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Cardiovascular and renal effects of cyclooxygenase inhibition in transgenic rats harboring mouse renin-2 gene (TGR[mREN2]27)

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Abstract

The present study examined the role of cyclooxygenase-synthetized prostanoids in the pathogenesis of angiotensin-II-induced inflammatory response and vascular injury in transgenic rats harboring mouse renin-2 gene (mREN2 rats). Five- to six-week-old, heterozygous mREN2 rats received the following drug regimens for 8 weeks: (1) controls; (2) cyclooxygenase-2 inhibitor (MF-tricyclic [3-(3,4-difluorophenyl)-4-(4-(methylsulfonyl) phenyl)-2(5*H*)-furanone], 14 mg kg^{-1} p.o.); (3) cyclooxygenase-1/cyclooxygenase-2 inhibitor (sulindac, 14 mg kg^{-1} p.o.); (4) angiotensin II receptor antagonist (losartan 40 mg kg^{-1} p.o.); (5) MF-tricyclic + losartan; (6) sulindac + losartan. Normotensive Sprague–Dawley rats served as controls. mREN2 rats developed pronounced hypertension, cardiac hypertrophy, and albuminuria as compared to normotensive Sprague–Dawley controls. mREN2 rats showed pronounced perivascular inflammation and morphological damage in the kidneys and the heart. Both MF-tricyclic and sulindac further increased blood pressure and albuminuria in mREN2 rats. Neither MF-tricyclic nor sulindac were able to prevent angiotensin-II-induced perivascular inflammation and morphological changes in the heart or in the kidneys. Myocardial and renal cyclooxygenase-2 mRNA expressions were decreased in mREN2 rats, whereas no difference was found in cyclooxygenase-1 mRNA expressions. Sulindac increased both cyclooxygenase-1 and cyclooxygenase-2 gene expressions, whereas MF-tricyclic increased only cyclooxygenase-2 gene expressions. Losartan normalized blood pressure, cardiac hypertrophy, albuminuria, inflammatory response and morphological changes in mREN2 rats, both in the presence and absence of cyclooxygenase inhibitors. Our findings indicate that cyclooxygenase does not play a central role in the pathogenesis of angiotensin-II-induced inflammatory response and vascular injury in mREN2 rats.

Keywords: Renin–angiotensin–aldosterone system; Angiotensin II; Prostanoid; Cyclooxygenase; Kidney; Heart

1. Introduction

The renin–angiotensin–aldosterone system plays a central role in the regulation of blood pressure and electrolyte homeostasis. Pharmacological blockade of renin–angiotensin–aldosterone system with angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists have proved to be effective in the treatment of cardiovascular diseases and diabetic vasculopathy (Burnier, 2001; Gavras and Brunner, 2001; Schoolwerth et al., 2001). There is now accumulating evidence to indicate that angiotensin II, the key effector of the renin–angiotensin–aldosterone system, is capable of inducing inflammatory response in the vascular wall (Griendling et al., 2000a,b; Romero and Reckelhoff, 1999). Angiotensin II induces vascular inflammation and upregulates the expression of adhesion molecules via angiotensin AT_1 receptor stimulation (Griendling et al., 2000a,b; Mervaala et al., 1999a; Romero and Reckelhoff, 1999). Angiotensin II may induce leukocyte infiltration even by blood-pressure-independent mechanisms (Mervaala et al., 2000). Previous studies have provided evidence that angiotensin-II-induced vascular inflammation is associated with increased formation of reactive oxygen species in the vascular smooth muscle cells and myocytes (Griendling et al., 2000a,b; Romero and Reckelhoff, 1999). Reactive oxygen species influence several cellular processes including cellular growth, hypertrophy, remodelling, lipid

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peroxidation, modulation of vascular tone, and inflammation (Alexander, 1995; Griendling et al., 2000a,b; Irani, 2000; Kunsch and Medford, 1999). Reactive oxygen species can also act as intracellular signalling molecules regulating the activation of transcription factors nuclear factor kappa B (NF-κB) and activating protein-1 (AP-1) and thereby induction of several pro-inflammatory cytokine genes (Griendling et al., 2000a,b; Irani, 2000; Kunsch and Medford, 1999).

Prostanoids are local mediators of inflammation and modulators of physiological functions, including the maintenance of gastric mucosal integrity, the regulation of renal microvascular hemodynamics, renin release, and tubular electrolyte and water reabsorption (Breyer and Harris, 2001; Harris, 2000). Prostanoids are synthesized from arachidonic acid by cyclooxygenase. Two isoforms of cyclooxygenase exist, namely cyclooxygenase-1 and cyclooxygenase-2. Cyclooxygenase-1 gene is constitutively expressed, whereas cyclooxygenase-2 is cytokine-inducible and glucocorticoid-inhibited gene that is highly expressed in many tissues in response to inflammation (Breyer and Harris, 2001; Harris, 2000). However, constitutive cyclooxygenase-2 expression also exists in the kidney (Breyer and Harris, 2001; Harris, 2000). Previous studies have provided evidence that cyclooxygenase-2 expression is increased in atherosclerotic lesions (Schonbeck et al., 1999) and in myocardium of patients with congestive heart failure (Wong et al., 1998). However, it remains to be established whether cyclooxygenase-2 overexpression in the vasculature is deleterious or protective. Whereas induction of cyclooxygenase-2 in macrophages and fibroblasts may significantly contribute to vascular inflammation, injury, and development of fibrosis, cyclooxygenase-2 overexpression in endothelial cells, leading to increased synthesis of vasoprotective prostacyclin, may represent a compensatory mechanism to defend against vascular injury (Wu, 1998).

Highly selective cyclooxygenase-2 inhibitors have been synthesized in order to cause fewer gastrointestinal adverse events than do the previous traditional nonselective cyclooxygenase inhibitors (FitzGerald and Patrono, 2001). However, only limited information is available on the cardiovascular and renal effects of these compounds and their interference with the cardiovascular drugs at the pharmacodynamic level. In fact, it has been claimed that the use of selective cyclooxygenase-2 inhibitors may even be associated with increased risk of myocardial infarctions (Mukherjee et al., 2001).

The present study aimed at exploring whether prostanoids are involved in the pathogenesis of angiotensin-II-induced vascular inflammation and injury. Cardiovascular and renal effects of selective cyclooxygenase-2 inhibitor MF-tricyclic, [3-(3,4-difluorophenyl)-4-(4-(methylsulfonyl) phenyl)-2(5*H*)-furanone], and a nonselective cyclooxygenase inhibitor sulindac were examined in transgenic rats harboring mouse renin-2 gene (TGR[mREN2]27, mREN2 rats). mREN2 rats develop hypertension, vascular inflammation, and end-organ damage apparently due to increased angiotensin II formation (Bohm et al., 1995; Lee et al., 1996; Mullins et al., 1990).

