General inhibition of renocortical cyclooxygenase-2 expression by the renin-angiotensin system

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Castrop, Hayo, Jürgen Klar, Charlotte Wagner, Klaus Höcherl, and Armin Kurtz. General inhibition of renocortical cyclooxygenase-2 expression by the renin-angiotensin system. Am J Physiol Renal Physiol 284: F518–F524, 2003.—Because across-the-board data indicate that renin and cyclooxygenase-2 (COX-2) expression in the kidney cortex are regulated in parallel and because ANG II can inhibit COX-2 expression, the purpose of our study was to characterize a potential general inhibitory feedback of the renin-angiotensin system on renocortical COX-2 expression in vivo. Rats were fed a high-, normal-, or low-salt diet or were chronically infused with furosemide (60 mg·kg⁻¹·day⁻¹) or the left renal artery was clipped, and the animals were treated in addition to or without the angiotensin-converting enzyme inhibitor ramipril (10 mg·kg⁻¹·day⁻¹). A high-salt diet reduced expression of COX-2, whereas a low-salt diet, furosemide infusion, and renal artery stenosis stimulated COX-2 expression. Additional angiotensin-converting enzyme inhibition led to further increases in renocortical COX-2 expression by 62, 136, 300, 50, and 70% for a high-, normal-, or low-salt diet and furosemide infusion, and renal artery stenosis, respectively. Thus our data suggest a general inhibitory effect of the renin-angiotensin system on renocortical COX-2 expression.

renin; angiotensin-converting enzyme inhibition; renal cortex; prostaglandins

CYCLOOXYGENASE-1 AND -2 (COX-1 and -2) are the two known isoforms of cyclooxygenases that convert arachidonic acid in a two-step reaction into PGH₂, which is then further processed to a variety of prostanooids by specific enzymes (14). COX-1 is designated as the housekeeping isoform of cyclooxygenase, whereas COX-2 is considered the inducible isoform that is induced, for example, under inflammatory conditions (5, 15).

In the renal cortex of the rat, COX-2 is not only constitutively expressed in the macula densa and neighboring cells of the thick ascending limb (TAL) of Henle (cortical TAL (cTAL)) (8, 20) but also its expression is influenced by a variety of parameters that are not related to inflammation. For example, COX-2 expression in the renal cortex is inversely related to the oral salt intake in such a way that high-salt intake leads to a reduced expression of COX-2 whereas low-salt intake stimulates the expression of COX-2 (8, 11, 22, 25).

Besides different salt diets, a reduced salt transport activity of the macula densa cells, as caused by loop diuretics, was recently shown to increase the expression of COX-2 in the renal cortex (13, 24). In this context, additional experiments with cultured cTAL cells have shown that both pharmacological inhibition of the Na-K-2Cl cotransport and reduction of the chloride concentration in the culture medium cause an upregulation of COX-2 (1, 24). It has been suggested that reduced salt transport activity of the cTAL cells under these conditions stimulates the expression of COX-2 via the p38 MAP kinase pathway (1, 24).

Not only salt intake and salt transport of the macula densa but also the renal perfusion pressure influences the expression of COX-2 in the renal cortex (9, 12, 21). In this context, it has been demonstrated that renal artery stenosis, which reduces the renal perfusion pressure, leads to an upregulation of the expression of COX-2, whereas an increased systemic blood pressure decreases the expression of COX-2 (9, 12, 21).

Under all of these conditions that influence the expression of COX-2 in the renal cortex, a striking parallel regulation of the synthesis and secretion of renin has been observed, suggesting a possible interdependency of the expression of both enzymes. A number of studies have already investigated the influence of COX-2 expression on the expression of renin (2, 3, 6, 7, 10–12, 16–19, 23), whereas less is known about the role of renin and ANG II for the control of renocortical COX-2 expression (2, 22).

