Renal cortical regulation of COX-1 and functionally related products in early renovascular hypertension (rat)

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Departments of 1Anatomy and 3Physiology, Charité Berlin, Berlin; 2Institut National de la Santé et de la Recherche Médicale U 702, Hôpital Tenon, Paris; 4Zentrum für Kinder u. Jugendmedizin, Marburg, Germany; 5Medizinische Poliklinik, Münster, Germany; and 6Institut National de la Santé et de la Recherche Médicale U 652, Université René Descartes, Paris, France

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Theilig, F., H. Debiec, B. Nafz, P. Ronco, R. Nüsing, H. W. Seyberth, H. Pavenstädt, N. Bouby, and S. Bachmann. Renal cortical regulation of COX-1 and functionally related products in early renovascular hypertension (rat). Am J Physiol Renal Physiol 291: F987–F994, 2006. First published June 20, 2006; doi:10.1152/ajprenal.00099.2006.—Renal volume regulation is modulated by the action of cyclooxygenases (COX) and the resulting generation of prostanoids. Epithelial expression of COX isoforms in the cortex directs COX-1 to the distal convolutions and cortical collecting duct, and COX-2 to the thick ascending limb. Partly colocalized are prostaglandin E synthase (PGES), the downstream enzyme for renal prostaglandin E2 (PGE2) generation, and the EP receptors type 1 and 3. COX-1 and related components were studied in two kidney-one clip (2K1C) Goldblatt hypertensive rats with combined chronic ANG II or bradykinin B2 receptor blockade using candesartan (cand) or the B2 antagonist Hoechst 140 (Hoe). Rats (untreated sham, 2K1C, sham + cand, 2K1C + cand, sham + Hoe, 2K1C + Hoe) were treated to map expression of parameters controlling PGE2 synthesis. In 2K1C, cortical COX isoforms did not change uniformly. COX-2 changed in parallel with NO synthase 1 (NOS1) expression with a raise in the clipped, but a decrease in the nonclipped side. By contrast, COX-1 and PGES were uniformly downregulated in both kidneys, along with reduced urinary PGE2 levels, and showed no clear relations with the NO status. ANG II receptor blockade confirmed negative regulation of COX-2 by ANG II but blunted the decrease in COX-1 selectively in nonclipped kidneys. B2 receptor blockade reduced COX-2 induction in 2K1C but had no clear effect on COX-1. We suggest that in 2K1C, COX-1 and PGES expression may fail to oppose the effects of renovascular hypertension through reduced prostaglandin signaling in late distal tubule and cortical collecting duct.

cyclooxygenase; nitric oxide synthase; bradykinin

RENOSVASCULAR HYPERTENSION (RVH) is a form of secondary hypertension. The two kidney-one clip rodent model of RVH (2K1C; Goldblatt model) is similar to unilateral RVH in humans. The defect is highly dependent on the enhanced activity of the renin-angiotensin system (RAS). Decreased renal perfusion pressure in the compromised kidney maximally activates the RAS, and its inability to balance the rise in blood pressure is partly the result of elevated ANG II levels. The nonstenotic kidney is subject to elevated perfusion pressure, responding with “pressure natriuresis” to lower blood pressure by the excretion of sodium. In the light of systemically elevated ANG II levels and the distinct constellation of the renal perfusion pressures, the RVH model is considered by some to be wholly one of increased peripheral vascular resistance, whereas others emphasize the role of volume retention (13; for a review, see Ref. 14). For the latter, elevated ANG II levels prevail in both kidneys in short-term and maintenance phase affecting both tubular transport and renal hemodynamics thereby causing long-term impairments in sodium balance regulation (29, 30).

Biological mechanisms with protective effects against hypertension and organ damage have received continuous interest and have therefore been studied also in RVH. In particular, paracrine and endocrine systems with protective potency have been analyzed for their role to oppose vascular and epithelial volume-retaining effects. Among these, cyclooxygenases (COX) and the prostaglandin system have received particular interest during the past decade for their involvement in the regulation of vascular tone and renal excretory function (for a review, see Ref. 9).