2. Materials and methods

2.1. Experimental animals, drug regimens, and sample preparation

Sixty 5–6-week-old male heterozygous mREN2 rats (body weight 212 ± 8 g) and 10 age-matched Sprague–Dawley rats purchased from M&B (Ejby, Denmark) were used. The protocols were approved by the Animal Experimentation Committee of the Medical Faculty, University of Helsinki, Finland, whose standards correspond to those of the American Physiological Society. The rats were housed five animals per cage in a standard experimental animal laboratory (illuminated from 6.30 a.m. until 6.30 p.m., temperature 22 ± 1 °C), and they had free access to tap water and chow (NaCl 0.8% w/w, R36, Finnewos Aqua, Helsinki, Finland) during the experiment. In the beginning of the study, the blood pressure- and body weight-matched mREN2 rats were divided into six groups (10 animals in each) to receive different drug regimens for 8 weeks: (1) control mREN2 rats; (2) mREN2 rats receiving a selective cyclooxygenase-2 inhibitor, MF-tricyclic [3-(3,4-difluorophenyl)-4-(4-(methylsulfonyl) phenyl)-2(5*H*)-furanone]; (3) mREN2 rats receiving a nonselective cyclooxygenase-1/cyclooxygenase-2 inhibitor sulindac; (4) mREN2 rats receiving angiotensin AT_1 receptor antagonist losartan; (5) mREN2 rats receiving MF-tricyclic + losartan; (6) mREN2 rats receiving sulindac + losartan. Normotensive Sprague–Dawley rats served as controls. MF-tricyclic, sulindac, and losartan were mixed in the food to produce approximate daily doses of 14 mg kg^{-1} MF-tricyclic (Buttar et al., 2002; Oshima et al., 1996), 14 mg kg^{-1} sulindac (Buttar et al., 2002; Oshima et al., 1996), and 40 mg kg^{-1} losartan (Makino et al., 1997). Systolic blood pressure and heart rate were measured every second week using a tail cuff blood pressure analyzer (Apollo-2AB, Blood Pressure Analyzer, Model 179-2AB, IITC Life Science, Woodland Hills, CA, USA). At the age of 8, 10, 12, and 14 weeks, food intake was recorded and urines collected over 24 h in metabolic cages. Rats were then decapitated, blood samples were taken using EDTA as anticoagulant, the heart and kidneys were removed, washed with ice-cold saline, blotted dry, and weighed. Tissue samples were snap-frozen in liquid nitrogen and samples for immunohistochemistry in isopentane (−35 °C). All samples were stored at −80 °C until assayed.

2.2. Tissue morphology

Samples for conventional morphology were fixed with 10% formalin and processed with routine techniques as described elsewhere (Pere et al., 2000). We relied on a quantitative grading scale for kidneys viewed by a patholo-

gist unaware of the regimens as follows: (0) Normal arteriologlomerular unit with open capillary lumens and a normal afferent arteriole. Arcuate and interlobular vessels normal. No tubular atrophy or interstitial fibrosis; (1) Slight thickening of the media of the afferent arteriole. Some mesangial thickening. Open capillary lumens in the glomerulus. Minimal medial/adventitial thickening in larger vessels. No signs of inflammation. (2) Marked mesangial thickening partly collapsed capillaries in the glomerulus. Sometimes arterial thickening. Mild peritubular fibrosis. Clear medial thickening in larger vessels. No signs of inflammation. (3) Focal glomerulosclerosis with medial thickening of the arteriolar wall. Diffuse tubular atrophy with proteinaceous casts, peritubular fibrosis. Sometimes fibrinoid necrosis of larger vessels. Occasional inflammatory infiltrate in the interstitium. (4) Focal glomerulosclerosis. Concentric hypertrophy and necrosis of afferent arteries. Fibrinoid necrosis of the interlobular and arcuate arteries. Atrophic and necrotic tubuli. Peritubular and interstitial fibrosis. Interstitial inflammation.

Furthermore, for the hearts, the arteries and ventricular fibrous tissue formation were also evaluated in a blinded fashion (Finckenberg et al., 2001). Each sample was scored from 0 to 3 according to morphologic changes as follows: (0) Epicardial vessels normal. No excessive ventricular connective tissue formation. (1) Normal intima. Media slightly thickened or normal. Slight increase of connective tissue around the epicardial and intramuscular arteries. (2) Normal intima. Clear medial thickening and adventitial scarring in epicardial and intramuscular arteries. Patchy increase of slender connective tissue bundles. (3) Normal intima. Clear medial thickening and adventitial scarring in epicardial and intramuscular arteries. Evident myocardial scars.

2.3. Immunohistochemistry

For immunohistochemistry, formalin-fixed kidneys and heart were processed as described in detail previously (Mervaala et al., 1999a, 2000). Primary monoclonal antibody against rat monocytes/macrophages (ED1) (1:300, Serotec, Oxford, England), primary polyclonal antibody against cyclooxygenase-2 (1:600, Cayman Chemical, Ann Arbor, MI, USA), secondary biotin-conjugated antibodies, and avidin biotin enzyme reagent (Vector Laboratories, Burlingame, CA, USA) were used. The sections were counterstained with hematoxylin before being examined under a light microscope. The relative amount of primary antibody positive label per sample was determined with computerized densitometry (Leica IM500 and Leica QWIN software; Leica Microsystems, Heerbrugg, Switzerland).

2.4. Reverse transcriptase polymerase chain reaction (RT-PCR) assay of cyclooxygenase-1 mRNA and cyclooxygenase-2 mRNA

Total RNA was extracted from the kidneys and heart with Trizol (GIBCO) reagent, according to the instructions of the manufacturer. After extraction and quantitation with spectrophotometry, a 2-μg aliquot of total RNA was reverse-transcribed to cDNA by incubation for 50 min at 45 °C in the presence of reverse transcriptase (Enhanced Avian RT-PCR kit, Sigma, USA). Subsequently, 4 μl of cDNA was subjected to a RT-PCR for the detection of cyclooxygenase-1 mRNA, cyclooxygenase-2 mRNA, and the GAPDH mRNA (housekeeping gene). Samples were amplified using AccuTaq DNA polymerase (29 cycles, 94 °C 2 min, 62 °C 30 s, 72 °C 45 s for cyclooxygenase-1, amplified product 304 bp in size; 94 °C 2 min, 60 °C 30 s, 72 °C 45 s for cyclooxygenase-2, amplified product 298 bp in size). GAPDH amplified product was 198 bp in size. The following RT-PCR primers for GAPDH, cyclooxygenase-1, and cyclooxygenase-2 were used: GAPDH forward, TGGGGCAGCCCAGAACATCA; GAPDH reverse, GCCGCCTGCTTCACCACCTT; cyclooxygenase-1 forward, CCCATCTGTTCCCCAGAGTA; cyoclooxygenase-1 reverse, GTGAGACCCCAAGTTCCAAA; cyclooxygenase-2 forward, AGTGCCTCCCACTCCAGACT; cyclooxygenase-2 reverse, CACTGGCTTATGCCGAAAAA (Sigma Genosys, United Kingdom). The reaction products were run simultaneously on a 2% agarose gel. The intensity of the resulting bands was measured by a computer-based imaging analysis system (Genetools, Syngene). cyclooxygenase-1 and cyclooxygenase-2 densities of cyclooxygenase-1 and cyclooxygenase-2 bands were expressed as relative to the density obtained for GAPDH.

