Role of Renocortical Cyclooxygenase-2 for Renal Vascular Resistance and Macula Densa Control of Renin Secretion

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Abstract. This study aimed to assess the role of cyclooxygenase-2 (COX-2)-derived prostanoids for the macula densa control of renal afferent arteriolar resistance and for renin secretion. For this purpose, studied were the effects of blocking macula densa salt transport by the loop diuretic bumetanide (100 μM) on renal perfusate flow and on renin secretion in isolated perfused rats, in which renocortical COX-2 expression was prestimulated in vivo by treatment with the angiotensin-converting enzyme inhibitor ramipril, with low-salt diet, or with a combination of both. These maneuvers stimulated COX-2 expression in an order of ramipril + low salt > low salt > ramipril > controls. Flow rates through isolated kidneys at a constant pressure of 100 mmHg were dependent on the pretreatment regimen, in the way that they went in parallel with COX-2 expression. The COX-2 inhibitor NS-398 (10 μM) lowered flow rates depending on the COX-2 expression level and was most pronounced therefore after pretreatment with low salt + ramipril. NS-398 did not change the increase of flow in response to bumetanide but attenuated the stimulation of renin secretion in response to bumetanide in a manner depending on the expression level of COX-2. These findings suggest that in states of increased renocortical expression of COX-2, overall renal vascular resistance and the macula densa control of renin secretion become dependent on COX-2–derived prostanoids.

Cyclooxygenase-2 (COX-2) catalyzes the formation of endoperoxides, leading to prostanoids (1). Although COX-2 generally is considered as an inducible enzyme, it is also expressed constitutively in some organs, including the kidney (2). There COX-2 immunoreactivity has been localized to renal arterioles, glomerular podocytes, cells of the thick ascending limb of Henle (TALH), macula densa cells, and inner medullary collecting duct cells (3–6). Within the kidney cortex, in particular in the late TALH and macula densa region, COX-2 expression can be enhanced further by low-salt intake (4), by renal hypoperfusion (7,8), and by inhibitors of the renin angiotensin system (9,10). The physiologic meaning of the constitutive expression of COX-2 in the kidney and its upregulation in the macula densa region under the aforementioned conditions is not well understood. Selective COX-2 inhibitors exert few renal effects in normal beings (11–14). In humans and in dogs kept on low-salt intake, COX-2 blockers have been reported to decrease urinary salt and water excretion and to lower renal blood flow (15–17). The role of COX-2 for renin secretion is also less clear. One the one hand, it has been reported that COX-2 blockers abrogate macula densa–mediated stimulation of renin secretion in the rabbit in vitro (18) and attenuate the stimulation of renin secretion in vivo induced by angiotensin-converting enzyme (ACE) inhibition (10) or by aortic coarctation in the rat (8). On the other hand, COX-2 blockers were without effect on plasma renin activity in humans (19) and dogs on low-salt intake (16). We also found no effect of the COX-2 blocker celecoxib on the stimulation of plasma renin activity by renal artery clipping and by the angiotensin II-AT1 receptor antagonist candesartan (Mann B, Hocherl K, unpublished observations) in rats.

In view of these conflicting data, it was of interest to us to study the possible role of COX-2 for macula densa–mediated effects such as renal vascular resistance and renin secretion under more defined experimental conditions. For this purpose, we examined the effects of specific COX-2 inhibition on macula densa–related actions on renal vascular resistance and on renin secretion in in vitro perfused kidneys isolated from rats with different degrees of prestimulated renocortical COX-2 expression. To stimulate COX-2 expression in the macula densa region, we used a low-salt diet, treatment with an ACE inhibitor, or a combination of both. The combination pretreatment regimen, which caused the strongest induction of renocortical COX-2 expression, was associated with a striking decrease in renovascular resistance in vitro. Only in this group of kidneys did selective COX-2 inhibition cause a significant increase of renovascular resistance and an attenuation of macula densa–mediated stimulation of renin secretion.