Apart from their vascular (20) and renal medullary interstitial action (42), renal prostaglandins have a tubular epithelial-bound focus in the cortical distal nephron and collecting duct system. Here, COX and downstream prostaglandin E synthase (PGES) show distinct cellular distribution, and prostaglandin E2 (PGE2) generation, the major prostaglandin produced along the nephron, may bind to colocalized EP1 and EP3 prostaglandin receptors to exert potent diuretic and natriuretic effects (2, 4, 5, 35). Because prostanoids are rapidly degraded, their action in the intimate vicinity of where they are produced must be assumed.

Expression of COX-2 within the thick ascending limb (TAL) is regulated by ANG II and probably modulates renal renin production (31, 37, 41) and may promote afferent vasodilation (19), although its role in juxtaglomerular signaling in RVH has been questioned (16). Less attention has been paid to COX-1 expression in RVH. COX-1, coexpressed with PGES, provides avid prostaglandin synthesis in the terminal distal convoluted tubule (DCT), connecting tubule (CNT), and cortical collecting duct (CCD) (5, 35). The EP1 and EP3 prostaglandin receptors have been colocalized to these segments as well, probably mediating reduced NaCl and water absorption, promoting natriuresis and diuresis (4), although acute inhibition experiments have questioned this role for COX-1 (34); a functional concept with the activities of COX-1 and COX-2 acting antagonistically has thus been stated. In contrast to COX-2, cortical
COX-1 has widely been considered as a constitutive enzyme with little or no adaptive response to variations in hemodynamics and renal urine concentrating functions (23, 26, 42). Together, the available evidence thus far has not reached consensus on the particular role of cortical COX-1 and the colocalized related components.

Given that the abundant expression of these components in the late distal epithelia would adapt to functional stimuli, this may demonstrate their functional significance. To test this, we chose the 2K1C model during early maintenance phase for its extremely divergent renal functional adaptations within one organism, before the rise of parenchymal alterations. Cortical expression of COX-1, PGES, and EP-1 and EP-3 receptors was assessed to consider their potential role in natriuresis and diuresis. The status of the cortical L-arginine-NO system was studied in parallel, as interference and synergistic effects with COX can be assumed (10, 24, 40). The possible interaction of ANG II with these parameters in 2K1C was monitored using candesartan. To test whether the topographically colocalized related components.

Together, the available evidence thus far has not reached consensus that reduced cortical epithelial COX-1 metabolism of cortical COX-1 and functionally related parameters in early RVH, with minor changes on RAS or KKS inhibition. We conclude that reduced cortical epithelial COX-1 metabolites could be related to impaired volume handling in RVH.

**MATERIALS AND METHODS**

**Animal preparation.** Young male Sprague-Dawley rats weighing 140 ± 7 g were obtained, in part, from the local animal facility of the Charité hospital, Berlin, and in part from Iffa Credo, Paris. All animals had free access to standard chow and tap water. The experimental protocol was approved by an independent state government review committee under registration no. 0357/97 (Landesamt Berlin). A total of 60 rats were subdivided into six groups by random selection (each n = 10, half and half divided for morphological and biochemical evaluation, receiving the following treatments: 2K1C; sham; age-matched, sham-operated controls): 1) sham, maintenance during 4 days before death with no further treatment; 2) 2K1C, maintenance during 4 days with no further treatment; 3) sham + candesartan (cand); maintenance during 4 days receiving the AT1 receptor antagonist cand (AstraZeneca, Molndal, Sweden; 10 mg·kg body wt⁻¹·day⁻¹ per os); 4) 2K1C + cand; maintenance during 4 days receiving cand; 5) sham + Hoe; maintenance during 4 days receiving 2.5% Hoechst 140 (Hoe; Merck, Darmstadt, Germany; 500 mg·kg body wt⁻¹·days⁻¹ sc); 6) 2K1C + Hoe; maintenance during 4 days receiving Hoechst 140. Drugs were generally applied beginning with the first day after surgery. The 2K1C treatment was designed to study the initial phase since after 4 days the RAS is expected to be maximally stimulated, yet without concomitant secondary tissue damage. For surgery, animals were anesthetized with pentobarbital sodium (50 mg/kg ip), then the left renal artery was isolated via laparotomy; a 0.2-mm silver clip was placed around the left renal artery using 330 mosmol sucrose/PBS (pH 7.4) during 20 s for blood rinsing, followed by 3% paraformaldehyde/PBS for 5 min. Fixative was subsequently removed by sucrose/PBS for 1 min. The left (clipped or sham) and the right kidneys (nonclipped or sham) were then removed, immersed in 800 mosmol sucrose/PBS (pH 7.4) for 12 h, shock-frozen in liquid nitrogen-cooled isopentane, and stored at −70°C. For Western blotting, kidneys were rinsed in situ with PBS (pH 7.4), removed, dissected into cortex and medulla, and shock-frozen in liquid nitrogen.