During a normal- and low-salt diet, an inhibitory effect of ANG II on the expression of COX-2 was suggested in the rat in such a way that both angiotensin-converting enzyme (ACE) inhibitors and angiotensin AT₁ receptor antagonists were found to moderately increase basal COX-2 expression and to enhance the expression of COX-2 stimulated by a low-salt diet (2, 22).
Whether these stimulatory effects of ANG II antagonists on COX-2 expression really reflect direct involvement of ANG II in the control of COX-2 expression or are more secondary to the aggravation of salt deficiency is less clear. We have previously found that ANG II antagonists also increase renocortical COX-2 expression in rats on a normal- and low-salt diet during a mineralocorticoid clamp, which prevents changes of systolic blood pressure and of glomerular filtration (11). It appears conceivable from these data that COX-2 expression in the renal cortex is, at least in part, negatively controlled by ANG II in vivo. If so, any alteration in the activity of the renin system and in consequence of ANG II formation would be expected to modulate COX-2 expression. Because there exists a striking parallel modulation of renocortical COX-2 expression and the activity of the renin system by salt intake, by renal perfusion pressure, and by loop diuretics, it is possible that changes of COX-2 expression are limited by concomitant changes of ANG II formation. In consequence, the apparent stimulation of COX-2 expression under a given condition would lead to an underestimation of the strength of the stimulus driving COX-2 expression. In turn, one would expect a general potentiation of COX-2 expression by ANG II antagonists, depending on the renin activity.

To address the question of whether such a general negative-feedback mechanism of the renin-angiotensin system on the expression of renocortical COX-2 exists, we have systematically investigated the influence of ACE inhibition on the expression of COX-2 under the aforementioned different conditions that lead to a parallel regulation of the expression of both COX-2 and renin.

We found an increased expression of renocortical COX-2 during ACE inhibition under all conditions examined that was most pronounced during a low-salt diet.

**MATERIALS AND METHODS**

**Animal Experiments**

Male Sprague-Dawley rats (180–200 g) were used in the experiments. All animal experiments were conducted in accordance with Guide for the Care and Use of Laboratory Animals (Washington, DC: National Academy Press, 1996) and German laws on the protection of animals.

Ten groups of rats (n = 7 each) were treated as follows.

**Group I.** Rats were fed a normal-salt diet [0.6% NaCl (wt/wt), Altromin, Lage, Germany] over a period of 7 days.

**Group II.** Rats were fed a low-salt diet [0.02% NaCl (wt/wt), ssniff special diets, Soest] over a period of 7 days.

**Group III.** Rats were fed a high-salt diet [8% NaCl (wt/wt), Altromin, Lage, Germany] over a period of 7 days.

**Group IV.** Rats were infused with furosemide (60 mg·kg⁻¹·day⁻¹) via subcutaneously implanted osmotic pumps (model 2ML1, Alza, Palo Alto, CA) for 7 days. These animals had access to tap water and a salt solution (0.9% NaCl, 0.1% KCl). Rats with empty osmotic pumps served as controls.

**Group V.** In rats with kidney artery stenosis, the left renal arteries were clipped (0.2-mm-inner-diameter silver clips, Degussa) under pentobarbital sodium anesthesia. In controls, the left renal artery was only touched with forceps (sham clipped).

Animals in groups VI–X were treated similarly to groups I–VI but also received the ACE inhibitor ramipril (10 mg·kg⁻¹·day⁻¹) via drinking water during the last 3 days of the experiment.

**Organ Sampling**

Animals were killed by decapitation and the kidneys were removed. Blood samples were taken for determination of plasma renin activity (PRA). The kidneys were cut in longitudinal halves. From one of these halves, the cortex was dissected under a stereomicroscope. Pieces of kidney cortex were frozen in liquid nitrogen and stored at −80°C until isolation of total RNA or protein. The second half was used for immunohistochemistry.

**Extraction of RNA**

Total RNA was extracted from the cortex basically according to the acid-guanidinium-phenol-chloroform protocol of Chomczynski and Sacchi (4). RNA pellets were dissolved in diethylpyrocarbonate-treated water, the yield of RNA was quantified by spectroscopy at 260 nm, and samples were placed in aliquots and stored at −80°C until further processing. The quality of extracted RNA was confirmed by the observation of intact 18S and 28S rRNA bands after gel electrophoresis in an ethidium bromide-stained agarose gel.

