rats were produced and bred in the Department of Pharmacology, Heidelberg, as previously described [20]. Heterozygous hypertensive males and females (200 to 500 g body weight, age 2 to 8 months) that had been analyzed for the presence of transgene construct by tail biopsy and Southern blot assay were used in this study. For the bilateral adrenalectomy experiment, five-month-old male TGR were used. Age-matched transgene-negative littermates were used as controls. Systolic blood pressure was measured by tail plethysmography under light ether anesthesia using the tail cuff method. Values ranged between 200 and 250 mm Hg in transgenic males, 160 to 220 mm Hg in transgenic females and 110 to 140 mm Hg in normotensive controls. No pharmacological intervention was performed to lower the blood pressure of the hypertensive animals.

Tissue preparation for morphology

For conventional morphological investigation using light and electron microscopy, animals were anesthetized by an i.p. injection of Nembutal (40 mg/kg body wt) and perfusion-fixed by cannulation of the abdominal aorta as described [23]. Animals were perfused at a pressure of 240 mm Hg for 2 minutes with a mixture containing 1.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. Total osmolality of the fixative was 800 mOsm/kg H2O. After perfusion, kidneys, adrenals, heart and thoracic aorta were removed and postfixed for an additional 24 hours in the same fixative. Subsequently, small tissue cubes were prepared and rinsed in cacodylate buffer adapted to the osmolality of the fixative with sucrose. The tissue was then postfixed in 1% osmium tetroxide solution and plastic-embedded after dehydration in a series of graded alcohols. Epon 812 was used as embedding medium. For light microscopy, semithin sections (1 μm) were cut and stained in Richardson's solution. Ultrathin gray sections were contrasted in uranyl acetate and lead citrate and viewed in a Philips 301 electron microscope.

Immunohistochemistry

Immunohistochemical studies were performed for the localization of renin and angiotensin II in the kidney and adrenal gland. For this purpose, hypertensive transgenic animals as well as normotensive controls of both sexes were anesthetized and perfusion-fixed as described above, using 2% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4, at room temperature. Total osmolality of the fixative was 800 mOsmol. After perfusion, the kidneys and adrenals were removed and postfixed in the same solution for two hours. Tissue was rinsed in PBS adapted to the osmolality of the fixative by addition of sucrose and then routinely embedded in Paraplast; 5 μm sections were mounted on acid-cleaned chrome-alum coated glass slides. Immunohistochemical staining for renin was performed using a polyclonal rabbit antibody against purified mouse submandibular gland renin ([24], from Dr. T. Inagami, Vanderbilt University, Nashville, Tennessee, USA). This antibody was used at dilutions of 1:500, 1:5000 and 1:10000, depending on the tissue. Detection of the bound antibody was performed using a peroxidase-antiperoxidase detection system (PAP; Dakopatts, Denmark). Sections were subsequently exposed to 0.1% diaminobenzidine and 0.02% H2O2 as a source of peroxidase substrate. For comparison, a rabbit polyclonal antibody against purified rat kidney renin was used at dilutions between 1:5000 and 1:50000 (antibody was from Dr. E. Hackenthal, Dept. of Pharmacology, Heidelberg, Germany). For detection of angiotensin II, a rabbit polyclonal antibody against angiotensin II was used at dilutions between 1:5000 and 1:10000. Control experiments were made by omitting the first antibody step (incubation with 1% normal nonimmune rabbit serum instead). Sections were not counterstained, and were viewed in a Reichert Polyvar microscope using interference contrast optics.

In situ hybridization

For in situ hybridization experiments, hypertensive rats as well as normotensive controls of both sexes weighing between 200 and 400 g were anesthetized with diethylether. After opening of the peritoneal cavity, kidneys and adrenal glands were quickly removed and tissue was treated as described [25]. To generate the hybridization probes, a SacI/PstI fragment of the mouse Ren-2d cDNA was subcloned into a pSP65 transcription vector to generate cRNA and into the pSP64 vector to generate mRNA. The plasmids were linearized using either AccI for the pSP65 plasmid to generate an appropriate template for in vitro transcription of renin antisense RNA (template pSLM-mRE251), or KpnI for the pSP64 plasmid to obtain a template for the renin sense RNA (template pMLS-mRE248) using established techniques. The probe size was approximately 330 base pairs. The plasmids were from Dr. K. Gross (Buffalo, New York, USA) [21].