**Laboratory parameters.** Urinary sodium and potassium concentrations were determined by flame photometry (FLM3, Radiometer) or by ion-selective electrode (Konelab microlyt 3+2). Urinary creatinine levels were determined in 10-µl probes by an Ortho Diagnostics system and read by a Kodak Biolayer. Urinary kallikrein was measured colorimetrically (405 nm) using glandular kallikrein as a standard (S2266 Chromogenix Instrumentation, Coger). Urinary prostaglandins were measured by gas chromatography and tandem mass spectrometry; prostaglandin E2 (PGE2), 6-keto-prostaglandin F1α (6kPGF1α), and thromboxane B2 (TXB2) were determined as described (38).

**Immunohistochemistry.** Cryostat or paraffin sections (5 µm) were used. Paraffin sections were dewaxed, rehydrated, immersed in citrate buffer (pH 6.0), heated in a microwave oven for 20 min, incubated with 5% skim milk in PBS (pH 7.4) containing 0.5% Triton X-100 overnight, followed by primary antibody for 2 h at room temperature and then overnight at 4°C. Signal was generated using an amplification kit containing biotinylated IgG and streptavidin-peroxidase (DAKO, Hamburg, Germany) signal reaction. For immunofluorescence, cryostat sections were treated with blocking medium (DAKO), incubated with primary and a suitable fluorochrome-linked secondary antibodies (DAKO).

The following primary antibodies were used: rabbit polyclonal anti-COX-1 (dilution 1:7,000; batch no.160109, Cayman Chemicals), rabbit polyclonal anti-COX-2 (1:500, no. 160126, Cayman Chemicals), rabbit polyclonal anti-microsomal PGES (1:5,000, no. 160140, Cayman Chemicals), mouse monoclonal anti-THP (1:100), and rabbit polyclonal anti-EP1 and anti-EP3 (1:100) (27).

**Quantification of histochemical COX-2 signal.** Histochemical signals of COX-2 were semiquantitatively evaluated in an area of a total of 400–600 glomeruli/animal as described (3). The mean number of COX-2-immunoreactive cells of TAL segments in the vicinity of the JGA and macula densa was counted to determine changes in COX-2 protein abundance.

**Quantification of histochemical NOS1 signal.** Histochemical signals of NOS1 were similarly evaluated as for COX-2 (3). The mean number of NOS1-immunoreactive cells of the macula densa area was evaluated.

**Western blot analysis.** For membrane fractionation, kidney cortex was minced and homogenized in 10-ml dissecting buffer containing 0.3 M sucrose, 25 mM imidazole, 1 mM EDTA and proteinase inhibitor cocktail, pH 7.2. Homogenates were centrifuged for 15 min at 4,000 g (4°C). The pellet was rehomogenized to increase yields. Supernatants were pooled and centrifuged at 17,000 g for 30 min to obtain a fraction enriched for plasma membranes and part of the microsomal fraction. The pellet was resuspended in PBS containing 2.5% Triton X-100. Protein concentration was assayed for rough estimation by spectrophotometric analysis at 280 nm, and Bradford’s assay using BCA kit (Pierce) was performed throughout for definitive measurements. Equal loading was controlled by Coomassie staining of the SDS-PAGE gels, and Ponceau staining was applied on membranes after blotting.

Protein fractions were solubilized in Laemmli buffer, incubated for 10 min at 85°C and separated in 10–12% SDS-PAGE. Proteins were transferred onto PVDF membranes (Bio-Rad, Richmond, VA). Membranes were incubated overnight at 4°C with 10% skim milk/PBS containing 0.1 mM levamisole for 1 h at 37°C and probed with anti-COX-1 (dilution 1:500), anti-mPGES (1:500), anti-EP1...
(12,000), anti-EP3 (dilution 1:1,000), or monoclonal mouse anti-β-actin (1:20,000; Sigma) used for normalizing data with housekeeping β-actin expression. After being washed in 0.5% Tween/PBS membranes were incubated with alkaline phosphatase-conjugated secondary antibody (Promega, Heidelberg, Germany) followed by signal revelation using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate complex. Membranes were evaluated by measuring density of pixels/area using PDQuest two-dimensional image analysis system (Bio-Rad).