2.5. Biochemical determinations

Urinary albumin was measured by enzyme-linked immunosorbent assay (ELISA) using rat albumin as a standard (Celltrend, Luckenwalde, Germany). Serum lactate dehydrogenase (LDH) concentrations were measured enzymatically (United Laboratories, Helsinki, Finland).

2.6. Drugs

MF-tricyclic [3-(3,4-difluorophenyl)-4-(4-(methylsulfonyl) phenyl)-2(5*H*)-furanone], sulindac, and losartan were generous gifts from Merck & Co. (Rahway, NJ, USA).

2.7. Statistical analysis

Data are presented as means ± S.E.M. Statistically significant differences in mean values were tested by analysis of variance (ANOVA) and the least significant difference (LSD) test. ANOVA for repeated measurements was applied for data consisting of repeated observations at successive time points. The differences were considered significant when $P < 0.05$. Regression lines were calculated by the least squares method. The data were analyzed using SYSTAT statistical software (SYSTAT, Evanston, IL, USA).

3. Results

3.1. Blood pressure, cardiac hypertrophy, and heart rate

Systolic blood pressure was markedly higher in mREN2 rats compared to normotensive Sprague–Dawley rats (186 ± 5 vs. 112 ± 4 mm Hg, $P<0.05$, Fig. 1A). Both MF-tricyclic and sulindac further increased blood pressure in mREN2 rats (215 ± 12 and 207 ± 7 mm Hg, respectively, $P<0.05$ compared to mREN2 controls). There was no difference in blood pressure values between MF-tricyclic and sulindac groups. Losartan completely normalized blood pressure in mREN2 rats. Neither MF-tricyclic nor sulindac interfered with the antihypertensive effect of losartan.

mREN2 rats showed cardiac hypertrophy, estimated as absolute heart weights (Fig. 1B), or heart weight-to-body weight ratio (Fig. 1C) (both $P<0.05$ compared to Sprague–Dawley rats). MF-tricyclic and sulindac tended further to increase cardiac hypertrophy; however, the differences reached statistical significance only when absolute heart weights were used as estimate of cardiac hypertrophy. Losartan completely prevented the development of cardiac hypertrophy, both in the presence and in the absence of cyclooxygenase inhibitors ($P<0.05$ compared to mREN2 controls). There was a close correlation between systolic blood pressure and the degree of cardiac hypertrophy ($P<0.001$, $R=0.881$, $n=60$, Fig. 1D).

There was no difference in heart rate between mREN2 and Sprague–Dawley rats (ANOVA $P=0.62$). MF-tricyclic, sulindac, or losartan did not influence heart rate in mREN2 rats (data not shown).

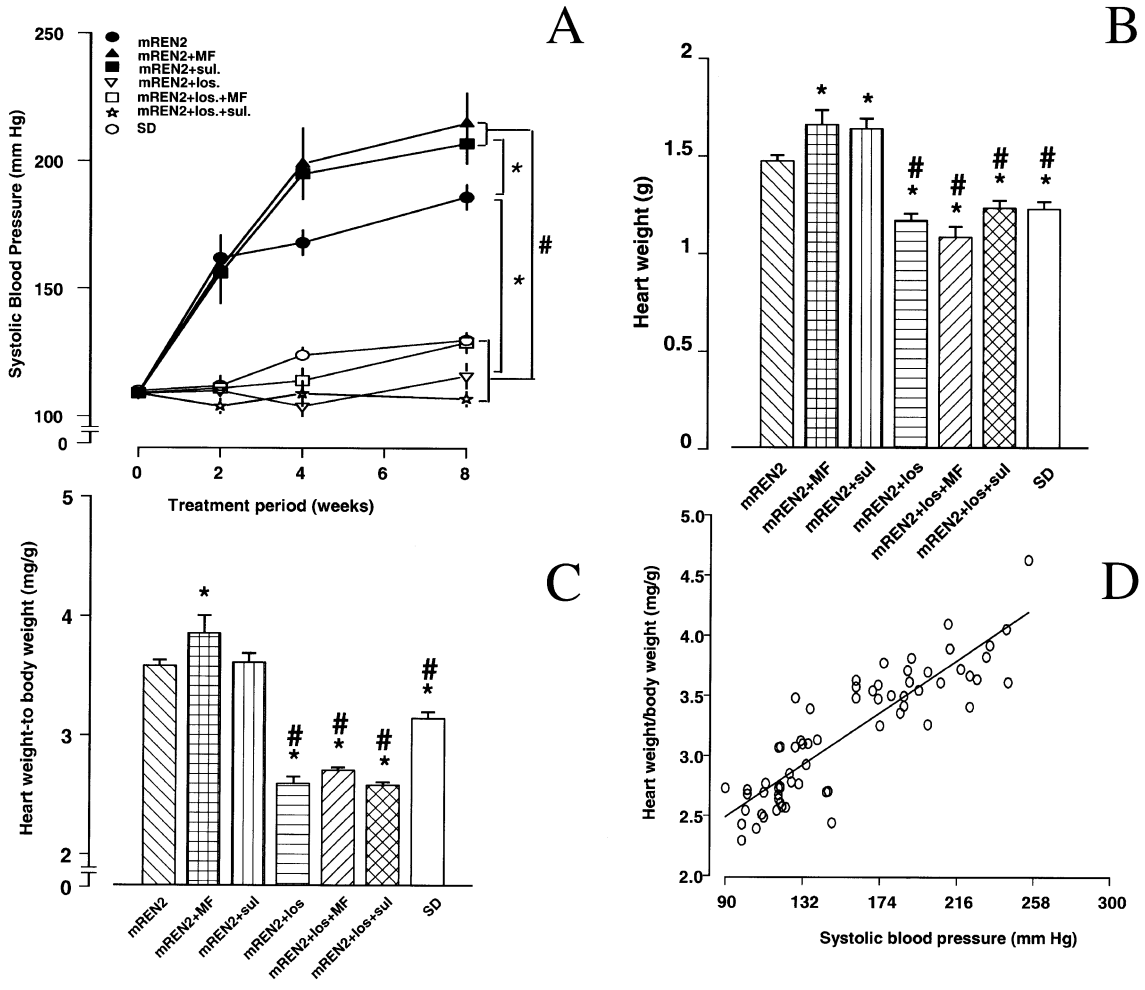

Fig. 1. Effects of 8-week treatment with MF-tricyclic, sulindac, and losartan on systolic blood pressure (Panel A), absolute heart weight (Panel B), and heart weight-to-body weight ratio (Panel C) in transgenic rats harboring mouse renin-2 gene (mREN2 rats). mREN2 denotes untreated mREN2 rats; MF, MF-tricyclic; sul, sulindac; los, losartan; SD normotensive Sprague–Dawley control rats. MF-tricyclic and sulindac further increased blood pressure and augmented heart weight in mREN2 rats. Losartan treatment, alone and in combination with cyclooxygenase inhibitors, completely prevented angiotensin-II-induced hypertension and cardiac hypertrophy in mREN2 rats. Means ± S.E.M. are given, $n=10$ in each group. *$P<0.05$ compared to untreated mREN2 rats, #$P<0.05$ compared to MF-tricyclic-treated and sulindac-treated mREN2 rats. A very close correlation was found between blood pressure and cardiac hypertrophy ($P<0.001$, $R=0.881$, $n=60$) (Panel D).