The in vitro transcription was carried out using SP6 polymerase (Promega; 1 U/μl to generate cRNA and mRNA, respectively, in the two plasmids) at 37° for 90 minutes. For transcription, one μg of linearized plasmid DNA, 48 pmol [35S]-α-labeled UTP (Dupont NEN; sp. act. 1350 Ci/mmol), 40 nmol ATP, CTP and GTP, respectively, and 240 pmol UTP were incubated for one hour at 40°C in a mixture containing 40 mM Tris-HCl (pH 7.5), 6 mM MgCl2, 2 mM spermidine, 10 mM NaCl, 100 μM DTT
and 1 U/μl RNase inhibitor (Promega). The DNA template was then digested by adding 20 ng/μl RNase-free DNase I for 15 minutes at 37°C in the presence of 1 U/μl RNase inhibitor. The transcripts were then purified in phenol and chloroform-isooamyl-alcohol (each at 1:1 vol/vol) and separated from non-incorporated nucleotides by ethanol precipitation, checked by denaturing formaldehyde gel electrophoresis, and used directly for the in situ hybridization experiments. The specific activity of the probes was in the order of 5 × 10^8 dpm/μg RNA.

For in situ hybridization, alternate sections were used for sense and antisense probe hybridization. Sections were washed two times for 10 minutes in sterile water followed by a deproteinization in 0.1 M HCl for 10 minutes. After two short washing steps (3 min) in PBS the slides were acetylated for 20 minutes in 0.1 M triethanolamine/0.25% acetic anhydride (pH 8.0). Next the slides were washed two times (5 min) in PBS, dehydrated in graded ethanol and air dried. Subsequently, 150 μl of a prehybridization mixture containing 50% deionized formamide, 50 mM Tris-HCl (pH 7.6), 25 mM EDTA, 20 mM NaCl, 0.25 mg/ml yeast tRNA, 2.5× Denhardt’s solution (100× = 0.05% ficoH, 0.05% polyvinylpyrolidone, 0.05% bovine serum albumin) was applied to the sections; they were incubated at 37°C for two to four hours in a humidified chamber. Next the slides were drained and 10 μl hybridization buffer (50% deionized formamide, 20 mM Tris-HCl, pH 7.6, 1 mM EDTA, pH 8.0, 0.3 mM NaCl, 0.1 M DTT, 0.5 mg/ml yeast tRNA, 0.1 mg/ml poly-A, 1× Denhardt’s solution, 10% dextran sulphate) containing either 5 or 20 pg of labeled cRNA or mRNA were μl were applied per section. Activity of the final probe was approximately 3000 and 10000 dpm/μl, respectively. The sections were covered with small pieces of Paraflim and incubated in a humidified chamber at 37°C for 16 to 18 hours. After hybridization, the Paraflim was removed in 2× standard saline citrate (SSC) at 48°C. Next, the slides were washed in 1× SSC/50% formamide at 48°C for six hours. The washing solution was changed every 90 minutes. Final high stringency washes were carried out with either 1× or 0.5× SSC, each at room temperature two times for 10 minutes. Next, the slides were dehydrated in a series of graded ethanol and air dried. The sections were dipped in a 50% NTB-2 emulsion (Eastman Kodak, Rochester, New York, USA) in water at 45°C, exposed at 4°C in bakelite boxes containing desiccant, developed with D19 developer (Kodak) and fixed with Unifix (Kodak). The time of exposure of the slides was either 5, 10 or 30 days.

**Bilateral adrenalectomy (ADX)**

For the ADX experiment, two groups of rats were established: one group of normotensive nontransgenic littermates (N = 5), and one group of hypertensive transgenic rats (N = 5). In both groups, ADX was carried out under ether anesthesia by opening of the abdominal cavity. After operation, all animals were supplemented with 0.9% saline as the only drinking source. In all animals, blood pressure was measured and blood was taken three days before surgery and four days after surgery. Blood specimens were collected in EDTA for determination of active renin and prorenin as described [26]. Values are given as nanograms of angiotensin I (ng Ang I) per milliliter plasma per hour. Statistically, changes were analyzed using ANOVA followed by paired t-test. Differences of a level of P < 0.05 were considered significant. The values are given as means ± standard deviation.