Materials and Methods

Pretreatment of Animals

Male Sprague-Dawley rats (250 to 300 g) were used for the experiments. Before the kidneys were isolated for ex vivo perfusion, the rats were subjected to four pretreatment protocols that lasted for 1...
Kidney perfusion was performed in a recycling system (20). In brief, the animals were anesthetized with 150 mg/kg 5-ethyl-(1'-methyl-propyl)-2-thiobarbituric acid (Inactin; Byk Gulden, Konstanz, Germany). Volume loss during the preparation was substituted by intermittent injections of physiologic saline via a catheter inserted into the jugular vein. After the abdominal cavity was opened by a mid-line incision, the right kidney was exposed and placed in a thermostated (37°C) reservoir, consisted of a modified Krebs medium (200 to 220 ml). The basic perfusion medium, which was taken from the renal venous effluent via a metal cannula back into a reservoir (Transducer P 10 EZ), and the pressure signal was used for feedback control of a peristaltic pump. The perfusion circuit was closed by a double-barreled cannula was inserted into the abdominal aorta and placed close to the origin of the right renal artery. After ligation of the aorta proximal to the right renal artery, the aortic clamp was removed quickly and perfusion was started in situ with an initial flow rate of 8 ml/min. The right kidney was excised, and perfusion at constant pressure (100 mmHg) was established. The renal artery pressure was monitored through the inner part of the perfusion cannula (Statham pressure), and the pressure signal was used for feedback control of a peristaltic pump. The perfusion circuit was closed by draining the venous effluent via a metal cannula back into a reservoir (200 to 220 ml). The basic perfusion medium, which was taken from a thermostated (37°C) reservoir, consisted of a modified Krebs-Henseleit solution containing all physiologic amino acids in concentrations between 0.2 and 2.0 mmol/L, 8.7 mmol/L glucose, 0.3 mmol/L pyruvate, 2.0 mmol/L l-lactate, 1.0 mmol/L α-ketoglutarate, 1.0 mmol/L l-malate, and 6.0 mmol/L urea. The perfusate was supplemented with 60 g/L bovine serum albumin, 10 ml/U vasoressin, l-lysine, and freshly washed human red blood cells (10% hematocrit). Ampicillin (3 mg/100 ml) and floxacillin (3 mg/100 ml) were added to inhibit possible bacterial growth in the medium. To improve the functional preservation of the preparation, the perfusate was dialyzed continuously against a 25-fold volume of the same composition but lacking erythrocytes and albumin. For oxygenation of the perfusion medium, the dialysate was gassed with a 95% oxygen, 5% carbon dioxide mixture. Under these conditions, both glomerular filtration and filtration fraction remain stable for at least 90 min at values of approximately 1 ml/min × g and 7%, respectively (21). Perfusion flow rates were obtained from the revolutions of the peristaltic pump, which was calibrated before and after each experiment. Renal flow rate and perfusion pressure were monitored continuously by a potentiometric recorder. After reperfusion loop was established, perfusate flow rates usually stabilized within 15 min. Stock solutions of the drugs to be tested were dissolved in freshly prepared perfusate and infused into the arterial limb of the perfusion circuit directly before the kidneys at 3% of the rate of perfusate flow.

For determination of perfusate renin activity, aliquots (approximately 0.1 ml) were drawn from the arterial limb of the circulation and the renal venous effluent, respectively. The samples were centrifuged at 1500 × g for 15 min, and the supernatants were stored at −20°C until assayed for renin activity. Samples for the determination of renin activity were taken in 3-min intervals. Renin secretion rates were calculated from the arteriovenous differences of renin activity and the perfusate flow rate. The perfusate samples were incubated for 1.5 h at 37°C with plasma from bilaterally nephrectomized male rats as renin substrate. The generated angiotensin I was determined by RIA (Sorin, Biomedica, Düsseldorf, Germany).

