Cyclosporine A Suppresses Cyclooxygenase-2 Expression in the Rat Kidney

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Abstract. On the basis of recent evidence that the cyclooxygenase-2 (COX-2) gene promoter contains functional binding sites for the nuclear factor of activated T cells (NFAT) and that COX-2 is expressed in a regulated fashion in the kidney, this study aimed to assess the effect of immunosuppressants on COX-2 expression in the kidney. Therefore, Wistar-Kyoto rats were treated with cyclosporine A (CsA; 15 mg/kg per day) or tacrolimus (5 mg/kg per day) for 7 d each. Both drugs markedly lowered COX-2 expression while COX-1 expression remained unaltered. Furthermore, CsA blunted the increase of renocortical COX-2 expression in response to low salt intake or a combination of low-salt diet with the ACE inhibitor ramipril (10 mg/kg per day), which strongly stimulates renocortical COX-2 expression. At the same time, calcineurin inhibitors moderately enhanced basal as well as stimulated renin secretion and renin gene expression. These findings suggest that inhibition of calcineurin could be a crucial determinant for the regulated expression of COX-2 in the kidney. Inhibition of COX-2 expression may therefore at least in part account for the well-known adverse effects of immunosuppressants in the kidney. Moreover, our data suggest that the stimulation of the renin system by low salt and by ACE inhibitors is not essentially mediated by COX-2 activity.

The family of cyclooxygenases (COX) comprises two members, COX-1 and COX-2, which convert arachidonic acid into endoperoxides, which are the direct substrates for prostanooid formation (1). Whereas COX-1 is considered to be a widely distributed and constitutively expressed enzyme, COX-2 is regarded as the more inducible form, which, for example, plays a major role in inflammation (1). It turned out, however, that COX-2 is already constitutively expressed in some organs, including the kidney (2). In renal tissue, COX-2 is significantly expressed in glomeruli, in the thick ascending limbs of Henle (TALH), including the macula densa regions and in medullary interstitial cells (2,3). There is convincing evidence to suggest that COX-derived prostanooids could be involved in the regulation of renin synthesis and secretion in the juxtaglomerular apparatus, as well as in the tubular salt and water handling (4,5). The involvement of COX-derived prostanooids in the control of the renin system was made even more intriguing by the findings that the expression of COX-2 in the TALH and in macula densa cells is physiologically regulated by salt intake, renal perfusion pressure, and AngII (2,6–8), and this expression of COX-2 is strikingly paralleled by the expression of renin. However, the molecular signaling pathways triggering COX-2 expression in TALH cells and also in medullary interstitial cells in response to the above mentioned stimuli are only poorly understood. Preliminary evidence has been elaborated for possible involvements of the MAP-kinase and NF-kB pathways (9–11). In fact, the COX-2 gene promoter contains, apart from a classical TATA box, an E-box and a CRE-element, both representing binding sites for transcription factors such as NF-kB (12–15). A series of recent reports has provided evidence that the COX-2 promoter also contains binding sites for the nuclear factor of activated T cells (NFAT) (16–19) and that activation of this factor stimulates COX-2 expression in several cell lines (16–19). The activation of NFAT requires calcineurin phosphatase, which in turn is inhibited by immunosuppressants such as Cyclosporine (CsA) (20–22); it is therefore conceivable that immunosuppressive agents could also interfere with the regulated expression of COX-2 in vivo, particularly in the kidney. It is well known that the kidney is vulnerable toward adverse effects of CsA, including decrease of GFR, tubular dysfunction, glomerulosclerosis, and renal interstitial fibrosis (23,24). In view of the involving role of COX-2 for normal kidney function (25) and the demonstration of NFAT binding sites on its promotor, it appeared of interest to us to determine the effects of calcineurin inhibitors on renal COX-2 expression as well as on the presumably COX-2–dependent regulation of renin synthesis and renin secretion (25).

Using the calcineurin inhibitors CsA and tacrolimus, we found that both drugs selectively suppressed renal COX-2 expression without attenuating the regulation of the renin system.