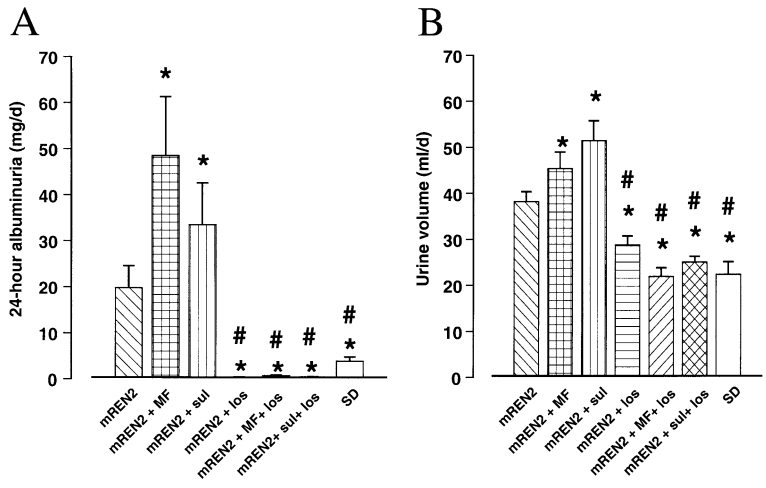

Fig. 2. Bar graphs showing the effects of 8-week treatment with MF-tricyclic, sulindac, and losartan on 24-h albuminuria (Panel A), and urine excretion rate (Panel B) in mREN2 rats. Twenty-four hour albuminuria and urine volume were significantly higher in mREN2 compared to Sprague–Dawley rats. MF-tricyclic and sulindac increased albuminuria in mREN2 rats. Losartan treatment, alone and in combination with cyclooxygenase inhibitors, completely prevented angiotensin-II-induced albuminuria as well as angiotensin-II-induced increase in urine volumes. Means ± S.E.M. are given, $n = 10$ in each group. *$P < 0.05$ compared to untreated mREN2 rats, #$P < 0.05$ compared to MF-tricyclic-treated and sulindac-treated mREN2 rats. For abbreviations, see figure legend 1.

3.2. Body weight, food intake, and calculated drug dosages

Rats gained weight steadily throughout the study. Final body weight was similar in untreated mREN2 and SD rats (412 ± 8 vs. 392 ± 14 g, $P = 0.27$). At the end of the experimental period, body weight in sulindac-treated (455 ± 17 g), losartan-treated (452 ± 19 g), and MF-tricyclic + losartan-treated mREN2 rats (457 ± 15 g) were slightly, but significantly higher compared to untreated mREN2 ($P < 0.05$). There were no differences between

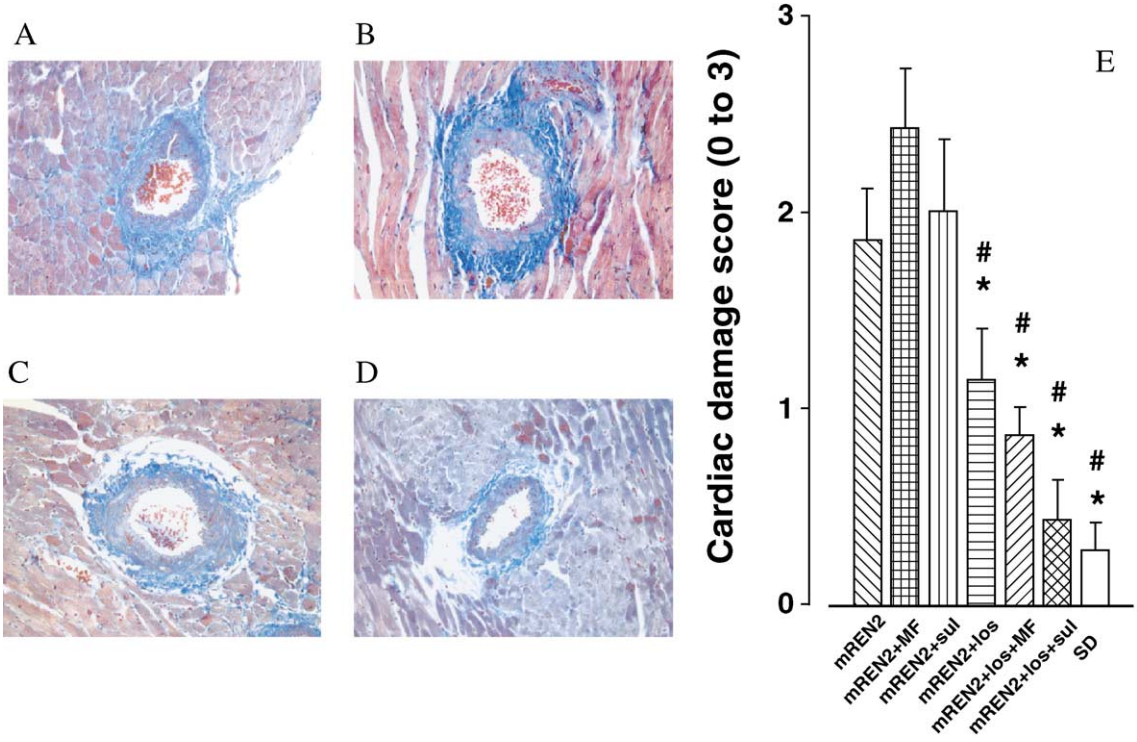

Fig. 3. Representative photomicrographs from the heart of untreated mREN2 rat (Panel A), mREN2 rat treated with MF-tricyclic (Panel B), mREN2 rat treated with sulindac (Panel C), and mREN2 rat treated with losartan (Panel D). Profound vascular damage with inflammation and vast myocardial infarcts was detected in the heart of mREN2 rats. Losartan, but not cyclooxygenase inhibitors, prevented angiotensin-II-induced myocardial and vascular damage in mREN2 rats. Cardiac damage scores are shown in Panel E. Values are means ± S.E.M., $n = 10$ in each group. *$P < 0.05$ compared to untreated mREN2 rats, #$P < 0.05$ compared to MF-tricyclic-treated and sulindac-treated mREN2 rats. For abbreviations, see figure legend 1.

the treatment groups in the 24-h food intakes at 2, 4, 6, and 8 weeks (ANOVA $P>0.05$). The average daily food consumption was approximately 25 g per rat. In the 8-week experimental period, the daily dosage of MF-tricyclic varied from 22 to 10 mg/kg, that of sulindac from 25 to 9 mg/kg, and that of losartan from 67 to 27 mg/kg.