RT-PCR analysis. Total RNA from kidney cortex was extracted using the guanidinium thiocyanate method. All samples were quantified by spectrophotometric analysis at 260 nm. Five micrograms of total RNA were reverse transcribed. The following sets of oligonucleotide primers were used to amplify cDNA: 5’-TATCCGGTTGGA TACGAC-3’ and 5’-GTCTAGAATGGCCACATTTTC-3’ (GAPDH; 300 bp); and 5’-CCTCCGTTGCGCAGATTA-3’ and 5’-GGCTTG CCGCTAAACTCATCC-3’ (COX-1; 475 bp) and 5’-ACACT CTTAGAATGGCCACATTTTC-3’ and 5’-GAGGAGACACCTTT ACCAT-3’ (COX-2; 585 bp). Amplification was performed using Taq polymerase for 30–35 cycles with an automated thermal cycler (PerkinElmer, Weiterstadt, Germany). Each cycle consisted of the following steps: denaturation at 98°C, annealing at 63°C, and extension at 72°C. Semi-quantitative PCR was performed using specific primers for COX-1 or COX-2, as well primers for GAPDH in the respective tube identified by the localization near a cortical interlobular vein (*).

Immunoreactive COX-1 was localized to the extraglomerular mesangium and DCT, CNT, and CCD with exception of 2K1C kidnneys compared with the sham controls. Parameters were measured on day 4 of the study. 2K1C, 2 kidney-1 clip Goldblatt hypertensive rats; cand, candesartan; Hoe, Hoechst 140; BP, blood pressure; UV, urinary volume excretion; UNa, urinary sodium excretion; FENa, fractional sodium excretion; UK, urinary potassium excretion; UKallikrein, urinary kallikrein excretion; PG, urinary excretion of prostaglandins. *P < 0.05; n = 5 for each group.

Values are means ± SE; all changes refer to untreated sham controls. Parameters were measured on day 4 of the study. 2K1C, 2 kidney-1 clip Goldblatt hypertensive rats; cand, candesartan; Hoe, Hoechst 140; BP, blood pressure; UV, urinary volume excretion; UNa, urinary sodium excretion; FENa, fractional sodium excretion; UK, urinary potassium excretion; UKallikrein, urinary kallikrein excretion; PG, urinary excretion of prostaglandins. *P < 0.05; n = 5 for each group.

Results

Physiological parameters. Blood pressure was significantly increased in the 2K1C group, equally high in the 2K1C + Hoe group, and near normal in the 2K1C + cand group (Table 1). Urine flow was significantly increased in all 2K1C groups, whereas no significant changes in fractional sodium excretion levels were registered. Kallikrein excretion was diminished in 2K1C and 2K1C + cand groups, but unchanged in the 2K1C + Hoe group; referring the latter to the sham + Hoe group, however, a diminution was evident as well (P < 0.05). Cand and Hoe treatments in the sham groups caused no significant changes.

COX-1. Immunoreactive COX-1 was localized to the extraglomerular mesangium and DCT, CNT, and CCD with exception of 2K1C kidneys compared with the sham controls. Parameters were measured on day 4 of the study. 2K1C, 2 kidney-1 clip Goldblatt hypertensive rats; cand, candesartan; Hoe, Hoechst 140; BP, blood pressure; UV, urinary volume excretion; UNa, urinary sodium excretion; FENa, fractional sodium excretion; UK, urinary potassium excretion; UKallikrein, urinary kallikrein excretion; PG, urinary excretion of prostaglandins. *P < 0.05; n = 5 for each group.