Materials and Methods

Materials

CsA was purchased from Sigma (Deisenhofen, Germany). Tacrolimus was a gift from Fujisawa (Munich, Germany), and ramipril was from AstraZeneca (Mölndal, Sweden).
Animals

Male Wistar-Kyoto (WKY) rats (Charles River, Sulzfeld, Germany), initially 7 wk of age, were given vehicle, CsA (15 mg/kg body wt per day) or tacrolimus (5 mg/kg body wt per day) orally by a stomach tube and fed a normal-salt diet (0.6% NaCl, wt/wt; Altromin, Lage, Germany) for 1 wk. The dosage of the drugs has been chosen in accordance with previous studies in which acute nephrotoxicity of these drugs has been studied in rats (26–29). Additional groups of animals were treated with vehicle or CsA and received in addition either a low-salt diet (0.02% NaCl, wt/wt; Ssniff special diets, Soest, Germany) or a low-salt diet in combination with ramipril (10 mg/kg body wt per day) in the drinking water for 7 d. Body weight was monitored daily before drug administration. Rats were divided into groups consisting of eight rats and were killed by decapitation during anesthesia with sevoflurane (3% vol/vol). Blood was collected into EDTA tubes. The kidneys were quickly removed and were dissected into cortex, outer medulla, and inner medulla with a scalpel blade under a stereomicroscope, frozen in liquid nitrogen, and stored at −80°C until extraction of total RNA (30).

Ribonuclease Protection Assays for β-Actin, Renin, COX-1, and COX-2

β-Actin, renin, COX-1, and COX-2 mRNA levels were measured by specific RNase protection assays as described (8). In brief, cRNA probes (5 × 10⁵ cpm) were hybridized at 60°C overnight with 50 μg of total RNA (COX-1 and COX-2), 20 μg of total RNA (renin), 1 μg of total RNA (β-actin), and 20 μg of t-RNA (negative control). They 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 were separated on 8% polyacrylamide gel. The gel was dried for 2 h, and bands were quantitated in a Phosphoimager (Instant Imager 2024, Packard). Autoradiography was performed at −80°C for 1 to 3 d.

Real-Time PCR Analysis of TGF-β₁ mRNA

Total RNA of cortex and outer and inner medulla samples were reverse transcribed into cDNA (20 μl) according to standard protocols. In brief, cDNA probes were synthesized in a 20-μl reaction with 2 μg of total RNA, 0.5 μg of oligo(dT)₁₂₋₁₈, 20 U of RNasin (Promega, Madison, WI), 4 μl of 5× RT Buffer, 0.5 mM dNTP, and 20 U of M-MLV reverse transcriptase enzyme (Life Technologies, Gaithersburg, MD).

Real-time PCR was performed in a Light Cycler (Roche, Mannheim, Germany). All PCR experiments were done using the Light Cycler DNA Master SYBR Green I kit provided by Roche Molecular Biochemicals (Mannheim, Germany). Each reaction (20 μl) contained 2 μl of cDNA, 3.0 mM MgCl₂, 1 pmol of each primer (TGF-β₁ upstream primer: cgggatccatcgacatggagctggtga, downstream primer: ggaattcttgtcatagattgcgttg), and 2 μl of Fast Starter Mix (containing buffer, dNTPs, Sybr Green dye, and Taq polymerase). The amplification program consisted of 1 cycle of 95°C with 10-min hold (hot start) followed by 40 cycles of 15 s at 95°C, 5 s at 60°C, and 20 s at 72°C. Amplification was followed by melting curve analysis to verify the correctness of the amplicon. A negative control with water instead of

Figure 1. Influence of cyclosporine A (CsA) and tacrolimus on basal renal cyclooxygenase-1 (COX-1) and COX-2 gene expression. COX-1 mRNA (A) and COX-2 mRNA (B) abundance was highest in the rat inner medulla followed by outer medulla and renal cortex. CsA or tacrolimus decreased COX-2 mRNA (B) but not COX-1 mRNA (A) in kidney zones. Immunoreactive COX-2 (C) increased from renal cortex to outer and inner medulla. CsA or tacrolimus treatment decreased immunoreactive COX-2 (C) in all kidney zones. Zonal prostaglandin E₂ (PGE₂) formation (D) was highest in the rat inner medulla and was decreased by CsA or tacrolimus in all kidney zones (mean ± SEM; n = 8). Inset shows representative blots. *P < 0.05 compared with control.
cDNA was run with every PCR to assess specificity of the reaction. To verify the accuracy of the amplification, PCR products were further analyzed on ethidium bromide–stained 2% agarose gel. Analyses of data were performed using Light Cycler software version 3.5.3. Standard curves for TGF-β1 and β-actin were generated by using cDNA of rat cortex as template, which was diluted 1:5, 1:10, 1:50, 1:100, and 1:1000. For each sample, the ratio of the amount of TGF-β1 mRNA to that of the β-actin mRNA was calculated.