For demonstration of sufficient blockade of prostanoid formation, urinary prostaglandin E$_2$ (PGE$_2$) excretion was determined. To this end, urine was collected for two 5-min periods during the control period as well as after the administration of the COX inhibitors. Urine samples were stored on ice, and PGE$_2$ concentrations were determined by PGE$_2$ monoclonal enzyme immunoassay (Cayman Chemical, Ann Arbor, MI) immediately after the end of each experiment. PGE$_2$ excretion was calculated from the urine flow and the PGE$_2$ concentration.

**Isolated Perfused Rat Kidney**

Kidney perfusion was performed in a recycling system (20). In brief, the animals were anesthetized with 150 mg/kg 5-ethyl-(1'-methyl-propyl)-2-thiobarbituric acid (Inactin; Byk Gulden, Konstanz, Germany). Volume loss during the preparation was substituted by intermittent injections of physiologic saline via a catheter inserted into the jugular vein. After the abdominal cavity was opened by a mid-line incision, the right kidney was exposed and placed in a thermostated (37°C) reservoir, consisted of a modified Krebs-Henseleit solution containing all physiologic amino acids in concentrations between 0.2 and 2.0 mmol/L, 8.7 mmol/L glucose, 0.3 mmol/L pyruvate, 2.0 mmol/L l-lactate, 1.0 mmol/L α-ketoglutarate, 1.0 mmol/L l-malate, and 6.0 mmol/L urea. The perfusate was supplemented with 60 g/L bovine serum albumin, 10 ml/U vasoressin, l-lysine, and freshly washed human red blood cells (10% hematocrit). Ampicillin (3 mg/100 ml) and floxacillin (3 mg/100 ml) were added to inhibit possible bacterial growth in the medium. To improve the functional preservation of the preparation, the perfusate was dialyzed continuously against a 25-fold volume of the same composition but lacking erythrocytes and albumin. For oxygenation of the perfusion medium, the dialysate was gassed with a 95% oxygen, 5% carbon dioxide mixture. Under these conditions, both glomerular filtration and filtration fraction remain stable for at least 90 min at values of approximately 1 ml/min × g and 7%, respectively (21). Perfusion flow rates were obtained from the revolutions of the peristaltic pump, which was calibrated before and after each experiment. Renal flow rate and perfusion pressure were monitored continuously by a potentiometric recorder. After reperfusion loop was established, perfusate flow rates usually stabilized within 15 min. Stock solutions of the drugs to be tested were dissolved in freshly prepared perfusate and infused into the arterial limb of the perfusion circuit directly before the kidneys at 3% of the rate of perfusate flow.

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**Determination of COX-2 Immunoreactivity**

The left kidneys were also removed and cut into two longitudinal halves. One half was fixed and processed further for COX-2 immunoreactivity as described previously (9).

**Determination of COX-2 mRNA, Renin mRNA, and β-actin mRNA in Kidney Cortex**

Cortex slices from the second half of the kidneys were snap-frozen in liquid nitrogen and stored at −80°C until isolation of total RNA, which was performed according to the method by Chomczynski and Sacchi (22). The abundance of COX-2 mRNA, renin mRNA, and β-actin mRNA was determined by specific RNase protection as described previously by us (9).

**Determination of COX-2 mRNA, Renin mRNA, Brain-Type Nitric Oxide Synthase mRNA, and β-Actin mRNA in Afferent Arterioles**

Cortex slices of the second half of the kidney were transferred to solution 1 (5 mM KCl, 2 mM CaCl$_2$, 130 mM NaCl, 10 mM glucose, 20 mM sucrose, 10 mM Tris [pH 7.4]) and microdissected immediately. The cortex was removed and thoroughly minced with a sharp scalpel for 2 min. After a 20- to 25-min digestion in 25 ml of solution 1 containing 0.1% collagenase A (Boehringer Mannheim, Mannheim, Germany) and 0.1% bovine serum albumin (Boehringer Mannheim) at 37°C, the preparation was centrifuged (3 min for 2500 × g) and washed two times with solution 1 to remove collagenase. Afferent arterioles with attached glomeruli were collected under a Zeiss binocular zoom microscope (Oberkochel, Germany) with a 10-μl pipette. Batches of 100 arterioles were pooled and total RNA was isolated using the Qiagen RNaseasy Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Isolated total RNA was dissolved in 30 μl of diethylpyrocarbonate-treated water. Total RNA content was determined photometrically.