**Results**

**Renin-angiotensin system in the adrenal gland**

For in situ hybridization using a mouse renin mRNA probe, adrenal glands of male and female transgenic and control rats of an age ranging from two to six months were examined. The renin probe used in the assay cross-hybridized with both mouse and rat renin mRNA and was therefore applied to both the tissues of TGR and controls.

Renin gene transcripts were 10 to 100 times more frequent in the TGR adrenal cortex, than in controls (Fig. 1 a-c). Labelling was enhanced in both sexes. The hybridization signals were restricted to the adrenal cortex in both transgenic and control animals. No specific autoradiographic grains were detected in the medulla or in the organ capsule (Fig. 1 a-e). In TGR, the strongest density of renin transcripts was found at inner layers of the zona glomerulosa and in the outermost region of the zona fasciculata at the border to the zona glomerulosa. In this region, scattered single cells or groups of two to four cells revealed a particularly strong hybridization signal. Particle density in these cells outnumbered the surrounding basic grain density by approximately fivefold (Fig. 1a). In some cells, the hybridization signal over the nuclei was enhanced. Hybridization signal over the inner zona fasciculata was less strong and autoradiographic grains were more evenly distributed. In the zona reticularis, patches of 30 to 100 cells bordering the adrenal medulla showed an increased signal about twice as strong as in the inner zona fasciculata (Fig. 1d). Hybridization signal in the normotensive control adrenal glands was generally low in the cortex with no recognizable zonal differences (Fig. 1 c,e). Background labelling was minimal or nonexistent under the high-stringency washing conditions employed.

For detection of renin protein, deparaffinized sections of the adrenal glands of TGR and control rats were incubated with a polyclonal anti-mouse renin antibody which, however, was not totally species-specific but cross reacted to some degree with the rat antigen. The TGR adrenal glands revealed stronger antibody labelling than the controls; labelling was localized at the outer zona fasciculata in close analogy to the in situ hybridization signal (Fig. 2a). The inner zona fasciculata in TGR was almost devoid of renin whereas in the zona reticularis, similar to renin mRNA distribution, patches of reactive cells could be observed. In control adrenal glands, antibody staining in the same concentration range applied for the TGR adrenal glands was so weak that the label could barely be visualized (Fig. 2c). In more detail, cellular immunoreactivity disclosed small labelled granules grouped around the nucleus (Fig. 2b). No cellular immunoactivity could be detected in the adrenal gland of TGR or controls using an antibody against angiotensin II. Comparison of the light and electron microscopical structure of perfusion-fixed adrenal glands from both groups revealed a striking morphological heterogeneity between individual animals, but it was not possible to assign a specific pattern to one or the other animal group.

**Influence of the adrenal renin expression on plasma prorenin level**

To test whether the overexpression of adrenal renin mRNA is related to the elevated plasma prorenin levels in TGR, a bilateral adrenalectomy (ADX) was performed in two groups of rats, one composed of TGR, the other of normotensive controls.
Fig. 1. In situ hybridization analysis of renin mRNA expression in the adrenal gland using a mouse-renin riboprobe. a. In TGR, the adrenal cortex reveals strongly enhanced expression in the zona glomerulosa and the outer zona fasciculata (arrowheads mark the approximate border between these zones). Note that single cells and smaller groups of cells show a particularly enhanced expression (antisense probe). b. In the same tissue, a control renin probe (sense probe) does not show significant labelling. c. The outer cortex of a normotensive control adrenal gland reveals a low level of renin mRNA expression (antisense probe). a-c. Hematoxylin-eosin, × 600. d. The zona reticularis (ZR) in a TGR adrenal gland reveals areas of enhanced renin expression at the border to the adrenal medulla (M). e. Same localization as in d; the zona reticularis of a normotensive control shows expression no higher than background level. d,e: The cortico-medullary border is indicated by dashed lines. Antisense probe, hematoxylin-eosin, × 600.