3.3. Albuminuria and urine volume

Twenty-four hour urine volume and urinary albumin excretion rate were markedly higher in mREN2 rats as compared to Sprague–Dawley rats ($P<0.05$, Fig. 2A,B). MF-tricyclic and sulindac further increased albuminuria in mREN2 rats ($P<0.05$ compared to mREN2 controls, Fig. 2A). Cyclooxygenase inhibitors slightly increased urine volume. Losartan, both in the absence and presence of cyclooxygenase inhibitors, completely prevented the development of albuminuria, as well as the angiotensin-II-induced increase in urinary output in mREN2 rats (all $P<0.05$ compared to mREN2 controls).

3.4. Heart and kidney morphology

Myocardium of mREN2 rats showed severe histopathological changes (Fig. 3A). In larger epicardial arteries, there were notable adventitial fibrosis and medial hypertrophy. There was an increase of inflammatory cells in the perivascular area. Smaller, mostly intramuscular arteries were even more severely damaged, and showed concentric hypertrophy occluding the vessels. Signs of myocardial infarctions were noted. Neither MF-tricyclic nor sulindac had clear beneficial or detrimental effects on cardiac morphology (Fig. 3B,C). In contrast, losartan completely prevented the angiotensin-II-induced morphological changes in mREN2 rats, both in the presence and in the absence of cyclooxygenase inhibitors (Fig. 3D,E). Serum lactate dehydrogenase concentrations between mREN2 and Sprague–Dawley rats were similar ($P=0.97$), and the drugs used did not influence it (data not shown).

The kidneys of mREN2 rats showed focal glomerulosclerosis with mesangial expansion, and apparent tubular atrophy with mild peritubular/interstitial fibrosis (Fig. 4A). The mid-sized arteries had notable adventitial and medial thickening whereas the smaller arteries had also intimal hyperplasia leading to narrowing of the luminal space. Inflammatory cells were found in the periarterial and peritubular areas. Neither MF-tricyclic nor sulindac were able to prevent angiotensin-II-induced changes in renal morphology (Fig. 4B,C). In contrast, some samples of cyclooxygenase inhibitor groups showed necrosis of the tubular epithelium.

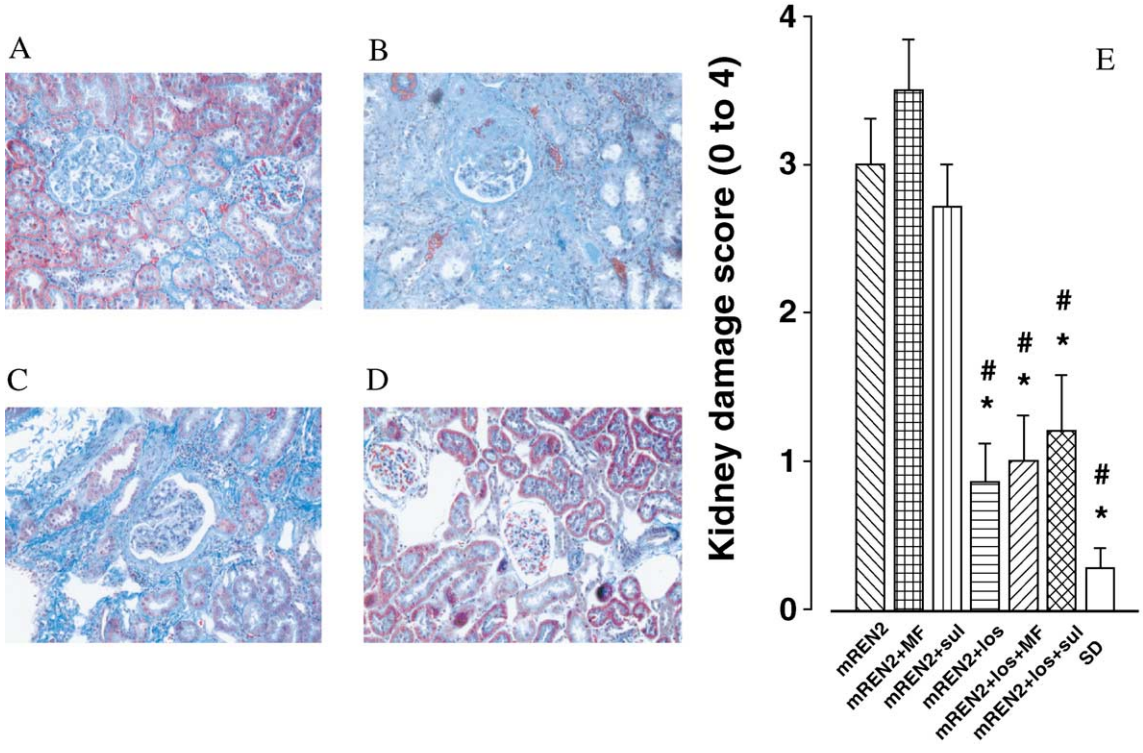

Fig. 4. Representative photomicrographs from the kidneys of untreated mREN2 rat (Panel A), mREN2 rat treated with MF-tricyclic (Panel B), mREN2 rat treated with sulindac (Panel C), and mREN2 rat treated with losartan (Panel D). The kidneys of untreated mREN2 rats showed severe glomerular and vascular damage with mesangial proliferation, glomerular sclerosis and necrosis, increased intimal and media thickness, and deposition of matrix. Losartan, but not cyclooxygenase inhibitors, prevented angiotensin-II-induced renal damage in mREN2 rats. Kidney damage scores are shown in Panel E. Values are means ± S.E.M., $n=10$ in each group. *$P<0.05$ compared to untreated mREN2 rats, #$P<0.05$ compared to MF-tricyclic-treated and sulindac-treated mREN2 rats. For abbreviations, see figure legend 1.

Renal morphology in mREN2 rats treated with losartan was indistinguishable from that of Sprague–Dawley rats (Fig. 4D,E).

3.5. Immunohistochemistry

mREN2 rats showed perivascular monocyte/macrophage infiltration (ED-1 immunopositive cells) especially in the kidneys (Fig. 5), and to a lesser extent in the heart (ED-1 immunopositive surface area $0.031 \pm 0.008\%$ in mREN2 rats compared to $0.015 \pm 0.003\%$ in Sprague–Dawley rats, $P < 0.05$). Neither MF-tricyclic nor sulindac ameliorated inflammatory response in mREN2 rats (Fig. 5). Losartan completely prevented leukocyte infiltration in mREN2 rats (Fig. 5).

