Role of prostanoids in regulation of the renin-angiotensin-aldosterone system by salt intake

KLAUS HÖCHERL,1 MARTIN C. KAMMERL,2 KARL SCHUMACHER,3 DIRK ENDEMANN,2 HORST F. GROBECKER,1 AND ARMIN KURTZ4
Institut für 1Pharmakologie, 2Anatome, und 4Physiologie der Universität Regensburg, D-93040 Regensburg; and 2Innere Medizin II, Universitätsklinikum Regensburg, D-93053 Regensburg, Germany

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Höcherl, Klaus, Martin C. Kammerl, Karl Schumacher, Dirk Endemann, Horst F. Grobecker, Armin Kurtz. Role of prostanoids in regulation of the renin-angiotensin-aldosterone system by salt intake. Am J Physiol Renal Physiol 283: F294–F301, 2002. First published February 26, 2002; 10.1152/ajprenal.00347.2001.—We investigated the effect of cyclooxygenase (COX) activity on the regulation of the renin-angiotensin-aldosterone system by salt intake. Therefore, Sprague-Dawley rats were subjected to different salt diets [0.02, 0.6, and 8% NaCl (wt/wt)] and treated with the COX-2 inhibitor rofecoxib (10 mg·kg body wt$^{-1}$·day$^{-1}$) or with ketorolac at a dose selective for COX-1 inhibition (2 mg·kg body wt$^{-1}$·day$^{-1}$) for 3, 7, 14, and 21 days. Rofecoxib and ketorolac caused a similar reduction of renocortical PGE2 formation with a low-salt diet. Rofecoxib did not change plasma renin activity or renocortical renin mRNA abundance with any of the diets but clearly lowered plasma aldosterone concentration. In contrast, ketorolac delayed the increase in plasma renin activity and of renin mRNA in response to low salt intake but did not change plasma aldosterone concentration. Prolonged treatment with rofecoxib but not with ketorolac caused an upregulation of COX-2 expression while COX-1 mRNA abundance remained unchanged. These findings suggest that COX-1-derived, but not COX-2-derived, prostanoids are of relevance for the regulation of the renin system by salt intake.

cyclooxygenase; kidney

THE ACTIVITY OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS) is determined by several factors, including the rate of salt intake (12). It is activated in states of low salt intake and suppressed by high salt intake (1, 32). The regulation of the RAAS by salt intake depends on the regulation of renin synthesis and renin secretion, because the protease renin is regarded as the rate-limiting enzyme of the RAAS. However, the mechanisms linking renin with salt intake have long remained obscure (12). Several studies have shown that the stimulation of the renin system by low salt intake is attenuated by COX inhibitors, suggesting a possible mediator role for prostanoids in the stimulation of the renin system by low salt intake (6, 10). However, COX inhibitors also cause volume retention, particularly in states of sodium depletion (2, 27, 35), and the effect of COX inhibitors on the renin system could therefore be related to volume changes rather than to interference with a direct action of prostanoids in the juxtaglomerular apparatus (29, 31). The demonstration that a low-salt diet enhances the expression of COX-2 in the macula densa cells (15) has reinforced the concept that COX-2-derived prostanoids in the macula densa may directly mediate the effect of salt intake on renin secretion and of renin gene expression in neighboring juxtaglomerular epithelioid cells (7, 24).

Such a concept is in good agreement with the idea of a direct stimulatory effect of PGE2 and PGI2 on juxtaglomerular epithelioid cells (22). It is further supported by reports that selective COX-2 inhibitors attenuate the stimulation of the renin system by low salt intake (13) and that a low-salt diet fails to stimulate the renin system in COX-2-deficient mice (39). At first glance, these data support the concept; however, they are hampered by some peculiarities. Thus administration of preferential COX-2 inhibitors only partially inhibited renin secretion (13, 23). Furthermore, acute intravenous administration of a COX-2 inhibitor had no effect on plasma renin activity (PRA) in rats (13) or dogs (9) kept on a low-salt diet, suggesting that COX-2 inhibition has to last for days to attenuate the stimulation of the renin system by a low-salt diet. Moreover, in COX-2-deficient mice, not only renin expression but also the structure of the kidney is markedly altered (5, 26), suggesting that the altered response of the renin system to low salt intake may not necessarily be linked to the lack of COX-2-generated prostanoids. In fact, there are studies questioning a causal involvement of COX-2-derived prostanoids in the regulation of the renin system by salt intake (18, 23, 31). In view of this unclear situation regarding the role of prostanoids for mediating the influence of salt intake on renin secretion and renin synthesis, it appeared reasonable to us to characterize systematically the involvement of COX-derived prostanoids for the regulation of the RAAS by

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salt intake. Because different published findings may have resulted from different time protocols of experiments and from different isoform selectivities of the COX inhibitors used, our purpose was to determine time-dependent effects of treatment with three COX inhibitors, including a COX-2-selective inhibitor, a nonselective COX inhibitor, and a preferential COX-1 inhibitor on the RAAS.