(Fig. 3). Plasma prorenin was determined before and four days after ADX. Prior to ADX, prorenin was approximately twelve-fold higher in TGR than in normotensive controls (27 ± 29 ng Ang I/ml/hr vs. 318 ± 79 ng; P < 0.005; Fig. 3). Blood pressure was 128 ± 5 mm Hg in controls versus 210 ± 13 mm Hg in TGR (P < 0.005). After ADX, prorenin levels in TGR were lowered
to 70 ± 43 ng Ang I/ml/hr (P < 0.005) whereas in controls, prorenin remained unchanged (26 ± 55 ng Ang I/ml/hr). Blood pressure in TGR was significantly lowered after ADX (163 ± 23 mm Hg, P < 0.005), but was unchanged in controls (128 ± 6 mm Hg). Plasma active renin in TGR was lower than in controls (14 ± 9 ng Ang I/ml/hr vs. 45 ± 41; P < 0.05). After ADX, active renin levels were raised in both strains; in TGR however, values were not significantly increased (78 ± 75 ng Ang I/ml/hr), whereas in controls, the increase was significant (177 ± 87 ng Ang I/ml/hr; P < 0.05).

**Renin-angiotensin system in the kidney**

For renin mRNA localization by in situ hybridization, kidneys from male and female TGR and controls aged between three and eight months were investigated using the same probe as for the adrenal glands. Renin mRNA expression at the juxtaglomerular apparatus in TGR was generally suppressed to a strong degree (Fig. 4a). As an exception, just a few glomeruli exhibited a clearly visible grain clustering at the afferent arteriole that was comparable to the autoradiographic signal in controls. It appeared, however, that the suppression of renin expression was depending on the degree of hypertension. Female TGR or older male TGR which occasionally revealed blood pressure values near normotension exhibited an almost normal level of renin gene expression, whereas in strongly hypertensive specimens numerous glomeruli did not reveal any expression signal at the vascular pole. Renin expression in the normotensive control kidneys was limited to the arterial tree at the regular juxtaglomerular site (Fig. 4b). Tubular epithelia, interstitial cells and capillaries never showed a hybridization signal.

Immunocytochemical labelling of the kidneys using the anti-mouse renin antibody revealed a reaction pattern analogous to that obtained from in situ hybridization. In the hypertensive rats, numerous afferent arterioles did not reveal any immunostaining at all at the juxtaglomerular apparatus (Fig. 4c), and in others, only few reactive cells per longitudinally-sectioned afferent arteriole were visible. Controls revealed strong antibody labelling over the juxtaglomerular granular cells (Fig. 4d). For comparison, the sections were stained with an anti-rat renin antibody which specifically recognized rat but not mouse renin.
Fig. 4. Renin in situ hybridization (a,b), renin immunohistochemistry (c,d) and angiotensin II immunohistochemistry (e,f) of TGR (left side of the panel) and control kidneys (right side of the panel). a. No renin in situ hybridization signal is visible at three glomerular vascular poles (arrows) indicating suppressed renin expression in the TGR kidney; for better visibility, glomeruli as well as afferent arterioles are outlined by dashed lines. b. In the control kidney, normal renin mRNA-expression is localized to the vascular poles of three glomeruli (arrows). a,b: Antisense mouse renin probe, hematoxylin-eosin, × 100. c. No renin-immunoreactivity is found in the wall of an afferent arteriole (A) of a TGR glomerulus; efferent arteriole (E), macula densa (arrow). d. In the control kidney, normal renin-immunostaining is seen in the wall of the afferent arteriole. c,d: Anti-mouse renin antibody, interference contrast microscopy, × 450. e. No angiotensin II-immunoreactivity is found in the wall of an afferent arteriole (arrow) of a TGR kidney. f. In the control kidney, angiotensin II-immunoreactivity is concentrated in the wall of two afferent arterioles at the glomerular vascular pole. e,f: Anti-angiotensin II antibody; interference contrast microscopy, × 115.
Staining with this antibody was much the same as with the mouse antibody (results not shown). Immunostaining with an antibody directed against angiotensin II showed the same pattern of reactive cells in the wall of the afferent arteriole, as observed with the renin antibody (Fig. 4 e,f).