Fifty ng of total RNA were reverse-transcribed using 200 U of Moloney murine leukemia virus–reverse transcriptase (Life Technologies/BRL, Eggenstein, Germany) according to standard protocols. To allow the quantification of COX-2 mRNA, renin mRNA, nitric oxide synthase-1 mRNA, and β-actin mRNA from one cDNA sample, we used oligo dT (12–18) for priming the reverse transcription.

Primers for PCR were designed according to the published sequences in the European molecular biology laboratory GenBank. Primers used for amplification of COX-2 (accession no. L20085) were as follows: 5′gctgctgagaagggagtgt3′(forward) and 5′ggttgactgtgtctcaca3′(reverse) for renin (accession no. X07033), 5′aagtctctgccagccg3′(forward) and 5′ttacacaccttgctga3′(reverse) for brain-type nitric oxide synthase (bNOS; accession no. X59949), 5′gaatccagctcagctc3′(forward) and 5′tggagcggtgccagc3′(reverse) and for β-actin (accession no. U01217),
and 5’ccaactgggacgacatgg3’ (forward) and 5’tggcgtgagggagagcat3’ (reverse). Twenty-three cycles were performed for
β-actin, 27 for renin, 28 for COX-2, and 30 for bNOS according to the following protocol:
denaturation at 94°C for 30 s, annealing at 60°C for 60 s, and extension
at 72°C for 30 s. After PCR, the amplification products were separated by
agarose gel electrophoresis.

Determination of Renal Renin Content
Renal renin content was determined according to a modified
method described by Norling et al. (23). In brief, 100 mg of cortex
tissue were homogenized in 1 ml of ice-cold homogenization solution
(5% glycerol [vol/vol], 0.1 mM phenylmethylsulfonyl fluoride, 10
mM ethylenediaminetetraacetic acid, 0.1 mM 4-(2-aminoethyl)benzene-
sulfonyl fluoride) for 20 s (Ultra-Turrax T25; IKA Labortechnik,
Staufen, Germany). After homogenization, samples were centrifuged
for 3 min at 4°C at 14000 g. Aliquots of the supernatant were
incubated for 1.5 h at 37°C with plasma from bilaterally nephrecto-
mized male rats as renin substrate. The generated angiotensin I was
determined by RIA (Sorin, Biomedica).

Statistical Analyses
For evaluation of significance of a certain experimental maneuver
on renin secretion from isolated kidneys, all renin secretion rates
obtained within this experimental period (four values) were averaged
and compared with the average values of renin secretion of an ad-
jointing experimental period. Paired t test was used to calculate levels
of significance within individual kidneys. P < 0.05 was considered
significant. For comparisons between animals, ANOVA with Bonfer-
noni’s reductions for multiple comparisons was used. P < 0.05 was
considered significant.

Results
Pretreatment of the rats in vivo with a low-salt diet, with the
ACE inhibitor ramipril (10 mg/kg per d), or with a combination
of both for 1 wk led to, respectively, 60%, 80%, and 230%
increases of renocortical COX-2 mRNA levels (Figure 1A) and
to parallel increases of COX-2 immunoreactivity in the macula
densa region (Figure 1B). Even under the strongest stimulation
of COX-2 expression by pretreatment with low salt + ramipril,
detectable COX-2 immunoreactivity in the renal cortex re-
mained restricted to the late TALH, including the macula densa
regions (Figure 2). All maneuvers also increased renin mRNA,
however, not in proportion with renocortical COX-2 expres-
sion (Figure 1C). Renocortical renin contents increased moder-
ately from 1.92 ± 0.11 μg of angiotensin I/g (no pretreat-
ment) to 2.71 ± 0.38 (ramipril), 2.60 ± 0.29 (low salt), 2.65 ±
0.28 (low salt + ramipril). Systolic BP fell from 120 ± 7
mmHg in animals without pretreatment to 98 ± 4 mmHg in
ramipril-treated rats and to 89 ± 5 mmHg in animals that
received the combination of both low salt and ramipril. Low salt alone did not change BP (123 ± 8 mmHg).