**MATERIALS AND METHODS**

*Treatment of animals.* Male Sprague-Dawley (SD) rats, 190–210 g, were used for this study (Charles River, Sulzfeld, Germany).

To examine the effect of the COX-2 inhibitor rofecoxib on the modulation of the RAAS by salt intake after a 3-wk treatment, groups of eight rats were treated with either a low-salt diet [0.02% NaCl (wt/wt)], a normal-salt diet [0.6% NaCl (wt/wt)], or a high-salt diet [8% NaCl (wt/wt)] in combination with vehicle or 10 mg·kg body wt⁻¹·day⁻¹ rofecoxib (VIOXX, MSD), orally by a stomach tube.

For the determination of time-dependent changes during low salt intake, 8 rats/group received a low-salt diet and were treated with ketorolac (2 mg·kg body wt⁻¹·day⁻¹; Cayman Chemical, Ann Arbor, MI), meclofenamate (10 mg·kg body wt⁻¹·day⁻¹; Cayman Chemical), or rofecoxib (10 mg·kg body wt⁻¹·day⁻¹) for 3, 7, 14, or 21 days. Along with low salt intake, we investigated time-dependent changes during normal salt intake and treatment with ketorolac or rofecoxib for 3, 7, 14, or 21 days. Each treatment period started in such a way that at the end of the study period all rats weighed ~290–310 g. Body weight was monitored daily before drug administration. Blood pressure measurements were performed (tail cuff method) before treatment was initiated and at days 3, 7, 14, and 21. Measurements were always performed 18–24 h after administration of drugs or vehicle.

At the end of the experiments, the rats were killed by decapitation during sevoflurane anesthesia. Blood was collected into tubes containing EDTA, and kidneys were quickly dissected, frozen in liquid nitrogen, and stored at −80°C until extraction of total RNA according to the protocol of Chomczynski et al. (3).

*Selection of drugs.* Drugs were primarily selected on the basis of the IC₅₀ ratios (whole blood assay-COX-2/COX-1) reported previously (37). These data suggest that rofecoxib has a >50-fold COX-2 selectivity, with a low affinity to COX-1, when COX-2 is inhibited by 80%. Meclofenamate is regarded as a dual COX inhibitor, and ketorolac shows the greatest selectivity for COX-1 of the nonsteroidal anti-inflammatory drugs on the market. Recently, it has been shown that the dose of ketorolac used in our study (2 mg·kg body wt⁻¹·day⁻¹) inhibits COX-1 for >90% without a significant effect on COX-2 activity (36) and that rofecoxib seems to be selective for COX-2 at the dose used in our study (10 mg·kg body wt⁻¹·day⁻¹) (11).

*Ribonuclease protection assays for β-actin, COX-2, COX-1, and renin mRNA.* Cytoplasmatic β-actin (1 μg of total RNA) and renocortical COX-1 (100 μg), COX-2 (50 μg), and renin mRNA (50 μg) were determined by specific RNase protection assays, as described previously (25, 38).

* Determination of PRA, plasma aldosterone concentration, and renocortical PGE₂ concentration.* PRA and plasma aldosterone concentration (PAC) were determined with commercially available radioimmunoassays (Sorin Biomedica, Düsseldorf, Germany). Concentration of renal cortical PGE₂ was assayed by using a monoclonal enzyme immunoassay kit (Cayman Chemical). One hundred milligrams of renal cortical tissue were homogenized with ice-cold isotonic NaCl solution and centrifuged at 10,000 g for 10 min. A 1:30 dilution of the supernatant was used for the determination of PGE₂ and also for the determination of protein according to the method of Lowry.