The light microscopical findings were corroborated by ultrastructural studies of aldehyde-perfused transgenic kidneys; it was found that in the classic juxtaglomerular position of the afferent arteriole, the number of granular cells was strongly reduced and a total absence of these cells was encountered in some of the arterioles (Fig. 5 a,b). Only few afferent arterioles were encountered containing small numbers of renin-type granules. Control kidneys of the same preparation exhibited the normal aspect of granular cells arranged in several layers in the tunica media of the afferent arteriole near the glomerulus; the cells contained abundant renin-type granules (Fig. 5 c,d).

### Pathological alterations of the kidney

A number of striking morphological alterations was found in the kidneys of the transgenic animals (Figs. 6 a-g, 7 a-c). The changes were by far more evident in males than in females, and were seen to increase considerably between the fourth and sixth month of age. A general pattern present in all hypertensive rats investigated irrespective of sex and age, was a prominent increase of the arterial wall-thickness-to-lumen diameter ratio of the large kidney arteries, that is, the arcuate and interlobular arteries and, to a lesser extent also the more proximal portions of the glomerular afferent arterioles (Fig. 7 a-c). The increase in media thickness was regularly paralleled by an enlarged interstitial space surrounding the individual smooth muscle cells; this space was filled with basement membrane-like material, collagen fibers and debris of vesicular structures (Fig. 7c). However, signs of myocyte degeneration or fibrinoid necrosis were absent.

Lesions of the nephron were inconspicuous both in male and female TGR at an age of four months. At six and eight months of age, however, male kidneys had regularly developed glomerular lesions as well as focal tubular atrophy together with interstitial accumulations of inflammatory cells. In six-month-old kidneys, roughly 10% of the glomeruli were damaged to varying extent. In eight-month-old kidneys the proportion of damaged nephrons was considerably increased and end-stage sclerotic glomeruli were frequent (Fig. 6). Thickening of Bowman's capsule was observed in many glomeruli. Most strikingly, glomeruli with a strongly widened Bowman's capsule occurred in midcortical and juxtamedullary position; these glomeruli were dominated by a dilated urinary space that dwarfed the glomerular tuft (Fig. 6b). The tuft morphology in these glomeruli, however, appeared to be intact. Other glomerular damages included mesangial expansion (Fig. 6a) as well as subendothelial hyalin deposits occluding the capillary lumen.
Fig. 6. Hypertension-induced lesions of the renal corpuscle. a. A glomerulus of a TGR kidney revealing attenuation of the visceral epithelium with pseudocyst-formations (arrowheads) and focal expansion of mesangial elements (arrows), × 330. b. Widely dilated urinary space of a TGR glomerulus with a fairly normal glomerular tuft structure, × 240. c. Hyalin deposition in a totally collapsed TGR glomerulus, × 310. a-c: Semithin sections, toluidine blue. d. Ultrastructure of pseudocyst formation in TGR glomerular visceral epithelium. Distended podocytes may bridge two or more capillary loops. Note the increased number of lysosomes, × 3500. e. Sclerotic end stage of a collapsed TGR glomerular capillary loop; note the folded glomerular basement membrane, × 7200. f. Retraction of podocyte foot processes in a TGR glomerular visceral epithelium, × 11800. g. Normal aspect of glomerular podocyte foot processes in a control glomerulus, × 18700. d-g: Ultrathin sections.
Fig. 7. Hypertension-induced morphological alterations of the renal arcuate artery (a-c) and of intramyocardial coronary arterioles of the heart (d,e). Compared with a control (a), the tunica media of the renal arcuate artery in TGR (b) is significantly thickened and there is expansion of the interstitial matrix. Note the difference in thickness of the internal elastic lamina (arrows). There is no obvious size difference of media myocytes between the hypertensive and the normotensive. a,b: Ultrathin sections × 2280. c. Higher magnification of a TGR renal arcuate artery revealing increase in basement membrane-like material and collagen fibres between myocytes of the tunica media. Ultrathin section, × 10900. Coronary arterioles of a similar external diameter in a control (d) and a TGR heart (e); note the increase in tunica media thickness as well as the obvious myocyte hypertrophy in the TGR arteriole. The TGR arteriole reveals perivascular fibrosis. d,e: Semithin sections, toluidine blue, × 256.