Table 1. Clinical parameters and renal function

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>2K1C</th>
<th>Sham + cand</th>
<th>2K1C + cand</th>
<th>Sham + Hoe</th>
<th>2K1C + Hoe</th>
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<tr>
<td>BP, mmHg</td>
<td>136±6</td>
<td>151±4*</td>
<td>126±6</td>
<td>127±6</td>
<td>138±3</td>
<td>155±8*</td>
</tr>
<tr>
<td>UV, μl/min</td>
<td>7.1±0.3</td>
<td>10.6±0.5*</td>
<td>6.6±0.2</td>
<td>9.7±0.3*</td>
<td>7.9±0.3</td>
<td>10.7±0.3*</td>
</tr>
<tr>
<td>USn, mmol/day</td>
<td>1.57±0.18</td>
<td>1.85±0.12</td>
<td>1.75±0.14</td>
<td>1.68±0.10</td>
<td>1.92±0.10</td>
<td>1.70±0.10</td>
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<td>FErenal, %</td>
<td>0.96±0.33</td>
<td>1.01±0.13</td>
<td>1.23±0.21</td>
<td>1.16±0.13</td>
<td>0.78±0.07</td>
<td>0.96±0.13</td>
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<td>UK, mmol/day</td>
<td>2.65±0.19</td>
<td>2.73±0.09</td>
<td>2.53±0.05</td>
<td>2.59±0.11</td>
<td>2.89±0.06</td>
<td>2.65±0.17</td>
</tr>
<tr>
<td>UKallikrein, mmol/day</td>
<td>1.25±0.09</td>
<td>0.78±0.10*</td>
<td>1.37±0.14</td>
<td>0.78±0.08*</td>
<td>1.52±0.15</td>
<td>1.15±0.10</td>
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<tr>
<td>PGE2, pg·ml⁻¹·min⁻¹</td>
<td>11.2±1.6</td>
<td>3.9±0.3*</td>
<td>7.4±0.8</td>
<td>5.0±0.8</td>
<td>10.8±1.4</td>
<td>4.5±0.3*</td>
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<td>Thromboxane B2, pg·ml⁻¹·min⁻¹</td>
<td>0.83±0.03</td>
<td>0.60±0.05</td>
<td>1.23±0.09</td>
<td>1.32±0.07</td>
<td>1.87±0.29</td>
<td>0.61±0.03</td>
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<tr>
<td>6-keto-PGF-1α, pg·ml⁻¹·min⁻¹</td>
<td>0.63±0.03</td>
<td>0.56±0.05</td>
<td>0.69±0.01</td>
<td>0.49±0.04</td>
<td>1.11±0.12</td>
<td>0.56±0.04</td>
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tion of the intercalated cells. Changes in signal intensity among the different conditions could be qualified and have been documented exemplarily (Fig. 1, A–C). Densitometric evaluation of Western blot analysis revealed a decrease in both kidneys of all 2K1C groups compared with the untreated sham group except for the contralateral 2K1C + cand condition (clipped vs. nonclipped kidneys, changes ± SE: 2K1C, -53 ± 3 vs. -34 ± 5%; 2K1C + cand, -79 ± 4% vs. no change; 2K1C + Hoe, -48 ± 4 vs. -32 ± 4%), whereas the kidneys of the sham groups receiving cand or Hoe were unchanged (Fig. 2); values have been normalized for β-actin. Results were verified at the mRNA level using semiquantitative RT-PCR from extracts of the kidneys of sham and 2K1C groups; changes were similar to the Western blot data (not shown).

**COX-2.** Immunoreactive COX-2 was localized in macula densa area and in scattered TAL cells. Numerical evaluation of these immunoreactive cells revealed the following changes in COX-2 signal in the 2K1C groups compared with the untreated

![Fig. 2](http://ajprenal.physiology.org/)

Fig. 2. Cortical abundances of COX-1 (A), COX-2 (B), PGES (C), prostaglandin E receptor 1 (EP-1; D), prostaglandin E receptor 3 (EP-3; E), nitric oxide synthase 1 (NOS1; F), and cyclic guanosine monophosphate (cGMP; G) production. Western blots (insets) show representative results of single individual kidney extracts run simultaneously in the same gel as positioned above the bar graphs, showing changes from densitometric evaluation (A, C, D, E). Histochecmical cell counting (B, F). Enzymelinked immunoassay (G). Means ± SD (A–F) and ±SE (G). *P < 0.05.
sham group (clipped vs. nonclipped kidneys): 2K1C, +107 ± 3 vs. −68 ± 3%; 2K1C + cand, 494 ± 51 vs. +344 ± 15%; 2K1C + Hoe, +49 ± 9 vs. −51 ± 2% (Fig. 2). The kidneys of the sham + cand group showed a +420 ± 32% change, the sham + Hoe group −26 ± 3%. Results were verified at the mRNA level using semiquantitative RT-PCR from extracts of the kidneys of the sham and 2K1C groups; changes were similar to the immunohistochemical data (not shown).