**COX-2 immunoreactivity.** COX-2 immunoreactivity was demonstrated as described previously (25). In brief, sections were layered with the primary antibody (dilution 1:500; M19, Santa Cruz Biotechnology, Santa Cruz, CA) and incubated at 4°C overnight. After addition of the second antibody (dilution 1:500; biotin-conjugated, rabbit anti-goat IgG), the sections were incubated with avidin-D horseradish peroxidase complex (Vectastain diaminobenzidine kit; Vector Labs) and exposed to 0.1% dianinobenzidine tetrahydrochloride and 0.02% H₂O₂ as the source of peroxidase substrate. Each slide was counterstained with hematoxylin-eosin. As a negative control, we used the same dilutions of preimmune goat serum (for the primary antibody) or normal rabbit IgG (for the secondary antibody).

**Statistical analysis.** The data are presented as means ± SE. The level of significance was calculated by ANOVA followed by Student’s t-test. A P value < 0.05 was considered significant.

**RESULTS**

*Effect of the COX-2 inhibitor rofecoxib on modulation of the RAAS by salt intake after a 3-wk treatment.* PRA was inversely related to salt intake. Compared with normal salt intake, PRA increased to ~225% during the low-salt diet and decreased to ~25% during the high-salt diet. A 3-wk treatment with rofecoxib (10 mg·kg body wt⁻¹·day⁻¹) had no influence on PRA for any of the diets (Fig. 1).

In the same fashion as PRA, renin mRNA abundance changed inversely with the salt content of the diet. Thus renin mRNA abundance increased to ~175% during the low-salt diet and decreased to ~25% of the control value during the high-salt diet. Treatment with rofecoxib decreased PAC values to about one-half of the control levels for any of the diets (Fig. 1).

Similarly PAC increased to ~400% during the low-salt diet and decreased to ~40% of the control value during the high-salt diet. Treatment with rofecoxib decreased PAC values to about one-half of the control levels for any given diet (Fig. 1).

Because COX-2 inhibition did not influence the regulation of renin secretion and renocortical renin gene expression by salt intake in this study, we determined the influence of different salt diets on renocortical PGE₂ tissue concentration. Tissue PGE₂ levels, as an indirect indicator for the activity of COX, decreased with increasing salt intake. The low-salt diet increased PGE₂ concentrations to ~140%, and the high-salt diet decreased PGE₂ to ~30% of the control value. Treatment with rofecoxib decreased renocortical PGE₂ concentrations to about one-half of the control levels for any diet (Fig. 2).

Similar to tissue PGE₂ concentrations, the low-salt diet increased COX-2 mRNA to ~190% and the high-salt diet decreased COX-2 mRNA to ~40% of the control value, whereas COX-1 mRNA remained unaltered. Treatment with rofecoxib markedly enhanced salt-trig-
gered COX-2 mRNA expression. Thus rofecoxib increased COX-2 mRNA abundance 3-, 2.8-, and 1.8-fold for the low-, normal- and high-salt diets, respectively. COX-1 mRNA levels were not affected by treatment with rofecoxib (Fig. 2).

Time dependency of COX inhibition on the RAAS during low salt intake. In view of the lack of effect of the COX-2 inhibitor on renin secretion and renin gene expression despite significant changes in COX-2 expression and tissue PGE₂ concentrations, we considered the temporal pattern of the effects of rofecoxib and compared it with the effects of a preferential inhibition of COX-1 activity. For this purpose, we determined the effects of rofecoxib, the nonselective COX inhibitor meclofenamate, and ketorolac (at a dose selective for COX-1) on the RAAS during low salt intake 3, 7, 14, and 21 days after the start of the treatment.

Renocortical PGE₂ concentrations increased with time during low salt intake and reached a plateau of 1.5-fold over the control values after 2 wk of salt restriction (Fig. 3). All three COX inhibitors lowered renocortical PGE₂ after 3 days of treatment, which was...
the first time point examined. The strongest effects were seen with ketorolac, which reduced PGE$_2$ by $\sim 60\%$ (Fig. 3). Compared with normal salt intake, PRA increased with time and reached a plateau of about threefold over the control values after 7 days of low salt intake (Fig. 4). Treatment with ketorolac clearly attenuated the increase of PRA at days 7 and 14 but had no inhibitory effect after 3 wk. Similar results were obtained with meclofenamate, which appeared less effective than ketorolac. Rofecoxib had no effect on PRA at any time point (Fig. 4).