(Fig. 6c). These lesions were segmental in some glomeruli and generalized in others. Microaneurysms were infrequent. Alterations of glomerular epithelial cells comprised the formation of large cytoplasmic vacuoles, a strong increase in large secondary lysosomes, as well as the development of pseudocysts of extended podocytes that appeared to bridge two or more capillary loops (Fig. 6 a,d). Retraction of podocyte foot processes occurred in many glomeruli with a concomitant loss of tertiary structure and a narrowing of the filtration slit (Fig. 6b). Degradation of the capillary wall or separation of the epithelium from the underlying basement membrane were rarely seen. End-stage sclerotic glomeruli were characterized by a total collapse of capillary lumen and a dense accumulation of heavily folded and thickened basement membrane surrounded by degenerated and fused podocytes (Fig. 6e). Some areas of damaged nephrons revealed shrunken glomeruli as well as atrophic dilated tubules with luminal casts and a thickened tubular basement membrane.

Pathological alterations of the heart

An increase in heart size was regularly observed in all transgenic rats when compared to age-matched normotensive littermates. Apart from hypertrophy, pathological changes of the heart were rather inconspicuous in the transgenic animals
with respect to myocardial and coronary morphology. Myocardial arterioles and coronary arteries clearly revealed an increase in media thickness in the transgenic males, and the media myocytes were hypertrophied revealing a strong increase of the cytoplasmic portion occupied by myofibrillae (Fig. 7 d,e). Focally, perivascular fibrosis occurred in the form of enlarged areas of extracellular connective tissue matrix encircling myocardial arterioles. In these areas, the surrounding myocardial cells were separated from each other by fibrous interstitial matrix. Pathologic changes in female TGR hearts were by far less evident than in males, as the females presented an overall intact tissue aspect. A systematic statistical analysis of the hypertensive changes has not been achieved as yet due to the small number of animals available for this study.

Pathological alterations of the aorta

Wall thickness of the thoracic aorta was strongly increased in transgenic animals of both sexes when compared to their normotensive littermates. Thickness of the tunica media in males was almost doubled at an age of six months (Fig. 8 a,b). Strikingly, in TGR a change in the overall structure of the media layers was observed: in controls, five to seven layers of circularly-oriented elastic laminae separating the smooth muscle layers were arranged regularly. In the hypertensive animals, however, this regular arrangement was disturbed by the formation of elastic fibrous bridges that formed an interweaving network of additional layers between the myocytes with no recognizable specific orientation. Ultrastructurally, the extracellular connective tissue matrix was enlarged proportionally to the hypertrophy of the smooth muscle cells. Strongly enlarged collagenous fiber bundles were intermingled with large elastic fibers (Fig. 8 c,d). There were no obvious signs of damage at the level of the endothelium and the internal elastic layer.

Discussion

This study presents morphological and functional data of a new hypertensive rat strain transgenic for the mouse Ren-2 gene. The localization and expression of RAS components were documented. Renal and cardiovascular morphology were investigated.

The most striking result obtained from the in situ hybridization experiments was the strongly-enhanced renin gene expression in the TGR adrenal gland. Even though the probe used in the in situ hybridization assay did not allow a specific distinction between mouse and rat renin transcripts, data presented by Mullins and coworkers allow the indirect conclusion that adrenal renin expression levels exceeding control levels were due to expression of the mouse transgene.