**RNase Protection Assays for COX-2, Renin, and Cytoplasmic β-Actin mRNA**

COX-2, renin, and β-actin mRNA levels were measured by RNase protection assays. In brief, after linearization and phenol/chloroform purification, the plasmids yielded radiolabeled antisense cRNA transcripts by incubation with SP6 polymerase (Promega) and [α-³²P]GTP (Amersham-Pharmacia) according to the Promega riboprobe in vitro transcription protocol. The lengths of the cRNA transcripts were 394, 347, and 351 bp for COX-2, renin, and β-actin, respectively. Five times 10⁵ counts/min of the cRNA probes were hybridized with 20 µg of total RNA (COX-2, renin), 1 µg of total RNA (β-actin), or 20 µg of tRNA (negative control) at 60°C overnight and were then digested with RNase A/T1 (RT/30 min) and proteinase K (37°C/30 min). After phenol/chloroform extraction and ethanol precipitation, protected fragments (370, 323, and 303 bp in length for COX-2, renin, and β-actin, respectively) were separated on an 8% polyacrylamide gel. The gel was dried for 2 h, bands were quantitated in a PhosphorImager (Instant Imager 2024, Packard), and autoradiography was performed at −80°C for 1–3 days.

**Determination of PRA**

PRA was determined utilizing a commercially available radioimmunoassay kit for ANG I (Sarin Biomedica, Düsseldorf, Germany).

**COX-2 Immunoreactivity**

After fixation in methyl-Carnoy’s solution (60% methanol, 30% chloroform, and 10% acetic acid), tissues were dehydrated by bathing in increasing concentrations of methanol, followed by 100% isopropanol. The tissue was embedded in paraffin and 4-µm sections were cut with a Leitz SM 2000R microtome (Leica Instruments, Oberkochel, Germany). After deparaffinization, endogenous peroxidase activity was blocked with 3% H₂O₂ in methanol for 20 min at room temperature. Sections were layered with the primary anti-
body (dilution 1:1,000; COX-2, Santa Cruz) and incubated at 4°C overnight. After addition of the second antibody (dilution 1:1,000; biotin-conjugated, rabbit anti-goat immunoglobulin G), the sections were incubated with avidin D horseradish peroxidase complex (Vectastain DAB kit, Vector Laboratory) and exposed to 0.1% diaminobenzidine tetrahydrochloride and 0.02% H₂O₂ as a source of peroxidase substrate. Each slide was counterstained with hematoxylin. As a negative control, we used the secondary antibody only, without incubation with the primary antibody.

**Statistics**

Data are presented as means ± SE. Levels of significance were calculated by ANOVA followed by Bonferroni’s test for multiple comparisons. P < 0.05 was considered significant.

**RESULTS**

**Influence of ACE Inhibition on the Expression of COX-2 and Renin in the Renal Cortex During Different Salt Diets**

To obtain information about the efficacy of the different maneuvers to modulate the intrarenal renin system, we measured renin mRNA in addition to COX-2 mRNA.

ACE inhibition by ramipril (10 mg·kg⁻¹·day⁻¹) during a normal-salt diet increased the renocortical mRNA abundance of COX-2 and renin by 125 ± 27 and 300 ± 30% compared with untreated controls (Figs. 1 and 2). In parallel with the mRNA, COX-2 protein immunoreactivity, semiquantitated as a percentage of immunopositive macula densa, also increased, from 5 ± 0.7% positive glomeruli in controls to 18 ± 2.1% positive glomeruli in ramipril-treated rats (Fig. 2).

A low-salt diet [0.02% NaCl (wt/wt)] led to a parallel stimulation of the mRNA expression of both COX-2 and renin, which increased by 75 ± 22 and 100 ± 25%, respectively, compared with rats on a normal-salt diet [0.6% NaCl (wt/wt)]. Additional ACE inhibition by ramipril further increased the mRNA levels of both COX-2 and renin by 325 ± 35 and 400 ± 17% compared with a low-salt diet without ramipril administration (Figs. 1 and 2).

In parallel with mRNA, COX-2 protein also increased during a low-salt diet to 17 ± 3 compared with a high-salt diet [8% NaCl (wt/wt)] decreased the mRNA abundance of both COX-2 and renin by 50 ± 5 and 70 ± 9% of the control levels, respectively. Ramipril in combination with a high-salt diet elevated the mRNA abundance of COX-2 and renin to values found during a normal-salt diet (Figs. 1 and 2).
Influence of ACE Inhibition on the Expression of COX-2 and Renin in the Renal Cortex During Chronic Furosemide Infusion with Salt Substitution

Loop diuretics were recently shown to increase the expression of COX-2 and renin in parallel in the renal cortex (13, 24). We therefore investigated the influence of ACE inhibition on the expression of COX-2 during chronic furosemide infusion with salt substitution. Furosemide infusion (60 mg·kg⁻¹·day⁻¹) increased the mRNA abundance of COX-2 and renin by 240±50 and 182±22% compared with control levels, respectively. We also found higher COX-2 protein levels of 34±5% immunopositive glomeruli compared with 5±1% in untreated animals. Both COX-2 mRNA and protein levels were further increased by ramipril (45±19 and 50±11%, respectively) compared with furosemide treatment without ACE inhibition. In parallel, renin mRNA levels were enhanced by 208±27% compared with furosemide infusion without ramipril treatment (Figs. 1 and 2).