Kidneys were isolated from the animals and perfused in vitro at a constant pressure of 100 mmHg without further addition to the perfusate (□, C) or with addition of bumetanide (■, 100 μM) or the COX-2 inhibitor NS-398 (□, 10 μM). Flow rates were determined in three consecutive 12-min periods with sampling every 3 min. Data are means ± SEM of six kidneys in each pretreatment group. * P < 0.05 between consecutive periods.

determined the effect of the L-type calcium channel blocker amlodipine on perfusate flow rates in some of these kidneys. As shown in the insert in Figure 4, amlodipine abolished the autoregulation of flow in the kidneys from animals that were pretreated with low salt + ramipril, indicating that the arterial vascular bed was not maximally dilated in those kidneys in the absence of amlodipine. This conclusion was supported further by the observation that inhibition of macula densa salt transport by the loop diuretic bumetanide (100 μM) further increased perfusate flow rates in those kidneys (Figure 3). Also, in kidneys that were isolated from control rats or animals that were treated with low salt or ramipril, bumetanide increased flow rates, albeit more moderately than in kidneys that were taken from rats that received the combination of low salt with ramipril (Figure 3).

Basal renin secretion rates from the kidneys of all experimental groups were relatively low and were in the same range (Figure 5). The loop diuretic bumetanide stimulated renin secretion approximately two- to threefold in all kidneys. Those similar renin secretion rates in vitro were in harmony with the similar renin contents but were in marked contrast to the renin mRNA levels, which varied markedly among the different experimental groups (Figure 1C).

In parallel experiments to those shown in Figures 3 and 5, we examined the effects of the COX-2 selective blocker NS-398 (10 μM) (24) on perfusate flow and on renin secretion in

Figure 3. Per fusate flow rates through kidneys of the different pretreatment groups at a constant pressure of 100 mmHg without further addition to the perfusate (□, C) or with addition of bumetanide (■, 100 μM) or the COX-2 inhibitor NS-398 (□, 10 μM). Flow rates were determined in three consecutive 12-min periods with sampling every 3 min. Data are means ± SEM of six kidneys in each pretreatment group. * P < 0.05 between consecutive periods.

Figure 4. Perfusate flow–perfusion pressure relationship for kidneys isolated from the different pretreatment groups. Values are given as average values for the different pretreatment groups (four rats in each group). Insert shows a representative perfusate flow–perfusion pressure relationship before and after the addition of the L-type calcium channel blocker amlodipine (5 μM) for a kidney isolated from a rat that was pretreated with low salt + ramipril.
the isolated kidneys. NS-398 significantly lowered basal flow rates in kidneys from animals with low salt pre-treatment only (Figure 3). The pattern of response of flow to NS-398 in those kidneys varied from more moderate to strong vasoconstriction (Figure 3). The vasoconstrictions occurred promptly with a delay time of less than 1 min (Figure 6).

Bumetanide again increased flow rates in all of the kidneys of all experimental groups (Figure 3). In view of the marked reductions of flow in response to the COX-2 inhibitor, we wondered whether the renal preglomerular vessels themselves were also targets for the COX-2 inhibitor. In fact, we found that in microdissected afferent arterioles with attached glomeruli, the COX-2 gene transcript was expressed basally and increased after pretreatment with low salt + ramipril but not after pretreatment with low salt or ramipril alone (Figure 7).

bNOS mRNA as a marker for macula densa cells was not detected in the preparation, indicating that the vessel preparation was not contaminated with macula densa structures.

NS-398 moderately lowered basal renin secretion rates in isolated kidneys from low salt–treated and low salt + ramipril–treated rats but not in others (Figure 5). NS-398 had no obvious effect on the stimulation of renin secretion by bumetanide in kidneys from control rats, low salt–treated rats, and ramipril–treated rats but attenuated the effect of bumetanide on renin secretion in kidneys from low salt + ramipril–treated rats (Figure 5).