PGES. Immunoreactive PGES was localized to the macula densa, DCT, CNT, and CCD with exception of the intercalated cells. Changes in signal intensity in the experimental conditions have been documented exemplarily (Fig. 1, D-F). Western blot analysis revealed a decrease in both kidneys of all 2K1C groups compared with the untreated sham group (clipped vs. nonclipped kidneys): 2K1C, −54 ± 2 vs. −31 ± 3%; 2K1C + cand, −93 ± 3% vs. no change; 2K1C + Hoe, −58 ± 5% vs. no change. The kidneys of the sham + cand group were unchanged and of the sham + Hoe group reduced (Figs. 1 and 2).

EP1. The EP-1 receptor was localized to the macula densa and neighboring TAL profiles, to CNT and CCD principal cells; signal was luminally enhanced (Fig. 1, G-I). Western blot analysis revealed a decrease of EP-1 abundance in the clipped kidneys of all 2K1C groups compared with the untreated sham group: 2K1C, −65 ± 4% vs. no change; 2K1C + cand, −93 ± 2% vs. no change; 2K1C + Hoe, no changes. The nonclipped kidneys were not different from the controls. The pooled kidneys (right and left kidney) of the sham groups receiving cand or Hoe were unchanged throughout (Fig. 2).

EP3. Immunohistochemistry localized EP-3 immunoreactivity mainly in TAL (Fig. 1, K-M). Western blot analysis revealed changes in EP-3 abundance in the 2K1C groups compared with the untreated sham group as follows (clipped vs. nonclipped kidneys): 2K1C, no change vs. +156 ± 16%; 2K1C + cand, −55 ± 3 vs. +200 ± 16%; 2K1C + Hoe, no change vs. +109 ± 13%. The kidneys of the sham groups receiving cand or Hoe were increased by 172 ± 25 and 100 ± 2%, respectively (Fig. 2).

Urinary prostaglandin excretion. Prostaglandin E2 excretion was significantly reduced in the 2K1C group (−65 ± 3%) and the 2K1C + Hoe group (−61 ± 2%) compared with the untreated sham group, whereas the controls showed +6% (Table 1). Urinary excretion of thromboxane B2 and prostaglandin F1α revealed no changes either (Table 1).

Cortical NO parameters-NOS1. Juxtaglomerular NOS1 expression as the major source of cortical constitutive NO production was quantified. Numerical evaluation of the immunoreactive cells revealed the following changes in NOS1 signal in the 2K1C groups compared with the untreated sham group (clipped vs. nonclipped kidneys): 2K1C, +40 ± 1 vs. −22 ± 6%; 2K1C + cand, 129 ± 19 vs. +69 ± 14%; 2K1C + Hoe, +4 ± 14 vs. −32 ± 9%. The kidneys of the sham + cand group showed a +99 ± 15% change, the sham + Hoe group −26 ± 3% (Fig. 2).

Cortical NO parameters-cGMP. Cortical cGMP levels in the 2K1C groups compared with the untreated sham group (clipped vs. nonclipped kidneys) were as follows: 2K1C, −10 ± 4 vs. −36 ± 3%; 2K1C + cand, −33 ± 7 vs. −53 ± 4%; 2K1C + Hoe, −50 ± 5 vs. −53 ± 8%. The kidneys of the sham + cand group showed a −15 ± 5% change, the sham + Hoe group −27 ± 13% (Fig. 2).

A summary of the changes in the experimental groups is presented in Table 2. Tendencies for coordinate changes of the cortical products, grouped together according to their common localization or biochemical relationship, have been indicated in the 2K1C groups by selective shading to facilitate an overview of the major results.