Influence of ACE Inhibition on the Expression of COX-2 and Renin in the Renal Cortex During Unilateral Renal Artery Stenosis

Because not only salt intake and salt transport of the macula densa but also renal perfusion pressure is known to influence the expression of COX-2 in the renal cortex (9, 12, 21), we investigated the influence of renal perfusion pressure on the expression of COX-2 in the renal cortex during ACE inhibition.

By left renal artery stenosis (0.2-mm-inner-diameter silver clip), both COX-2 and renin mRNA were increased by 60±20 and 100±25% compared with the level in sham-operated rats, respectively. During ACE inhibition, the mRNA expression of both enzymes was further upregulated by 82±20 and 180±31%, respectively. COX-2 immunohistochemistry changed similarly to the mRNA. Thus COX-2 protein was also increased in the clipped kidney (28±4% of immunopositive glomeruli compared with 7±2% in sham-operated rats, not shown), and ramipril treatment further increased the COX-2 protein abundance to 35±4% immunopositive glomeruli (Figs. 1 and 2).

In the contralateral kidney, COX-2 and renin mRNA abundances were reduced to 70±10 and 45±6% of the level in sham-operated animals, respectively. COX-2 immunoreactivity in the contralateral kidney was only slightly reduced (Figs. 1 and 2).

Ramipril treatment during renal artery stenosis increased both cortical COX-2 mRNA expression and COX-2 immunoreactivity in the contralateral kidney (Fig. 2).

PRA and Relative Enhancement of COX-2 Expression by ACE Inhibition

In view of the different potencies of ACE inhibition to enhance COX-2 expression after ACE inhibition during the different experimental maneuvers, we considered the plasma renin activities (PRAs) under these different conditions to determine a possible interrelationship between the degree of activated renin system as reflected by PRA and the relative enhancement of COX-2 expression in response to ACE inhibition.
Average PRA was $5 \pm 1 \text{ ng ANG I h}^{-1} \text{ ml}^{-1}$ during a normal-salt diet. A high-salt diet reduced PRA to $35 \pm 5\%$, whereas a low-salt diet increased PRA to $300 \pm 20\%$ of the control level. Furosemide infusion elevated PRA sevenfold, which was the highest increase in PRA of all maneuvers examined. Renal artery stenosis increased PRA by $300 \pm 25\%$ compared with control levels.

Thus we found the following order of PRA: furosemide $>$ renal artery stenosis $>$ low-salt diet $>$ normal-salt diet $>$ high-salt diet (Fig. 4).

For the relative enhancement of COX-2 mRNA expression by ACE inhibition, we found an order of increases as follows: low-salt diet $>$ normal-salt diet $>$ renal artery stenosis, clipped kidney $>$ high-salt diet = furosemide $>$ renal artery stenosis, contralateral kidney (Fig. 5).

Thus despite the only moderately elevated PRA during a low-salt diet compared with chronic furosemide infusion, ACE inhibition during a low-salt diet led to a six times higher enhancement of COX-2 mRNA levels than ACE inhibition during chronic furosemide infusion. The enhancement of the COX-2 expression by ACE inhibition was by far highest during a low-salt diet.

As a consequence, we found a good positive correlation between PRA and the relative enhancement of COX-2 expression by ACE inhibition for the different salt diets only but not for furosemide treatment or for unilateral renal artery stenosis.

**DISCUSSION**

The purpose of our study was to investigate whether there exists a general negative effect of the renin system on the expression of COX-2 in the renal cortex of rats. For this purpose, we investigated the influence of ACE inhibition under a number of conditions that lead to a parallel regulation of the expression of both COX-2 and renin.