In the kidneys, a weak cyclooxygenase-2 immunopositive staining was found in the cortex and medullary interstitial cells. Renal cyclooxygenase-2 expression was decreased in mREN2 compared to Sprague–Dawley rats (cyclooxygenase-2 immunopositive surface area $1.2 \pm 0.17\%$ and $2.1 \pm 0.34\%$, respectively, $P < 0.05$). In the heart of mREN2 and Sprague–Dawley rats, only minimal cyclooxygenase-2 expression was found in endothelial cells (data not shown).

3.6. Cyclooxygenase-1 mRNA and cyclooxygenase-2 mRNA expressions

As compared to Sprague–Dawley rats, cyclooxygenase-2 mRNA expression in mREN2 rats was decreased by 80% in the heart (Fig. 6A) and by 60% in the kidneys (Fig. 6B). MF-tricyclic, sulindac, and losartan significantly increased myocardial and renal cyclooxygenase-2 mRNA expressions ($P < 0.05$ compared to mREN2 controls).

There was no difference in cyclooxygenase-1 mRNA expression in the heart (Fig. 6C) or kidneys (Fig. 6D) between

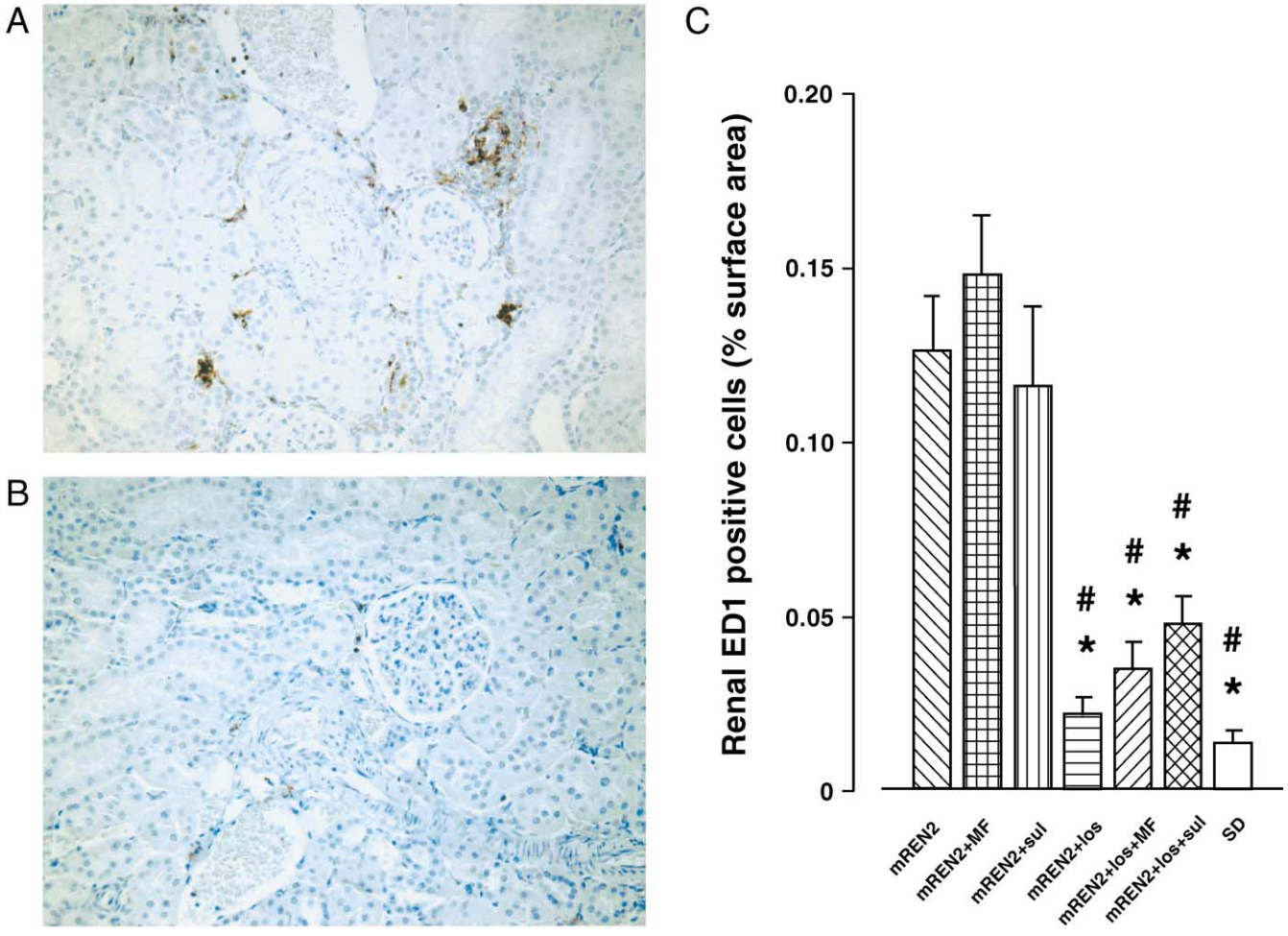

Fig. 5. Representative immunohistochemical photomicrographs of monocyte/macrophage infiltration (ED1-immunopositive cells) from the kidneys of untreated mREN2 rat (Panel A) and normotensive Sprague–Dawley rat (Panel B). Semiquantitative scoring of ED-1 immunopositive cells is shown in Panel C. The kidneys of untreated mREN2 rats kidneys showed severe perivascular inflammation. Neither MF-tricyclic nor sulindac significantly ameliorated angiotensin-II-induced monocyte/macrophage infiltration. In contrast, losartan completely prevented monocyte/macrophage infiltration in mREN2 rats. Values are means ± S.E.M., $n = 9–10$ in each group. *$P < 0.05$ compared to untreated mREN2 rats, #$P < 0.05$ compared to MF-tricyclic-treated and sulindac-treated mREN2 rats. For abbreviations, see figure legend 1.

