Role of macula densa cyclooxygenase-2 in renovascular hypertension

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Cyclooxygenases (COXs) are key enzymes in the generation of prostaglandins, which are involved in the regulation of renin in the kidney (10). In particular, renal expression of COX-2 has attracted the attention of many hypertensologists, as it is localized in the macula densa cells of the thick ascending limb of Henle (5, 6). Furthermore, macula densa COX-2 is upregulated by low-sodium intake (5), low perfusion pressure (6), and inhibition of the renin-angiotensin system (21), suggesting an involvement of COX-2 in the macula densa mechanism. Indeed, some authors have reported that selective inhibition of COX-2 reduced the increase in renal renin induced by salt depletion (4) and angiotensin inhibition (3). On the other hand, controversial findings were reported with other COX-2 inhibitors. Hypertension in 2K1C rats was not affected or slightly enhanced by COX-2 inhibition, as measured by plasma renin activity (17). Previous experiments with nonselective COX inhibitors were compatible with this notion (16). However, controversial findings were subsequently reported with specific blockers of COX-2: the increase in renin in the poststenotic kidney was blocked in aortic coarctation (20) but not in an acute model of renal artery stenosis (14). One possible explanation for these apparently controversial findings is that the role of the macula densa mechanism may differ relative to other mechanisms of renin regulation. For example, a strong stimulation of the baroreceptor mechanism in acute renal artery stenosis may overrule the macula densa mechanism.

To clarify the role of macula densa COX-2 in the maintenance phase of renovascular hypertension, we used a model in which the poststenotic kidney was blocked in aortic coarctation (20) but not in an acute model of renal artery stenosis (14). One possible explanation for these apparently controversial findings is that the role of the macula densa mechanism may differ relative to other mechanisms of renin regulation. For example, a strong stimulation of the baroreceptor mechanism in acute renal artery stenosis may overrule the macula densa mechanism.
does not occur immediately after the procedure but develops slowly with the growth of the animal over a period of at least 1 wk. Animals were only included into the 2K1C groups if systolic blood pressure was above 150 mmHg. Animals that failed to thrive or lost weight were excluded after 2 wk.

**COX-2 inhibition with NS-398 or rofecoxib.** Six 2K1C and six control rats then received the COX-2 inhibitor NS-398 for another 2 wk at a concentration of 10^{-6} M in the drinking water. Based on the animals’ drinking behavior, the approximate dose taken up was 1 mg·kg^{-1}·day^{-1}. Six 2K1C and six controls received solvent (0.3% ethanol) only. In a separate set of experiments, 2K1C hypertension was induced as described above. After 2 wk, six 2K1C and five sham-operated animals were treated with 10 mg·kg^{-1}·day^{-1} rofecoxib (Vioxx solution; MSD, Munich, Germany) by daily gavage for 14 days. Six 2K1C and five sham-operated controls received solvent (43 g sorbitol and xanthan gum in 100 ml tap water) by daily gavage. Both COX-2 blockers at the doses used have been shown to be effective in the kidney (11, 12, 18).

**Measurement of arterial blood pressure.** Four weeks after clipping, the animals were equipped with a femoral artery catheter under ketamine/xylazine anesthesia, and intra-arterial blood pressure was measured in conscious rats 4 h after anesthesia via a transducer connected to a polygraph (Hellige, Freiburg, Germany).

**Collection of blood and tissue material.** Blood was taken from arterial catheters for quantification of plasma renin activity. Animals were then killed by dissecting the abdominal artery and bleeding under deep ketamine/xylazine anesthesia. After kidney weight was measured, the organs were decapsulated. Kidneys were put in methyl-Carnoy’s solution (60% methanol, 30% chloroform and 10% glacial acetic acid) for fixation.

**Plasma renin activity.** Plasma renin activity was assessed by determination of the conversion of angiotensigen to angiotensin I. Angiotensin I was measured by radioimmunoassay after incubation at 37°C for 1 h (13).

**Salt depletion.** To test the effects of COX-2 blockade on a primarily macula densa-mediated stimulus, we studied salt depletion for comparison. Eight rats received a low-salt diet (0.06% NaCl), and four rats received a normal-salt diet (0.6% NaCl) for 10 days. At the same time, four of the low-salt-treated rats received −1 mg·kg^{-1}·day^{-1} of NS-398 via the drinking water. Plasma renin activity was also measured in these animals.