DISCUSSION

The rational of the present experiments was to characterize changes in the components of the renal cortical prostaglandin system and in potentially interfering products of the cortical L-arginine-NO system in RVH. The model was chosen for its highly divergent hemodynamic and regulatory configuration of the stenotic vs. contralateral kidneys. The potential influences of chronic inhibition of the RAS, which should improve the condition, and of the KKS, which should aggravate the condition, were assessed. In our setting of an initial short-term maintenance phase, the clipped kidney is expected to have an established low renal plasma flow, reduced glomerular filtration rate, stimulated macula densa response, and elevated juxtaglomerular parameters; the inverse constellation would characterize the contralateral kidney. Major parenchymal changes are not to be expected within the selected time span (3). Plasma RAS levels are typically high under this condition and renal ANG II levels are elevated in both kidneys, whereas renin levels are high in the clipped kidney and reduced in the

Table 2. Summary of changes in renal cortical protein abundance, cortical tissue cGMP levels, and urinary prostaglandin production

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<th>COX-1</th>
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<th>COX-2</th>
<th>NOS1</th>
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<td>Nonclipped</td>
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COX, cyclooxygenase; PGES, prostaglandin E synthase; EP, prostaglandin E receptor; NOS, nitric oxide synthase; cGMP, cyclic guanosine monophosphate; UPGE2, urinary prostaglandin E2 excretion. All changes refer to untreated sham controls and are indicated as ↑, significant increase; ↓, significant decrease; →, no significant change. Tendencies for coordinate changes of the cortical products, hypothetically grouped together including “no change” parameters, are indicated in the 2K1C groups by light versus dark shading. Note that contrary to the cortical parameters, UPGE2 may not only reflect cortical but also medullary prostaglandin output.
nonclip side (29, 30). By contrast, kallikrein activity is reduced principally in the clipped kidney, and normal or decreased in the nonclipped kidney (25). Changes of COX and related parameters, which we registered in the present experiments, were analyzed to permit new insights into their potential roles in the development of RVH.

Our results have, in part, corroborated, and, in part, extended available data on changes in inducible COX-2 of TAL and macula densa region as the major source of cortical PGE$_2$ synthesis (33, 40, 41). Chronic blockade of the ANG II AT$_1$ receptor had a marked additive effect resulting in a fourfold increase of COX-2 over control level on the stenotic side, similar to previous data on the coordinate changes of COX-2 and renin (18). It further led to an equally drastic rise in COX-2 signal in the contralateral kidney. COX-2 metabolite-dependent modulation of macula densa-mediated renin synthesis and release has been established, involving PGI$_2$- or PGE$_2$-mediated activation of local EP2 and EP4 receptors, and a causal relationship between these products is likely to play a role in RVH (20, 32, 36, 37). Chronic Hoe treatment prevented the induction of COX-2 expression in the stenotic kidney but had inconspicuous effects in the control and clipped conditions. RVH was earlier shown to present with increased B$_2$ receptor density in both kidneys (11), which may be explanatory to the fact that abrogation of kinin function has been worsening the development of RVH, possibly via reduced prostaglandin formation and consequent reduction in renal blood flow and sodium excretory ability (25). The full expression of COX-2 further appears to require intact B$_2$ receptor function (21). Alternatively, however, Hoe-induced reduction of NOS1 expression in RVH may have interfered with COX-2 (7), possibly through a p38 MAPK-dependent pathway as recently proposed (10). The conspicuous changes in NOS1 and COX-2 in the 2K1C groups with and without Hoe may support this view.

The major new finding of the present study, however, was that cortical COX-1 expression showed major changes as well in the present experimental conditions. Previous functional studies have highlighted substantial activity of COX-1 in the cortical vasculature, probably determining ANG II-stimulated increases in prostaglandins of the renal venous blood via calcium-liberating effects (for a review, see Ref. 36). Yet, in our hands, cellular localization indicated a clear prevalence of COX-1 in the terminal nephron and collecting duct system (5); it is further plausible that these sites in addition to the medullary sources of COX are suitably localized to determine urinary prostaglandin excretion (2). Unlike COX-2, COX-1 was downregulated in both, the clipped and the nonclipped kidneys in RVH. Changes in PGES expression were parallel to COX-1, and these parameters were further coordinated with an ~65% decrease in PGE$_2$ urinary output. Currently available data provide no specific mechanisms for these changes, and most data on COX-1 have come from studies in the renal inner medulla where tonicity appears to play a role in its regulation (6, 23). Yet, the present conditions cannot easily be related with cortical cell volume or transport status. In this regard, rats with central diabetes insipidus were compared with AVP-supplemented rats, showing no change in cortical COX-1 expression (23). Another COX-2-induced mechanism, involving transport-dependent activation of the p38 MAP kinase pathway in TAL and macula densa epithelium (8), to date has not been identified for COX-1 in the late distal tubule or collecting duct, and an attenuation of COX-1 expression by ANG II via the AT$_1$ receptor, as shown for COX-2 (18), is not entirely evident from the present data either. In controls, cand only numerically but not significantly increased COX-1, which is in contrast to the drastic increases in the juxtaglomerular parameters under this condition. In the clipped + cand kidneys, COX-1 was even further reduced by the ANG II blocker, whereas in the nonclipped + cand kidney, the decrease observed in the untreated clipped groups was blunted. Although the latter result would parallel changes seen with COX-2, the present data are not sufficiently clearcut to conclude that cortical COX-1 were under the same negative feedback control, as defined earlier for cortical COX-2.