To confirm the significance of these effects of the COX-2 blocker in kidneys from low salt + ramipril–treated rats, we performed additional experiments in which both the effects of the COX-1 selective inhibitor valeryl salicylate (1 mM) and of the COX-2 selective blocker NS-398 were examined sequentially. Although valeryl salicylate reduced urinary PGE2 excretion by 46 ± 6% (mean ± SEM; n = 3) from 28.8 to 15.6 pg/min, it did not change perfusate flow rates or renin secretion rates in kidneys from low salt + ramipril–treated rats during perfusion with bumetanide, whereas NS-398 significantly decreased both parameters (Figure 8). NS-398 generally did not inhibit renin secretion in kidneys from low salt + ramipril–treated rats because it did not change the stimulation of renin secretion by the β-adrenoreceptor agonist isoproterenol (not shown).

Discussion

The goal of this study was to assess the possible role of COX-2 in mediating the well known influences of the macula densa on renal vascular resistance and on renin secretion (25). We analyzed this question in an isolated perfused rat kidney system using kidneys with different degrees of COX-2 expression in the macula densa region. In accordance with previous data, COX-2 expression in this study was increased with a low-salt diet (4) or with ACE inhibition (9,10). An almost overadditive stimulation of COX-2 expression was achieved by
combining a low-salt diet with ACE-inhibitor treatment (9,10). To block macula densa salt transport function, we used the loop diuretic bumetanide (26), which inactivates the tubuloglomerular feedback (27), resulting in a fall of renovascular resistance, and which stimulates renin secretion, as also seen in this study (28,29).

A striking observation in the course of our experiments was that kidneys taken from rats that were pretreated with low salt + ACE inhibitor displayed markedly lower in vitro vascular resistance than those taken from untreated rats or rats that received either low salt or the ACE inhibitor alone. Nonetheless, these kidneys also showed good autoregulation of perfusate flow in vitro, indicating that the low resistance was not due to an alteration of the myogenic response. The marked vasoconstrictor response of flow through these kidneys toward selective COX-2 inhibition suggests that COX-2–mediated prostaglandin formation contributes substantially to the low vascular resistance in these kidneys. This conclusion fits with the strongly increased COX-2 expression levels in the cortices of these kidneys. A more moderate increase of renocortical COX-2 expression induced by a low-salt diet was associated with less-pronounced effects on vascular resistance, and also in these kidneys COX-2 inhibition moderately reduced perfusate flow. Pretreatment of the animals with an ACE inhibitor apparently was without impact for renovascular resistance, although renocortical COX-2 mRNA levels were somewhat increased. Similarly, inhibition of COX-2 had no influence on renal perfusate flow from normal kidneys. These data are in good accordance with previous observations in humans (15), dogs (13,16,17), and rats (14), indicating that COX-2 inhibitors have no effect on renal blood flow at normal perfusion pressure in normal beings but lower renal blood flow significantly in states of sodium depletion.

The onset of action of the COX-2 inhibitor on renovascular resistance was fast. It seems likely from our findings that apart from TALH cells, blood vessels also express COX-2 and are therefore a rapidly accessible target for COX-2 inhibitors.

Figure 7. (A) Reverse transcription-PCR (RT-PCR) analysis of COX-2, brain-type nitric oxide synthase (bNOS), renin, and β-actin mRNA in microdissected afferent arterioles pools from three rats without specific pretreatment and from three rats that were pretreated with low salt + ramipril. (B) RT-PCR analysis of COX-2, renin, and β-actin mRNA in pools of microdissected afferent arterioles from two rats without specific pretreatment and from two rats each pretreated with low salt or ramipril.

Figure 8. Perfusate flow rates (A) and renin secretion rates (B) in kidneys of low salt + ramipril–pretreated rats at a constant pressure of 100 mmHg with subsequent addition of bumetanide (100 μM), valeryl salicylate (1 mM), and NS-398 (10 μM) to the perfusate. Flow rates and renin secretion rates were determined in five consecutive 12-min periods with sampling every 3 min. Data are means ± SEM of four kidneys. * P < 0.05 between consecutive periods.