In accordance with previous reports, also in this study a low-salt diet (8, 11, 22, 25), chronic furosemide infusion (13), and renal hypoperfusion induced by renal artery stenosis (9, 12, 21) led to increases of renocortical COX-2 and renin expression, whereas a high-

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![Fig. 4. Plasma renin activity (PRA; ng ANG I h$^{-1}$ ml$^{-1}$) of rats after a high-, normal-, and low-salt diet, chronic furosemide infusion, and renal artery stenosis. *P < 0.05 vs. treatment without ramipril; n = 7 each.](http://ajprenal.physiology.org/)

![Fig. 5. Relative enhancement of COX-2 mRNA expression by ACE inhibition (compared with the respective treatment without ACE inhibition) in relation to PRA and respective pretreatment of a high-, normal-, and low-salt diet, furosemide infusion, and renal artery stenosis (clipped and contralateral kidney). A good correlation (y = 18 + 18x, $r^2 = 0.89$) between the relative COX-2 mRNA enhancement by ACE inhibition and the respective PRA during the pretreatment was observed for experiments in which dietary salt intake was modulated. No clear correlation between PRA and enhancement of COX-2 by ACE inhibition was found for the other conditions (y = 126 − 2.4x, $r^2 = 0.5$). Values are means ± SE.](http://ajprenal.physiology.org/)
salt diet and contralateral renal artery stenosis reduced the expression of both enzymes (9, 12, 25).

Under all of these conditions, ACE inhibition increased the expression of renocortical COX-2, regardless of whether the expression was suppressed, normal, or stimulated by the pretreatment maneuver. These data are in accordance with reports that COX-2 expression is increased in response to ACE inhibition in rats on a normal- or low-salt diet (2, 22). Our findings fit also with an in vitro study describing a direct inhibitory effect of ANG II on the COX-2 expression of cultured cells of the cTAL of the rabbit (2). Thus our data suggest a general negative effect of the renin-angiotensin-system on renocortical COX-2 expression.

Despite the general enhancement of COX-2 expression by ACE inhibition, both the relative and the absolute increases of COX-2 expression in response to ACE inhibition were different for the examined pretreatment conditions. Thus the relative enhancement of COX-2 expression during ACE inhibition was not related to the level of COX-2 expression during the pretreatment without ACE inhibition.

Further analysis revealed that the relative enhancement of COX-2 expression during ACE inhibition was correlated with PRA only during modulations of salt intake but not during treatment with loop diuretics nor during unilateral renal artery stenosis. Such a stimulus-dependent but PRA-unrelated efficacy of ACE inhibition renders the concept less likely that the general negative effect of renin activity on COX-2 expression is only mediated by a direct inhibitory effect of circulating ANG II on the COX-2-expressing cells. It appears as if COX-2 expression becomes more sensitive toward negative regulation by ANG II during modulations of salt intake than during changes of the renal perfusion pressure or during changes of salt transport in the loop of Henle. How salt intake, in particular low-salt intake, sensitizes cTAL and macula densa cells toward the actions of ANG II remains to be clarified. In any case, this mechanism is not specific for rats, because we also found a strong potentiation of COX-2 gene expression in the mouse kidney cortex by the combination of a low-salt diet with ACE inhibition (Wagner, unpublished observations).

For all experimental conditions, we found a striking, almost linear, correlation for the expression of both COX-2 and renin (Fig. 6). This correlation continued to exist during ACE inhibition; thus ACE inhibition enhanced the expression of both enzymes to the same extent. These data are in good accordance with a number of reports describing a parallel regulation of the expression of COX-2 and renin under a variety of experimental conditions (2, 9–13, 16, 19, 22, 25). It has therefore been proposed that COX-2-derived prostanooids may mediate a stimulation of renin expression and secretion. This concept is controversially discussed, because inhibition of COX-2 activity led in some studies to reduced renin expression (2, 3, 6, 7, 19, 21, 23) whereas in other studies COX-2 inhibitors did not influence renin expression (10, 12, 16–18). A second possible explanation for the finding that COX-2 and renin are regulated in parallel under various conditions would be the existence of a common, yet unknown, coregulator of COX-2 and renin expression, which is affected by the different treatments and controlled by a negative feedback of the renin system.

In summary, our findings suggest a general inhibitory effect of ANG II on the expression of COX-2 in the renal cortex, which becomes most apparent during modulations of salt intake.

A general enhancement of COX-2 expression by ANG II antagonists leading to an enhanced formation of prostanooids in the kidney cortex could be of clinical relevance, because prostanooids are relevant for renal perfusion (vasodilation) and for tubular salt and water transport (diuresis).

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REFERENCES


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