**Determination of Plasma Renin Activity (PRA) and of Renocortical PGE2 Concentration**

PRA was determined with a commercially available RIA (Sorin Biomedica, Düsseldorf, Germany). Concentration of tissue PGE2 was assayed by using a monoclonal EIA kit (Cayman Chemical, Ann Arbor, MI). In brief, tissues were homogenized with 10-fold ice-cold isotonic NaCl solution and centrifuged at 10000 × g for 10 min. The supernatant was used for the determination of PGE2 and for the determination of protein according to the method of Lowry.

**Immunoblotting for COX-1 and COX-2 Protein**

Ten micrograms for inner medulla and 100 μg for outer medulla and renal cortex of total protein were loaded per lane, separated by an 8% SDS-polyacrylamide gel and transferred onto a nitrocellulose membrane (Bio-Rad, Hercules, CA). Membranes were blocked overnight at 4°C and incubated for 2 h at room temperature with the primary antibody (COX-2 murine polyclonal AB [1:500], Cayman Chemicals; COX-1 murine polyclonal AB [1:500], Cayman Chemicals) and a horseradish peroxidase-labeled secondary antibody (goat anti-rabbit IgG [1:500], Santa Cruz Biotechnology, Ann Arbor, MI). Detection was achieved by enhanced chemiluminescence (Amerham). The band intensities were quantified densitometrically.

**COX-2 Immunoreactivity**

COX-2 immunoreactivity was demonstrated as described previously (31). In brief, sections were layered with the primary antibody (dilution, 1:500; M19; Santa Cruz Biotechnology) 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 streptavidin-D horseradish peroxidase complex (Vectastain DAB kit; Vector Laboratory, Burlingame, CA) and exposed to 0.1% diaminobenzidine tetrahydrochloride and 0.02% H2O2 as 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 second antibody).

Renin Immunoreactivity

For renin immunoreactivity, sections were layered with the primary antibody (dilution, 1:500; gift from Dr. C. Wagner, University of Regensburg, Regensburg, Germany) and incubated at 4°C overnight. After addition of the second antibody (dilution, 1:500; biotin-conjugated, chicken anti-goat IgG, ICN Pharmaceuticals, Frankfurt, Germany), 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 source of peroxidase substrate. Each slide was counterstained with hematoxylin-eosin.

Statistical Analyses

All values are presented as mean ± SEM. ANOVA and Bonferroni t tests were used for statistical analyses, and differences were considered significant when P < 0.05.

Results

Effect of CsA and Tacrolimus on COX Isoform Expression during Normal Salt Intake

Rats with normal salt intake were treated with vehicle, CsA (15 mg/kg per day) or tacrolimus (5 mg/kg per day) for 7 d, and the effects on the expression of COX-1 and COX-2 in the kidney were determined. Both COX-1 (Figure 1A) and COX-2 (Figure 1B) mRNA levels showed a clear gradient from the renal cortex to the inner medulla with ratios for COX-1 of 1:2.4:15.7 and for COX-2 of 1:2.2:4.6, respectively, in vehicle-treated animals. The ratio of COX-2 to COX-1 mRNA in these animals was 0.5, 0.5, and 0.2 for the renal cortex, outer medulla, and inner medulla, respectively. Basal COX-1 mRNA was not influenced by CsA or by tacrolimus in the rat renal cortex, the outer medulla, and the inner medulla (Figure 1A). In contrast, the levels of basal COX-2 mRNA of animals receiving either CsA or tacrolimus were markedly reduced (P < 0.05) in the renal cortex (to 15% and 12% of values found in vehicle-treated rats), in the outer medulla (to 14% and 9%), and in the inner medulla (to 8% and 7%) (Figure 1A). This decline of COX-2 mRNA abundance was paralleled by a decrease of COX-2 immunoreactive protein (P < 0.05). In animals receiving either CsA or tacrolimus COX-2 protein in the renal cortex fell to 20% and 25% of values found in vehicle-treated rats, to 17% and 14% in the outer medulla, and to 10% and 8% in the inner medulla (Figure 1C).

Similarly, renal tissue PGE2 levels fell (P < 0.05) in animals receiving either CsA or tacrolimus to 60% and 55% of vehicle controls in the cortex, to 68% and 65% in the outer medulla, and to 69% and 64% in the inner medulla (Figure 1D). Also COX-2 immunoreactivity in the TALH/macula densa structures decreased as well as in the medullary interstitial cells (Figure 2).