Renin mRNA expression was enhanced over the entire

Fig. 8. Hypertension-induced morphological alterations of the thoracic aorta (at the transition of the aortic arch to the straight part). a,b. Aorta of a 6-month-old male control (a) and of an age-matched male TGR (b); wall thickening in the TGR aorta is evident, and the muscle layers are interwoven with the elastic laminae. a,b: Semithin sections, toluidine blue, × 380. c,d. Normal control (c) and age-matched hypertensive aorta (d), age: 6 months. Note the regular arrangement of smooth muscle cells and elastic laminae of the tunica media in the control; in the hypertensive aorta, interstitial collagenous and elastic deposits are increased. c,d: Ultrathin sections, × 2660.
Adrenal expression of the mouse Ren-2 gene in transgenic animals has already been studied previously by Mullins and coworkers [21]. These authors found that a one-renin-gene mouse strain (Ren1-c/Ren1-c) made transgenic for the mouse Ren-2 gene revealed a strongly enhanced constitutive expression of the transgene in the female adrenal gland. In the male adrenal gland, however, transgene expression level was comparatively low and corresponded to the level of the wild type expression of Ren-2 in the DBA/2J strain from which the transgene was derived. These results differ from TGR adrenal renin expression which was strongly enhanced both in males and females; the discrepancy may be due to species-specific genomic differences.

Immunoreactive renin in the TGR adrenal gland was localized to the same adrenal cortical zones that revealed high gene expression levels. Compared to the high increase in mRNA expression in the TGR adrenal cortex, however, immunoreactive renin was only moderately enhanced over control levels. This finding suggests that renin or its inactive precursors are mostly secreted rather than stored in the adrenal gland. Both in TGR and in control adrenal cortex, the majority of immunoreactive cells were located in the outer cortical zone, that is, in the zona glomerulosa and the outer zona fasciculata. This agrees with other reports on rat adrenal renin immunoreactivity [13, 27, 28], whereas in the mouse adrenal gland, Naruse and coworkers [29] found immunoreactive renin to be preferentially localized in the inner adrenal cortex.

The mechanism of action of the increased adrenal renin mRNA level on the genesis and maintenance of hypertension in TGR is not clear. It is now generally acknowledged that, as for many other tissues, the adrenal gland contains the complete endogenous renin-angiotensin system and is involved in the regulation of blood pressure [30—32]. The adrenal RAS may act by a direct effect of angiotensin II or by controlling aldosterone biosynthesis [3, 6, 16, 27, 33] and by the angiotensin II-induced release of medullary catecholamines [34, 35]. However, plasma catecholamine levels (unpublished results) and plasma angiotensin II levels [20] were not significantly altered in TGR; urinary aldosterone levels in TGR were roughly twice above normal [20] but were probably not sufficiently elevated to fully account for the strongly-elevated blood pressure in these animals.

On the other hand, the most conspicuous value of the plasma RAS parameters in TGR is the high renin level which exceeds control values by approximately twofold. Prorenin is now recognized to be identical to plasma inactive renin, and it has recently been found that prorenin can be converted to active renin in the liver, kidney and adrenal gland [36]. To establish a relation between the elevated adrenal renin mRNA-expression and plasma prorenin levels, a bilateral adrenalectomy (ADX) was performed in hypertensive transgenic rats and in normotensive littermates. ADX significantly lowered plasma prorenin to about 22% of the initial value. Even though after ADX prorenin levels in TGR are still a little, but not significantly higher than in controls, this study provides evidence that the adrenal gland is likely to be the major source of prorenin. It may thus be concluded that the adrenal renin mRNA overexpression accounts for a major share of the elevated prorenin level in TGR. Whether prorenin secretion may be varied by changes in volume homeostasis, is unclear at present; generally, prorenin is known to be secreted constitutively [37]. Plasma active renin levels four days after ADX were increased in the control group but were not significantly raised in TGR; the reason for this discrepancy may well be based on the fact that a normal amount of renin producing cells present in the control kidneys may strongly react to a secretion stimulus, whereas in TGR, metaplastic transformation of smooth muscle cells into granular renin secreting cells probably has to occur before renal renin production reaches levels equivalent to the control group. This process may require a time span longer than four days.

Blood pressure in TGR was lowered after ADX; however, this cannot provide evidence for the adrenal prorenin production as the direct cause of hypertension, since the lack of mineralocorticoids induced by the bilateral ADX may equally well account for a lowered blood pressure. Thus, the role of prorenin in TGR on the genesis of hypertension will have to be further elucidated.