**Immunohistochemistry for renin and COX-2.** After overnight fixation in methyl-Carnoy’s solution, tissues were de-
hydrated by bathing in increasing concentrations of methanol, followed by 100% isopropanol. After being embedded in paraffin, 3-μm sections were cut with a Leitz SM 2000R microtome (Leica Instruments, Nussloch, Germany). Before any staining procedure was conducted, sections were deparaffinized and rehydrated, and endogenous peroxidase activity was blocked. Detection of renin and COX-2 was performed as described previously (6, 7). The Vectastain DAB kit (Vector Laboratories, Burlingame, CA) was used as a chromogen. The primary antibodies used were 1) polyclonal antiserum 8914 to detect rat renin (a generous gift from Walter Fischli, Actelion, Allschwil, Switzerland) in a dilution of 1:2,000 and 2) a polyclonal antibody to COX-2 (M-19, Santa Cruz Biotechnology, Heidelberg, Germany) in a dilution of 1:500. The specificity of the antibodies used was confirmed as described before (6).

Data analysis. Quantification of renin or COX-2 expression was performed by counting the number of glomeruli with an adjacent juxtaglomerular apparatus staining positive for COX-2 or renin, respectively (see Fig. 4). In each kidney, 100–200 glomeruli were counted, and the number of positive glomeruli was expressed as a percentage of the total number of glomeruli counted. These percent values were used for statistical analysis. Two-way analysis of variance, followed by a post hoc Newman-Keuls test, was used to test the significance of differences between groups. A P value < 0.05 was considered significant. Procedures were carried out using SPSS software (release 9.01, SPSS, Chicago, IL). Values are displayed as means ± SE.

RESULTS

Mean arterial pressure was significantly increased by renal artery clipping in both sets of experiments as measured 4 wk after placement of the clip (Fig. 1, A and B). This increase could be detected in vehicle-treated and COX-2 inhibitor-treated rats alike. Administration of NS-398 (estimated dose: 1 mg·kg⁻¹·day⁻¹) had no effect on mean arterial pressure, either in sham-operated or in 2K1C animals (Fig. 1 A). Treatment with rofecoxib even led to a slight elevation of mean arterial blood pressure, which did not reach statistical significance (Fig. 1 B).

Similar effects were observed with regard to plasma renin activity: 2K1C increased plasma renin activity significantly in both sets of experiments (Fig. 2, A and B). Administration of the COX-2 inhibitors NS-398 or rofecoxib had no significant effect on plasma renin

Fig. 3. Plasma renin activity (PRA) measured in samples from conscious rats after 10 days of a low-salt (0.08% NaCl) diet with or without treatment with NS-398 or a normal-salt (0.6% NaCl) diet. Values are means ± SE. *P < 0.05, NS-398-treated rats with a low-salt diet vs. vehicle-treated rats with a low-salt diet.

Fig. 4. Immunohistochemical localization of renin (A) or COX-2 (B) in clipped kidneys of 2K1C rats. Positive renin staining was observed in the media of afferent arterioles, while COX-2 was detected in cells of the macula densa. C: absence of renin immunoreactivity in most of the juxtaglomerular apparatus of contralateral kidneys.
activity, either in sham-operated or in 2K1C animals (Fig. 2, A and B).

As a control for the activity of the COX-2 inhibitor NS-398, plasma renin activity was determined in salt-depleted rats with and without treatment with NS-398. A low-salt diet for 10 days significantly increased plasma renin activity, which was partly reduced by treatment with the COX-2 inhibitor (Fig. 3).

The number of glomeruli with a renin-positive juxtaglomerular apparatus (Fig. 4A) was increased in clipped kidneys of 2K1C rats and decreased in contralateral nonclipped kidneys of 2K1C rats compared with sham-operated animals. Neither NS-398 nor rofecoxib had an effect on the number of glomeruli with a renin-positive juxtaglomerular apparatus (Fig. 5, A and B).

To rule out a potential effect of a COX-2 inhibitor on the abundance of COX-2 in the kidney, the number of glomeruli with COX-2-positive macula densa cells (Fig. 4B) was counted. As expected, COX-2 staining was increased in clipped kidneys compared with sham-operated rats. However, NS-398 had no significant effect on COX-2 immunoreactivity (Fig. 6).