Renin mRNA abundance increased in parallel with PRA, reaching plateau levels of $\sim 2.3$-fold over the control values after 7 days of the low-salt diet. Ketorolac and meclofenamate, but not rofecoxib, attenuated this characteristic increase in renin mRNA at days 2 and 7 but had no inhibitory effect after 2 and 3 wk.
Fig. 8. Representative distribution of COX-2 immunoreactivity in the rat kidney from SD rats receiving a normal-salt diet [0.06% NaCl (wt/wt)] and vehicle (A and B), low-salt diet [0.02% NaCl (wt/wt)] and vehicle (C and D), low-salt diet and ketorolac (2 mg·kg body wt⁻¹·day⁻¹; E and F), or low-salt diet and rofecoxib (10 mg·kg body wt⁻¹·day⁻¹; G and H) for 3 wk. Arrows, COX-2 immunostaining in the macula densa region. Magnification, ×25 (A, C, E, and G) and ×40 (B, D, F, and H).
PAC increased about fourfold, reaching plateau levels after 2 wk of the low-salt diet (Fig. 6). Rofecoxib blunted a further increase in PAC after 3 days of low-salt feeding. To a lesser extent than rofecoxib, meclofenamate attenuated PAC, particularly after prolonged treatment for 3 wk. Ketorolac had no significant effect on PAC at any time examined (Fig. 6).

Renocortical COX-1 mRNA abundance remained unchanged by treatment with low salt and by any drug at any time point analyzed (data not shown).

Renocortical COX-2 mRNA abundance increased time dependently and reached a plateau of about twofold over the control values after 7 days of low-salt feeding (Fig. 7). Rofecoxib further increased COX-2 mRNA after 2 wk of treatment. Meclofenamate exerted an effect similar to that of rofecoxib but was less effective. Ketorolac did not change the temporal pattern of COX-2 mRNA (Fig. 7).

**Immunohistochemistry for COX-2.** In view of the strong upregulation of COX-2 mRNA by prolonged treatment with rofecoxib, we were interested in the localization of COX-2 protein in these kidneys. Figure 8 shows representative examples of COX-2 immunoreactivity in the kidney of a control rat during normal salt intake (Fig. 8, A and B) and low salt intake (Fig. 8, C and D) and rats kept on a low-salt diet with ketorolac (Fig. 8, E and F) or rofecoxib (Fig. 8, G and H) treatment for 3 wk. In the kidneys of control rats, COX-2 immunoreactivity was scarce and was restricted to the cortex, in which it was mainly associated with juxtaglomerular regions. In animals on a low-salt diet, COX-2 immunoreactivity increased moderately without changing the distribution of COX-2 expression. Ketorolac treatment did not alter the localization of COX-2 protein compared with low salt intake. In animals on a low-salt diet with additional rofecoxib treatment, COX-2 immunoreactivity strongly increased but was still restricted to the cortex. Under this experimental condition, the cortical thick ascending limbs of Henle displayed COX-2 immunoreactivity over almost their entire lengths (Fig. 8, G and H).

**Time dependency of COX inhibition on systolic blood pressure during low salt intake.** Systolic blood pressure (sBP) of all animals was ~125–135 mmHg at the beginning of the treatment and was not altered in control rats with normal salt intake during the whole treatment period. During low salt intake, sBP was in the range of 120–130 mmHg and was not different compared with normal salt intake at all time points investigated. Low salt intake and rofecoxib treatment did not change sBP during the 3-wk treatment. In contrast, sBP increased in the range of 135–145 mmHg in rats subjected to low salt intake and ketorolac or meclofenamate after 14 days of treatment. However, sBP was not altered in these animals on days 3 and 7 (Fig. 9).

**Time dependency of COX inhibition on the RAAS during normal salt intake.** In view of the attenuation of renin gene expression and renin secretion by ketorolac during low salt intake, we considered the temporal pattern of the effects of rofecoxib and ketorolac on the renin system during normal salt intake. Neither ketorolac nor rofecoxib affected renocortical renin mRNA abundance or PRA at any time point investigated (data not shown). Furthermore, ketorolac did not influence PAC, COX-1, or COX-2 mRNA during normal salt intake, although it decreased basal renocortical PGE2 levels in the range of 40–50% compared with control rats. During normal salt intake, rofecoxib behaved very similarly to what was seen for the low-salt diet. Thus renocortical PGE2 content was decreased to ~50% of the control levels. COX-1 mRNA was not affected, whereas renocortical COX-2 mRNA abundance increased ~1.7-fold over the control values after 14 days of treatment with rofecoxib. PAC was attenuated by rofecoxib after 14 days of treatment in that rofecoxib lowered PAC to ~50% of basal levels.