Effect of CsA on Renocortical COX-2 Expression during Low Salt Intake or a Combination of Low Salt Intake and ACE Inhibition

We found similar effects of CsA and tacrolimus on basal COX expression; we therefore used CsA only for subsequent

Figure 3. Effect of CsA on COX-2 mRNA abundance during low salt intake or low salt intake combined with ramipril treatment. Low-salt diet or low-salt diet and ramipril treatment did not affect renocortical COX-1 mRNA abundance (A) but increased renal cortical COX-2 mRNA (B) abundance. Additional treatment with CsA abolished the rise in COX-2 mRNA (B) without affecting COX-1 mRNA (A) abundance (mean ± SEM; n = 8). Renocortical PGE2 concentration (C) was decreased by CsA treatment. *P < 0.05 compared with control. †P < 0.05 compared with normal salt intake. ‡P < 0.05 compared with low salt intake.
studies in which COX-2 expression was prestimulated. Renocortical COX-1 expression was not influenced by a low-salt diet for 1 wk or by a combination of low-salt diet with ramipril. Additional treatment with CsA did also not affect renocortical COX-1 expression during low salt intake or during low salt intake in combination with ramipril treatment (Figure 3A). In contrast, low-salt diet increased renocortical COX-2 levels 1.8-fold (P < 0.05) (Figure 3B), whereas the combination of low-salt diet with the ACE inhibitor ramipril (10 mg/kg per day) increased COX-2 mRNA levels 6.6-fold (P < 0.05) (Figure 3B). The ratio of COX-2 to COX-1 mRNA in the renal cortex for these rats was 0.9 for the low-salt diet group and 3.5 for rats treated with low-salt diet and ramipril. In rats treated with CsA, COX-2 mRNA levels failed to increase with low-salt diet (P < 0.05) or the combination of low salt intake with ramipril (P < 0.05) (Figure 3B). Low-salt diet led to a small 1.3-fold (P > 0.05) increase, and the combination of low-salt diet with ramipril to a 1.8-fold (P < 0.05) increase in renocortical PGE₂ levels. Additional treatment with CsA lowered (P < 0.05) renocortical PGE₂ levels to 71% of the respective controls during low salt intake and to 32% (P < 0.05) during the combination of low salt intake and ramipril treatment (Figure 3C).

The data on COX-2 mRNA levels were paralleled by respective changes of COX-2 immunoreactivity in the TALH/macula densa structures (Figure 4). The percentage of glomeruli with adjacent COX-2 immunoreactivity increased from 6% during normal salt intake to about 13% (P < 0.05) during low salt intake and to about 45% (P < 0.05) during low-salt diet in combination with ramipril treatment. Additional treatment with CsA decreased (P < 0.05) the percentage of glomeruli with adjacent COX-2 immunoreactivity to about 2% for all treatment groups (Figure 5A). Renocortical ir-COX-1 protein was not affected by low-salt diet or a combination of low-salt diet with ramipril (Figure 5B) or by additional treatment with CsA. Renocortical ir-COX-2 protein increased to about 130% (P > 0.05) by low salt intake and increased to about 300% (P <
0.05) by low salt intake in combination with ramipril. CsA clearly decreased ir-COX-2 during low salt intake to 27% (P < 0.05) and during low salt intake in combination with ramipril treatment to 10% (P < 0.05) compared with the respective control groups (Figure 5C).

### Effect of CsA and Tacrolimus on Renin Synthesis, Secretion, and Immunoreactivity

CsA and tacrolimus moderately increased PRA from 6.4 ± 1.0 Angl/h × ml to 11.7 ± 1.9 (P < 0.05) and to 10.8 ± 1.6 (P < 0.05), respectively (Figure 6A). Renin mRNA levels were increased 1.7-fold (P < 0.05) by CsA treatment (Figure 6B) or 1.6-fold (P < 0.05) by tacrolimus treatment under basal conditions. Low-salt diet and low-salt diet in combination with the ACE inhibitor ramipril clearly increased PRA (1.8-fold [P < 0.05] and 7.5-fold [P < 0.05]) and renin mRNA levels (1.8-fold [P < 0.05] and 6.4-fold [P < 0.05]), respectively. The increase in PRA was enhanced by additional CsA treatment to about 200% (P < 0.05) during low salt intake and to