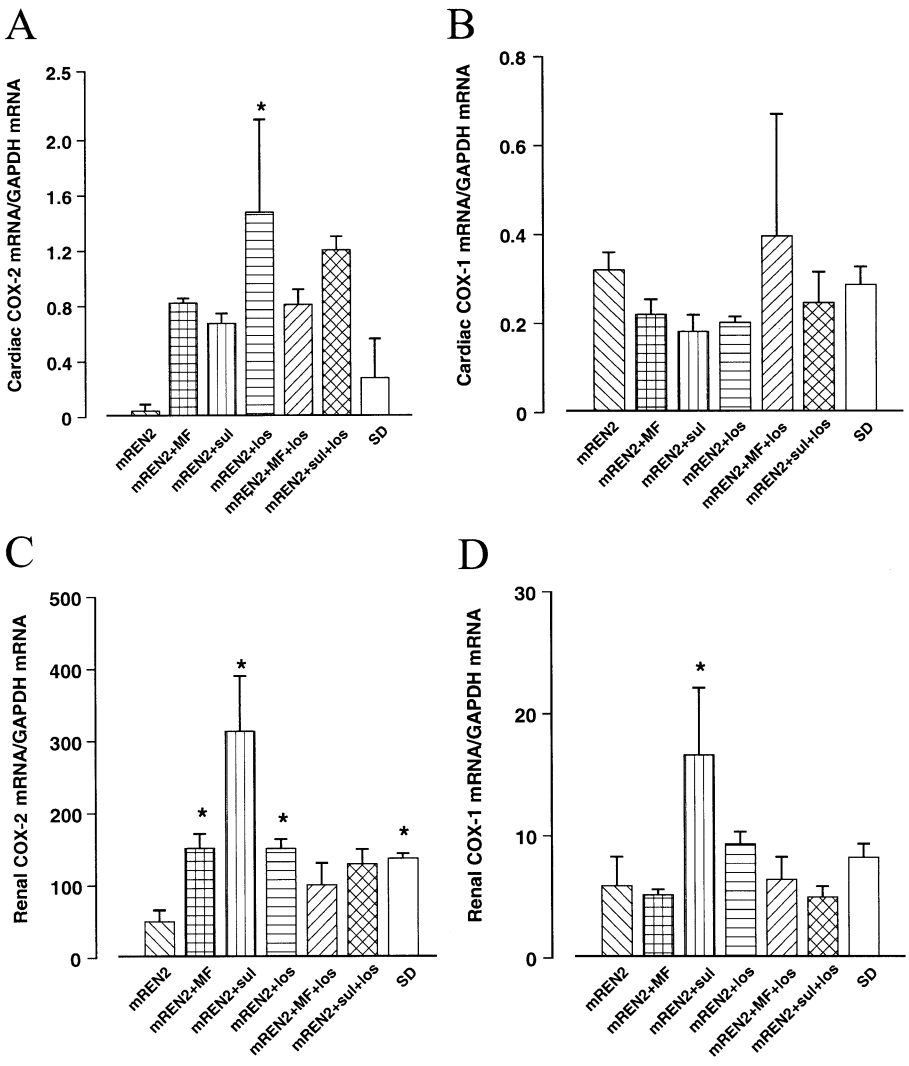

Fig. 6. Bar graphs showing the effects of 8-week-treatment with MF-tricyclic, sulindac, and losartan on cyclooxygenase-2 mRNA expression in the heart (Panel A) and kidney (Panel C), as well as on cyclooxygenase-1 mRNA expression in the heart (Panel B) and kidney (Panel D). Cyclooxygenase-2 mRNA expression both in the heart and kidneys were decreased in mREN2 rats. Selective cyclooxygenase-2 inhibition induced only cyclooxygenase-2 mRNA expression, whereas nonselective cyclooxygenase inhibition increased the gene expression of both cyclooxygenase-1 and cyclooxygenase-2. Values are means ± S.E.M., $n = 9-10$ in each group. *$P < 0.05$ compared to untreated mREN2 rats. For abbreviations, see figure legend 1.

mREN2 and Sprague–Dawley rats. Only sulindac increased cyclooxygenase-1 mRNA expression. Losartan did not influence cyclooxygenase-1 gene expression when given alone or in combination with cyclooxygenase inhibitors.

4. Discussion

Transgenic rats harboring mouse renin-2 (mREN2) rats are characterized by hypertension and angiotensin-II-induced tissue injury in the brain, heart, kidneys, and vasculature (Bohm et al., 1995; Lee et al., 1996; Mullins et al., 1990). There is accumulating evidence indicating that inflammatory response plays an important role in the pathogenesis of angiotensin-II-induced end-organ damage. The present study aimed at exploring whether cyclooxygenase-synthetized prostanoids are involved in the pathogenesis of angiotensin-II-induced inflammatory response and vascular injury in mREN2 rats. The important finding of the present study was that neither nonselective cyclooxygenase inhibitor sulindac nor cyclooxygenase-2 selective MF-tricyclic was capable of preventing angiotensin-II-induced inflammatory response or tissue damage. In contrast, both compounds showed clear blood-pressure-increasing effect and further aggravated angiotensin-II-induced renal damage. RT-PCR gene expression analysis revealed that cyclooxygenase-2 mRNA expression was downregulated by angiotensin II both in the kidney and heart. Although cyclooxygenase inhibitors exerted detrimental cardiovascular and renal effects in mREN2 rats, they did not attenuate the therapeutic effects of angiotensin AT_1 receptor antagonist losartan. Taken together, our findings indicate that cyclooxygenase

does not play a central role in the pathogenesis of angiotensin-II-induced vascular inflammation and injury in mREN2 rats.

Long-term clinical use of nonselective cyclooxygenase inhibitors is limited by gastrointestinal side effects such as dyspepsia, abdominal pain, and less frequently, duodenal or gastric ulcers. In contrast, selective cyclooxygenase-2 inhibitors have demonstrated improved gastrointestinal safety and tolerability (FitzGerald and Patrono, 2001). However, recent clinical studies have suggested that cyclooxygenase-2 inhibitors may increase the risk of cardiovascular events, e.g. through increased blood pressure or decreased endothelial prostacyclin production (FitzGerald and Patrono, 2001; Mukherjee et al., 2001). In the present study, we found that both the nonselective cyclooxygenase inhibitor sulindac as well as the cyclooxygenase-2-selective MF-tricyclic increased systolic blood pressure by 20 to 30 mm Hg in mREN2 rats. We were unable to detect any significant difference between the two drug treatments. The blood-pressure-increasing effect of cyclooxygenase inhibitors was noted already after 4 weeks of treatment. An important finding of the present study was the close correlation between systolic blood pressure and the degree of cardiac hypertrophy in mREN2 rats, suggesting that the development of cardiac hypertrophy in mREN2 rats is primarily mediated by increased pressure overload. Although cyclooxygenase inhibitors increased blood pressure and slightly augmented cardiac hypertrophy in mREN2 rats, cardiovascular mortality, myocardial morphology as well as serum lactate dehydrogenase concentrations were unaltered by the two cyclooxygenase inhibitors. Our findings thus do not provide any evidence for further aggravation of angiotensin-II-induced myocardial or vascular lesions by cyclooxygenase inhibitors.