**DISCUSSION**

The purpose of our study was to determine the relevance of prostanoids, in particular of COX-2-generated prostanoids, for the regulation of the RAAS by variation of salt intake. In agreement with previous studies (17, 19), we found a time-dependent increase in PRA, PAC, and renin mRNA during low salt intake and an inverse relationship between PRA, PAC, and renin mRNA and the salt content of the diet. Also, COX-2 gene expression in the renal cortex as an indicator for macula densa COX-2 expression (16) changed inversely with the rate of salt intake, thus confirming previous reports (21, 40).

Our data show that renocortical tissue levels of PGE2 are also inversely related to the rate of salt intake and that they can be markedly lowered by preferential COX-1 and COX-2 inhibitors. This finding
is compatible with the observation that COX-1 and COX-2 genes are expressed to a similar extent in the renal cortex. With regard to the relevance of PGs for the regulation of renin secretion and renin gene expression by salt intake, our findings would suggest that abolition of COX-1-derived rather than of COX-2-derived prostanoids transiently attenuates the stimulation of the renin system by low salt intake. This conclusion thus supports previous findings obtained with nonselective COX inhibitors (7, 8, 24) and furthermore suggests that macula densa-derived COX-2-mediated prostanoids are not relevant for the regulation of the renin system by salt intake. Several previous studies have suggested that the inhibition by COX inhibitors of renin secretion stimulated by low salt is related to volume retention (29, 31). The finding that COX-1 inhibition only transiently attenuates the stimulation of renin secretion and of renin gene expression indicates that COX-1-derived PGs are not permanently required for the stimulation of the renin system by low salt intake, indicating a more indirect effect of COX-1-derived prostanoids. In summary, we conclude from our findings that PGs are not the direct mediators of salt intake at the level of renal juxtaglomerular cells. This conclusion is in contrast to the concept that mainly COX-2-derived prostanoids should mediate the effect of salt intake on the renin system (14, 39). With regard to the previous observations of our laboratory (23), we found that the attenuation of PRA by COX-2 inhibition during a low-salt diet is a rather inconsistent phenomenon that could not be confirmed in further studies with rats of the Wistar-Kyoto and spontaneously hypertensive strains (Hocherl K, Endemann D, Kammerl MC, Grobecker HF, and Kurtz A, unpublished observations) or with the SD rats in this study.

Nonsteroidal anti-inflammatory drugs are thought to increase blood pressure, which in turn may have a suppressive effect on renin synthesis and renin gene expression, because it is known that blood pressure negatively affects PRA and renin mRNA (12). However, our data do not suggest a major influence of changes in blood pressure on PRA and renocortical renin mRNA abundance during a low-salt diet.

The role of the two COX isoforms in the generation of aldosterone appears to be quite different. It has been suggested that prostanoids are involved in the regulation of aldosterone release at the cellular level (4, 30). Our data would now suggest that mainly COX-2-derived prostanoids are relevant for aldosterone production. A moderate constitutive expression of COX-2 has already been reported for the adrenal cortex (20). From our findings, we conclude that aldosterone regulation by salt intake partially depends on the COX-2 mechanism, but other factors may be involved.

Prolonged administration of COX-2 inhibitors but not of COX-1 inhibitors strongly enhanced COX-2 gene expression in our study. This enhancement of COX-2 expression occurred in the same structures in which COX-2 is sporadically found in the kidneys of normal rats, namely, the cortical thick ascending limbs of Henle, including the macula densa regions (15, 34). This stimulation of COX-2 expression somewhat resembles the situation of adrenalectomized animals in which COX-2 expression in the cortical thick ascending limb of Henle is strongly increased because of mineralocorticoid deficiency (33). In fact, treatment with the COX-2 inhibitor attenuated aldosterone release. However, whether this lowering of PAC in conjunction with a low-salt diet is sufficient to cause such strong stimulation of COX-2 expression remains questionable. Another explanation could be that COX-2 inhibitors stimulate COX-2 expression by activating nuclear factor-κB, which has been demonstrated in vitro (28). Finally, a negative feedback of COX-2 expression by COX-2-derived prostanoids also appears conceivable in principle.

Taken together, our data indicate that in rats, COX-2-derived prostanoids are not essentially required for the regulation of renin secretion, renin synthesis, aldosterone production, and COX-2 expression in the macula densa by salt intake. COX-2-derived prostanoids exert an additional stimulatory effect that amplifies the regulation of aldosterone production by salt intake. Conversely, COX-2-derived prostanoids exert a direct negative effect on COX-2 gene expression.

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REFERENCES


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