Regarding the measured changes in PGES expression, available information on the physiological regulation of this terminal enzyme for PGE$_2$ biosynthesis (35) is scarce as well, with most data coming from an inflammatory background (22). The coordinate changes of COX-1 and PGES in the 2K1C as well as 2K1C + cand groups are obvious from the present results, but it remains to be discovered whether changes of COX-1 and PGES are coupled by common mechanisms. The changes of PGES under cand mirror those of COX-1, with an equally ambiguous indication for an influence by ANG II.

Because COX-1/PGE$_2$ signaling in the distal epithelia occurs mostly through the activation of EP1 and EP3 receptors (15, 27, 35), we quantified their expression as well. However, uniform changes in the two products were not detected, and only in the clip and the clipped + cand kidneys a downregulation of EP1 was paralleled by COX-1. EP3 expression showed no clear correlations, possibly since in our hands its localization was more focused to the TAL than to late distal segments, and in TAL, coupling to COX-isofoms would be less evident from the available data. The observed focal occurrence of coordinate changes of receptor and COX products thus has no clear implications on the mode of prostaglandin signaling in our experimental setting.

Comparing our data on COX-1 and PGES with the parameters of the L-arginine-NO system, we considered that NO of juxtaglomerular origin may potentially reach downstream nephron segments to possibly affect the expression of these products. Of note, other sources of NO, which have not been considered here, may be involved. In the clipped kidneys of 2K1C, there was no clear correlation between NO status and COX-1. In the nonclipped kidneys, however, changes in COX-1 and PGES paralleled those of juxtaglomerular NOS1 and cortical cGMP levels. Reductions in NOS1 levels in nonclipped kidneys of the 2K1C and also 2K1C + Hoe groups suggest functional relationships with COX-1 as outlined above for juxtaglomerular COX-2. Likewise, cGMP in these kidneys was lowered. This may suggest a potentially synergistic effect on distal volume handling by the two systems in the context of pressure natriuresis of the contralateral Goldblatt kidney (24).

Focusing on the tissue KKS and on kinins as potent enhancers of renal prostaglandin synthesis with particular respect to distal tubule and collecting duct function, we found marked attenuations in kallikrein excretion in RVH except for the Hoe-treated 2K1C group. The findings agree with published data on diminished KKS activity, contributing to the pathogenesis of RVH by the failure to oppose vasoconstriction and concomitant diminution in fluid and
electrolyte transport (17). ANG II blockade failed to alter the reduction in kallikrein, and an interdependence of local renal kallikrein synthesis and angiotensin signaling is not obvious (for a review, see Ref. 28). Treatment with Hoe normalized urinary kallikrein excretion compared with untreated sham values; intragroup comparison, however, revealed similar significant reduction of this enzyme as in the other groups. Also, the cortical quantity of COX-1 and the urinary PGE2 levels were largely unaffected by the B2 receptor blockade. In parallel, we further analyzed B2 receptor-deficient mice and controls under steady-state conditions but failed to detect any changes with respect to COX-1 and -2, PGES, EP1 or EP3 protein abundance between strains either (results not shown); the localization of these products in mouse in our hands was not principally different from that in rat kidney. The absence of cortical COX-1 changes in the knockouts agrees with previous data from whole kidney extracts (21), so that the evidence is against specific interactions between the KKS and COX-1 or its metabolites in the late distal and collecting duct epithelia in early RVH. In fact, most of the available data have ascribed to the B2 receptor function in early RVH an antihypertensive, protective role preferentially acting on the vascular side, buffering ANG II vasoconstrictor effects (7, 25).

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REFERENCES