![Figure 5](image1.png)

**Figure 5.** Effect of CsA on COX-1 and COX-2 protein expression during low salt intake or low salt intake combined with ramipril treatment. COX-2 immunoreactivity related to macula densa structures (A). CsA treatment decreased irCOX-2 in macula densa of most glomeruli of rats kept on normal-salt diet, on low-salt diet, or on a combination of low-salt diet with the ACE inhibitor ramipril for 7 d. Renocortical ir-COX-1 (B) was not affected by any treatment maneuver. Renocortical ir-COX-2 (C) was decreased by CsA treatment in rats kept on normal-salt diet, on low-salt diet, or on a combination of low-salt diet with the ACE inhibitor ramipril for 7 d. (mean ± SEM; n = 8). *P < 0.05 compared with control; †P < 0.05 compared with normal salt intake; ‡P < 0.05 compared with low salt intake.

![Figure 6](image2.png)

**Figure 6.** Influence of CsA on the renin system. Low-salt diet or low-salt diet and ramipril treatment increased plasma renin activity (PRA) (A) and renal renin mRNA abundance (B). PRA and renin mRNA were increased by CsA during normal and low salt intake, but not by low salt intake when combined with ramipril (B) (mean ± SEM; n = 8). *P < 0.05 compared with control; †P < 0.05 compared with normal salt intake; ‡P < 0.05 compared with low salt intake.
about 126% ($P > 0.05$) during low salt intake in combination with ramipril treatment (Figure 6, A and B). Renin mRNA levels were also enhanced 1.6-fold ($P < 0.05$) during low salt intake and 1.2-fold ($P > 0.05$) during low salt intake in combination with ramipril by additional CsA treatment. The changes of renin mRNA levels were paralleled by respective changes of renin immunoreactivity in the juxtaglomerular regions (Figure 7).

**Effect of CsA on TGF-β1 mRNA Levels**

TGF-β1 mRNA also showed a clear gradient from the renal cortex to the outer and the inner medulla (1:4.5:10) (Figure 8A). Treatment with CsA for 1 wk resulted in a 1.6-fold ($P < 0.05$) increase of TGF-β1 mRNA in the rat renal cortex, in a twofold ($P < 0.05$) increase in the outer medulla, but not in the inner medulla (1.1-fold) (Figure 8A). Low-salt diet alone or the combination of low-salt diet with ramipril did not influence renocortical TGF-β1 mRNA levels (Figure 8B). Additional CsA treatment enhanced renocortical TGF-β1 mRNA 1.7-fold ($P < 0.05$) during low salt intake, but it failed to increase of TGF-β1 mRNA if low-salt diet was combined with ramipril treatment.

**Discussion**

The data obtained in this study clearly demonstrate that calcineurin inhibitors not only decrease basal COX-2 gene expression in the renal cortex and medulla of rats but also attenuate the well-established noninflammatory induction of renocortical COX-2 expression, particularly in the TALH/macula densa structures, by low-salt diet or AngII antagonists (2,7,8). Inhibition of COX-2 gene expression was associated by a decline of COX-2 protein expression as well as by a fall of tissue PGE2 levels. Moreover, this effect of calcineurin inhibitors...
compared with control. (B) (means ± SEM; n = 8) in the rat renal cortex. *P < 0.05 compared with control.

Figure 8. Influence of CsA on basal renal TGF-β_1_ mRNA levels. TGF-β_1_ mRNA levels were highest in the rat inner medulla followed by outer medulla and renal cortex (A). CsA increased TGF-β_1_ mRNA in the renal cortex and the outer medulla but not in the inner medulla (A). Low-salt diet or low-salt diet in combination with ramipril treatment did not affect renocortical TGF-β_1_ mRNA levels (B). TGF-β_1_ mRNA levels were increased by CsA during normal and low salt intake, but not by low salt intake when combined with ramipril (B) (means ± SEM; n = 8) in the rat renal cortex. *P < 0.05 compared with control.

Inhibitors was specific for COX-2, because the expression of COX-1 was not affected.