COX-2 gene expression in preglomerular vessels also is inducible, as indicated by the upregulation of COX-2 mRNA in afferent arterioles of low salt + ACE inhibitor–treated rats. The protein levels of COX-2 in cells of the afferent arterioles of rats, however, must be markedly lower than in cells of the TALH, because we found COX-2 immunoreactivity in the renal cortex only in the TALH cells including the macula densa region. It should be noted in this context that COX-2 immunoreactivity has been demonstrated for human preglomerular vessels (6).

The absolute increase of flow by the loop diuretic bumetanide, which is thought to reflect inhibition of tubuloglomerular feedback, was in proportion to the basal flow rate. Despite the significant effects of COX-2 inhibition on basal flow in kidneys from low salt or low salt + ACE inhibitor–treated rats, however, COX-2 inhibition did not clearly attenuate the increase of perfusate flow by bumetanide, indicating that COX-2–related prostaglandins are not likely to be involved causally in the macula densa signaling regulating afferent arteriolar tone.

In previous studies, we and others demonstrated that each of the experimental maneuvers used in this study not only enhance renin mRNA but also increase plasma renin activity as an indicator of renin secretion in vivo (8,10). Our data now show that basal renin secretion rates from the isolated kidneys of all four pretreatment groups were similar, indicating that renin secretion in vivo in these animals was regulated by factors that were neutralized under conditions of the isolated perfused kidneys. Those factors likely compose the effect of angiotensin II in vivo, which is absent in the isolated kidney because of the lack of angiotensinogen in the system; a fall of BP in vivo, which is prevented by pressure-constant perfusion of the isolated kidney; increased sympathetic nerve activity in vivo, which plays no role in the isolated kidney, because renal nerves are dissected; and a yet unknown signal in vivo, which mediates the effect of oral salt intake on renin secretion. From our findings, we infer, therefore, that there is no preconditioning of renin secretion by low salt intake or by inhibition of angiotensin II formation at the cellular level in vitro, at least under our experimental conditions. This conclusion is corroborated further by the observations that not only basal secretion but also the stimulations of renin secretion by bumetanide and by the β-adrenoreceptor agonist isoproterenol in vitro were not dependent on the pretreatment regimen in vivo. Considering the marked differences of renocortical COX-2 expression in all kidneys therefore leads to the conclusion that COX-2–derived prostaglandins are of less relevance for general renin secretion, at least in vitro. This assumption is supported further by the findings that COX-2 inhibition did not attenuate clearly the stimulation of renin secretion by bumetanide in kidneys from untreated and low salt– or ACE inhibitor–treated rats, suggesting that in these kidneys COX-2–related prostaglandins are not likely to be involved causally in the macula densa signaling regulating renin secretion. In kidneys with the highest levels of renocortical COX-2 expression, COX-2 inhibition clearly attenuated bumetanide-stimulated renin secretion. This finding fits with the previous demonstration that COX-2 but not COX-1 inhibition abrogates macula densa–stimulated renin secretion in the rabbit in vitro (18).

We cannot provide a clear answer for the different sensitivity of bumetanide-stimulated renin secretion toward COX-2 inhibition between the different pretreatment regimens. Considering the semiquantification of COX-2 immunoreactivity in the juxtaglomerular macula densa region suggests that the macula densa signaling controlling renin secretion becomes significantly dependent on COX-2 activity only in states of a more generalized expression of COX-2 in the macula densa region.

Taken together, our data suggest that COX-2–derived prostaglandins likely are relevant for overall renovascular resistance. Our data do not indicate that they are essentially required for the macula densa–triggered dilation of afferent arterioles. Under conditions of moderate renocortical COX-2 expression, COX-2–derived prostaglandins also seem dispensable for the stimulation of renin secretion by the macula densa mechanism. Only in states of high renocortical COX-2 expression does macula densa–stimulated renin secretion become dependent on COX-2–derived prostaglandins in the rat.

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