The kidneys exhibit abundant constitutive expression of both cyclooxygenase-1 and cyclooxygenase-2 (Breyer and Harris, 2001; Harris, 2000). Mice with targeted disruption of the cyclooxygenase-2 gene, as well as mice treated with a selective cyclooxygenase-2 inhibitor, showed renal dysgenesis (Kömhoff et al., 2000), suggesting an important role of cyclooxygenase-2 in renal development. In the kidney, cyclooxygenase-2 is predominantly expressed in the macula densa, cortical thick ascending limb of Henle, as well as in the medullary interstitial cells. Although the physiological role of cyclooxygenase-2 in the kidney is not fully understood, several studies support the notion that renal cyclooxygenase-2 may regulate renin release as well as renal microcirculation in response to environmental factors such as salt and water depletion (Breyer and Harris, 2001; Harris, 2000). Previous studies have shown that angiotensin II is a central regulator of renal hemodynamics and renal electrolyte and water excretion (Cowley, 1992). We have shown previously in transgenic rats harboring both human renin and angiotensinogen genes that angiotensin II decreases renal blood flow and glomerular filtration rate and increases tubular sodium reabsorption via angiotensin AT_1 receptor stimulation (Mervaala et al., 1999b). Infusions of physiological amounts of angiotensin II also causes marked reduction in renal blood flow with or without any changes in glomerular filtration rate (Hall and Guyton, 1990). Recent studies have provided compelling evidence that endothelial- and vascular smooth muscle cell-derived vasodilatory prostanoids attenuate the renal vascular response to angiotensin II (Imig, 2000). Interestingly, Qi et al. (2002) demonstrated very recently that cyclooxygenase-1 and cyclooxygenase-2 exert opposite effects on the pressor response to angiotensin II, and that vasodilatory prostaglandins (prostaglandin E_2 and prostaglandin I_2) derived from cyclooxygenase-2, effectively counteract the pressure effect of angiotensin II. In the present study, both cyclooxygenase inhibitors markedly increased angiotensin-II-induced renal damage when estimated by 24-h albuminuria. We further evaluated the renal effects of cyclooxygenase inhibitors by scoring the tissue damage. Perivascular inflammation, glomerular sclerosis with mesangial expansion, and interstitial fibrosis were found in the kidneys of mREN2 rats. We did not notice any statistically significant increases in renal damage score by cyclooxygenase inhibitors. However, a tendency toward more frequent existence of evident tubular necrosis was noted in kidney samples taken from cyclooxygenase-treated mREN2 rats. Taking into consideration the major role of kidney in the long-term regulation of blood pressure, we speculate that the blood-pressure-increasing effect of cyclooxygenase inhibitors in mREN2 rats is likely to be due to derangement in renal microcirculation and tissue damage.

mREN2 rats develop hypertension, cardiac hypertrophy, and renal damage due to increased angiotensin II formation in tissues and circulation (Bohm et al., 1995; Lee et al., 1996; Mullins et al., 1990). We showed recently that angiotensin II induces tissue damage even by blood-pressure-independent mechanisms (Mervaala et al., 2000). Angiotensin II generates reactive oxygen species, activates redox-sensitive transcription factors NF-kB and AP-1, and induces severe perivascular inflammation and adhesion molecule overexpression through angiotensin AT_1 receptor stimulation (Luft et al., 1999). Both cyclooxygenase-1- and cyclooxygenase-2-derived prostanoids play an important role in mediating and terminating inflammatory response (Breyer and Harris, 2001; Imig, 2000; Harris, 2000; Mitchell et al., 1995). Cyclooxygenase-2 can be induced in endothelial cells, fibroblasts, smooth muscle cells, and macrophages by proinflammatory cytokines, endotoxin, prostanoids, tumor promoters, mitogens, and hypoxia (Mitchell et al., 1995; Wu, 1995). Interestingly, treatment with cyclooxygenase-2-selective inhibitors like NS398 and celecoxib, but not with indomethacin, severely diminished early and late events of T cell activation, partly through NF-κB- and nuclear factor of activated T cells (NFAT)-transcription pathways, supporting the notion that selective cyclooxygenase-2 inhibitors might act as modulators of the immune system (Iniguez et al., 1999). However, anti-inflammatory effects of cyclooxygenase-2-derived prostanoids have also been described (Gilroy

et al., 1999). Interestingly, it has also been claimed that cyclooxygenase-2 induction in endothelial cells may represent an important compensatory mechanism to defend against vascular injury (Wu, 1998). In the present study, we estimated the degree of angiotensin-II-induced inflammatory response by quantifying the ED-1-immunopositive cells. ED-1 antibody detects mainly monocytes and macrophages. Consistent with the previous studies (Mervaala et al., 1999a, 2000; Romero and Reckelhoff, 1999), angiotensin II induced a marked monocyte/macrophage infiltration in the kidneys and to a lesser extent in the heart. We were unable to detect any anti-inflammatory effects by cyclooxygenase inhibitors, suggesting that cyclooxygenase does not play a central role in the pathogenesis of angiotensin-II-induced inflammatory response.

In mREN2 rats, cyclooxygenase-2 mRNA expression both in the heart and kidneys were downregulated, whereas angiotensin AT_1 receptor blockade by losartan increased cyclooxygenase-2 gene expression. Regulation of cyclooxygenase-2 protein expression by renin–angiotensin–aldosterone system was also noted with cyclooxygenase-2 immunohistochemistry. Our findings are thus in good agreement with previous studies (Breyer and Harris, 2001; Cheng et al., 1999; Harris, 2000) and indicate an important role for angiotensin II in the regulation of cyclooxygenase-2 gene expression in vivo. Selective cyclooxygenase-2 inhibition induced only cyclooxygenase-2 mRNA expression, whereas nonselective cyclooxygenase inhibition increased the gene expression of both cyclooxygenase-1 and cyclooxygenase-2. Our findings thus indicate that cyclooxygenase genes are compensatory induced after chronic enzyme inhibition.

Previous studies have revealed that angiotensin-II-induced hypertension is associated with pronounced inflammatory response in the vascular wall (Griendling et al., 2000a,b; Luft et al., 1999; Mervaala et al., 1999a; Romero and Reckelhoff, 1999). In contrast, perivascular inflammation, adhesion molecule overexpression, and increased formation of reactive oxygen species are typically not found in spontaneously hypertensive rats, the most common animal model for essential hypertension, or in noradrenaline-induced hypertension (Hilgers et al., 2000; Pinto et al., 1997; Rajagopalan et al., 1996; Strawn et al., 1999). In fact, inflammatory response in mREN2 rats as well as in double transgenic rats harboring human renin and human angiotensinogen genes is partly mediated by blood-pressure-independent mechanisms through angiotensin AT_1 receptor pathway (Mervaala et al., 2000; Strawn et al., 1999). Although mREN2 rats offer a unique opportunity to study the role of cyclooxygenase-synthetized prostanoids in the pathogenesis of angiotensin-II-induced inflammatory response and vascular injury, the transgenic animal model used in the present study may also have some limitations. Since the transfected mouse renin-2 gene is not under physiological control, changes in dietary salt intake or cyclooxygenase inhibition are unlike to modulate the renin expression in mREN2 rats. It is therefore of importance to underline that the findings and conclusions of the present study should be limited only to mREN2 rats. Further clinical studies examining the cardiovascular and renal effects of cyclooxygenase inhibition, and in particular the interaction with increased renin–angiotensin–aldosterone system activity, are therefore warranted.

In conclusion, our findings indicate that cyclooxygenase does not play a central role in the pathogenesis of angiotensin-II-induced inflammatory response and development of angiotensin-II-induced end-organ damage in mREN2 rats.

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