Due to their action on calcineurin phosphatase, CsA and tacrolimus are well-characterized inhibitors of the NFAT signaling pathway (32). This signaling pathway has previously been reported to be of major importance for the regulation of COX-2 gene expression in vitro (16–19). Although our in vitro data cannot prove such in vitro data, they are in excellent congruence with them. One may speculate therefrom that the NFAT-pathway could be also of major importance for the expression of COX-2 in the kidney. An inhibition of COX-2 expression and consequently an inhibition of prostanoid formation by calcineurin inhibitors also fits well with in vitro and in vivo data. In this context, it has been found that CsA decreases PGE_2_ formation in cultures of vascular smooth muscle cells (33) and that calcineurin inhibitors decrease the renal excretion of vasodilatory prostanooids, like PGE_2_ and prosta cyclin, in rats and humans (34,35).

COX-2–derived prostanoids are of importance for renal blood flow and salt excretion (5,25); it is therefore conceivable that the inhibition of COX-2 expression contributes to the well-known adverse effects of calcineurin inhibitors (23,24). The precise mechanism of CsA-induced nephrotoxicity is not well understood, but it has been mainly attributed to afferent arteriolar vasoconstriction leading to an alteration of renal hemodynamics (36). Several vasoactive mediators have been suggested to be involved in this vasoconstriction, including activation of the sympathetic nervous system (37) and the renin system (38), but also a decreased formation of vasodilatory agents like nitric oxide (36,37). However, it has been suggested that the imbalance of vasodilator to vasoconstrictor prostanooids is partially involved in afferent arteriolar vasoconstriction (36). In line, additional treatment with prostanooid-analogous has been shown to prevent at least in part acute CsA-induced nephrotoxicity (39–41). A comparison of the renal effects of selective COX-2 inhibitors with the renal effects of immunosuppressants, in fact, reveals a number of similarities. Both groups of drugs cause vasoconstriction of afferent arterioles, reduce GFR, and cause potassium retention and sodium retention (23,24,42–44). All together, it does not appear far-fetched to attribute those yet-unexplained renal effects of calcineurin inhibitors to the suppression of COX-2–derived PGE_2_ formation.

It is thought that TGF-β_1_ mediates part of the renal adverse effects of calcineurin inhibitors, such as the development of interstitial fibrosis (36). In accordance with previous reports (26–29), we found that CsA increased TGF-β_1_ gene expression and that this stimulation was abrogated by an ACE inhibitor (29). CsA suppressed COX-2 expression both in the absence and in the presence of an ACE inhibitor; TGF-β_1_ is therefore unlikely to mediate this particular renal effect of CsA. Because PGE_2_ exerts antifibrotic effects, inhibition of PGE_2_ by CsA may in turn additionally enhance TGF-β_1_–induced interstitial fibrosis and chronic calcineurin inhibitor nephrotoxicity, which has been mainly linked to an increased formation of TGF-β_1_ (36).

The physiologic role of COX-2 expressed in the TaLH, including the macula densa region, is still a matter of debate. Due to the close vicinity of macula densa cells to the renin-producing juxtaglomerular cells, it has been hypothesized that COX-2–derived prostanoids could be involved in the control of renin secretion and renin gene expression (25). This concept has been supported by data obtained from experiments with certain COX-2 blockers (7,45,46) and with COX-2 knockout mice (47,48). However, conflicting data have also been recently reported, raising some doubts on this concept, because commonly available COX-2 blockers do not consistently influence the control of renin synthesis and secretion (31,44,49).

Our data now show that in the absence of calcineurin inhibitors low-salt diet and a combination of low-salt diet with an ACE inhibitor increase renin secretion as well as renin- and COX-2 mRNA expression in parallel, which is in accordance with previous studies (7,8,25). In the presence of CsA, low-salt diet and the combination of low-salt diet and ACE inhibitor stimulates renin secretion and renin expression, whereas...
COX-2 expression remains blunted. Calcineurin inhibitors even moderately enhanced renin secretion and renin gene expression in rats during normal or low salt intake, which has already been reported by others (38,50).

We infer from these data that the stimulation of renin secretion and of renin gene expression by low salt intake or by a combination of low-salt diet with ACE inhibition are not causally linked to an increased COX-2 expression and probably not linked to COX activity at all. Thus, our data would support those studies that failed to demonstrate an effect of selective COX-2 blockers on the control of renin secretion and renin expression.

In conclusion, our findings provide evidence that calcineurin inhibitors markedly suppress renal COX-2 expression and COX-2–dependent prostanoid formation. This effect may contribute to the adverse renal effects of such drugs. Furthermore, we conclude from our findings, that low-salt diet or AngII-mediated stimulation of the renin system is not causally mediated by a stimulation of macula densa COX-2 activity.

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