**DISCUSSION**

Our results show that in established 2K1C hypertension, COX-2 blockade had no effect on the increase in blood pressure or on plasma renin activity. Moreover, NS-398 or rofecoxib did not alter the increase in renin immunoreactivity in clipped kidneys or the decrease in renin immunoreactivity in the contralateral kidneys of 2K1C hypertensive rats. This observation was surprising, because in a previous study we had shown a regulation of COX-2, which correlated with renin in this model (6). Evidence against a causal link between COX-2 and renin was also reported from a model of acute renal artery stenosis. Treatment with the COX-2 inhibitor celecoxib did not change renin expression in this model (14).

In another model of renovascular hypertension, however, the COX-2 inhibitor SC-58236 was found to decrease renin production and release (20). We do not know whether the differences between that study and our findings are due to the different models of renovascular hypertension or to the different drugs used. Although we did not directly assess blockade of renal COX-2 activity, both inhibitors used in our study were applied in concentrations that had been shown before to inhibit renal COX-2 activity (11, 12, 18). A dose of 1 mg·kg⁻¹·day⁻¹ NS-398 has been shown to reduce prostaglandin E₂ release from rat kidney by 20–30%, which is approximately the percentage that can be ascribed to COX-2 (18). A dose of 10 mg·kg⁻¹·day⁻¹ rofecoxib has been shown to abolish the diuretic-induced, COX-2-mediated increase in urinary prostaglandin E₂ and F₁α (11). On the other hand, SC-58236, which was given by Wang et al. (20) for 1 wk, has a half-life of ~5 days in rats (15). A dose of 10 mg/kg for 7 days results in a cumulative dose that might well reach the IC₅₀ of SC-58236 for COX-1 in plasma (17.8 nmol/ml). Whether or not the substance further accumulates in the kidney is unknown, but additional inhibition of COX-1 cannot be ruled out after the dosage given by Wang et al. (20).

Stimulation of plasma renin activity by a low-sodium diet, however, was attenuated by the COX-2 inhibitor

![Fig. 5. Quantification of kidney renin staining 4 wk after renal artery clipping or sham operation with or without COX-2 inhibition by NS-398 (A) or rofecoxib (B). The no. of glomeruli with a renin-positive juxtaglomerular apparatus is expressed as a percentage of all counted glomeruli. Values are means ± SE. There were no significant differences between COX-2 inhibition and solvent. *Significant differences between 2K1C (clipped or contralateral kidney, respectively) and sham operation, P < 0.05.](image1)

![Fig. 6. Quantification of kidney COX-2 staining 4 wk after renal artery clipping or sham operation. The no. of glomeruli with a COX-2-positive juxtaglomerular apparatus is expressed as a percentage of all counted glomeruli. Values are means ± SE. There were no significant differences between NS-398 and solvent.](image2)
NS-398, indicating that NS-398 in fact exerted a functional effect on COX-2 activity, leading to a partial blockade of the macula densa mechanism. These data agree with some results on the role of COX-2 in the regulation of renin. An inhibitory effect of COX-2 blockade with NS-398 on low-salt-induced renin secretion was shown in a model of isolated perfused juxta-glomerular apparatus (19). Furthermore, inhibition of low-salt-induced renin activity by NS-398 was demonstrated by Harding et al. (4). Similarly, rofecoxib attenuated the stimulation of renin by a low-salt diet in the same concentrations that were used in our study (12). NS-398 was also efficient in attenuating the stimulation of renin secretion in response to the loop diuretic bumetanide, which blocks macula densa salt transport (2). In accordance with this concept, renin activation by a low-salt diet was markedly attenuated in COX-2 knockout mice (22). When these findings are taken together, there is now good evidence for a role of COX-2-derived prostaglandins in the regulation of renin in response to salt load.

Other mechanisms could be implicated in the regulation of renal renin in response to renal artery stenosis. The expression of the neuronal nitric oxide synthase in the macula densa was regulated in parallel to renin in the juxtaglomerular apparatus after renal artery stenosis (1). Two other possible mechanisms involved in the regulation of renin stimulation are the baroreceptor mechanism and renal nerve activity. Interestingly, the latter seems to be required for stimulation of cortical neuronal nitric oxide synthase expression during salt deficiency but not for stimulation of renin or COX-2 expression in this model (8).

We speculate that the most likely explanation of our findings is that the macula densa mechanism for renin release is blocked to some degree by COX-2 inhibition but that this mechanism is not absolutely required for the regulation of renin in renovascular hypertension. Other mechanisms, in particular the baroreceptor mechanism, may mediate renin regulation under these conditions even if the function of the macula densa is impaired.

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