The present study clearly underlines that hypertension in TGR is not due to renin overexpression in the kidney, since intrarenal renin protein and mRNA levels were suppressed. The results are in agreement with previous data which established a low renal renin activity in TGR [20]. Renin transcripts were rarely detectable by in situ hybridization at the vascular poles of glomeruli in TGR, whereas the controls revealed normal levels of renin expression at the juxtaglomerular afferent arteriole as previously described elsewhere [13, 38]. Immunohistochemically, the majority of the juxtaglomerular afferent arterioles was devoid of renin as well as angiotensin II immunoreactivity. Most of the afferent arterioles investigated did not show the typical renin producing granular cells but rather, at the site where granular cells are normally located, smooth muscle cells were encountered. Since it may readily suggest that granular cells had been present before the onset of hypertension, this finding indicates that an extensive transformation of renin containing cells into vascular smooth muscle cells must have occurred. The suppression of kidney renin synthesis is probably a secondary effect of the elevated blood pressure in TGR as has been shown in other hypertensive models [39]. On the other hand, the relation between renal renin status and hypertension in TGR differs from that in spontaneously hypertensive rats (SHR) which reveal renal renin levels similar to normotensive controls [40], even though systolic blood pressure elevation is similar in both SHR and TGR.

Structural lesions of the nephron in TGR were moderate in both sexes at an age of four months, except for an overall increase in wall thickness of the larger arterioles and arteries. Damages were seen to increase considerably in male TGR six to eight months of age. Damages in the (less hypertensive) females were much weaker and sometimes not observable. Systemic hypertension has long been recognized as a significant compound of progressive renal disease [41]. The observed patterns
of glomerular damage in TGR, that is, mesangial expansion, hyaline deposits, lesions in podocyte structure and finally glomerulosclerosis, are typical for experimental models developing glomerular capillary hypertension [42]. Since glomerular lesions in TGR were occurring at a relatively early age, it appears that the systemic hypertension, at least to some extent, is transmitted to the glomerulus via the glomerular afferent arteriole.

The increases of thickness of the tunica media observed in the muscular type arteries as well as in the aorta of the TGR are in agreement with those observed in other hypertensive rat strains [43—47]. In SHR, mass of the aortic media is known to increase proportionally with variations in systolic blood pressure [46]. Hadjiisky, Peyri and Grosgeat [44] reported a 26% increase in aortic wall thickness in seven-month-old SHR when compared to normotensive controls, and described similar modifications as found in TGR with respect to thickness of the interlamellary spaces and increase in collagen around myocytes and along the elastic laminae. The extent of thickening of the renal and coronary arteries and arterioles in TGR, accompanied by varying degrees of interstitial fibrosis, was comparable to SHR vascular morphology, exhibiting thicker walls than age-matched normotensive rats Wistar-Kyoto rats [45, 47]. Generally, vascular hypertrophy in adult TGR is probably based on myocyte hypertrophy and only to a small extent to hyperplasia, since in hypertension, hyperplasia is supposed to be completed at an early stage [46].

The cause leading to the vascular changes in TGR is unknown. The media hypertrophy may either be the result of an adaptive process in response to the elevated blood pressure [48], or blood vessel thickness may already be increased prior to hypertension development, as was observed in the renal vasculature of SHR [49]. The overall vascular hypertrophy in TGR may as well be the consequence of a local expression of the transgene or activation of prorenin and, consequently, locally acting angiotensin II, which is known to provoke myocyte hypertrophy [50] and to increase peripheral vascular resistance [51]. In view of the great complexity of mechanisms possibly acting on the increase of vascular resistance, however, these speculations must await further experimental data on TGR blood pressure regulation.

In conclusion, the construction of transgenic rats provides a new opportunity for investigating the pathogenesis of hypertensive damages. Even though the cause of hypertension in the mouse Ren-2 transgenic rats is not yet fully established, this new animal strain may serve as a genetically well defined, low plasma-renin hypertension model that will permit new insights in the mechanisms of the development of glomerulosclerosis and cardiovascular damage.

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