Angiotensin II–Independent Upregulation of Cyclooxygenase-2 by Activation of the (Pro)Renin Receptor in Rat Renal Inner Medullary Cells

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Abstract—During renin–angiotensin system activation, cyclooxygenase-2 (COX-2)-derived prostaglandins attenuate the pressor and antinatriuretic effects of angiotensin II (AngII) in the renal medulla. The (pro)renin receptor (PRR) is abundantly expressed in the collecting ducts (CD) and its expression is augmented by AngII. PRR overexpression upregulates COX-2 via mitogen-activated kinases/extracellular regulated kinases 1/2 in renal tissues; however, it is not clear whether this effect occurs independently or in concert with AngII type 1 receptor (AT_1R) activation. We hypothesized that PRR activation stimulates COX-2 expression independently of AT_1R in primary cultures of rat renal inner medullary cells. The use of different cell-specific immunomarkers (aquaporin-2 for principal cells, anion exchanger type 1 for intercalated type-A cells, and tenascin C for interstitial cells) and costaining for AT_1R, COX-2, and PRR revealed that PRR and COX-2 were colocalized in intercalated and interstitial cells whereas principal cells did not express PRR or COX-2. In normal rat kidney sections, PRR and COX-2 were colocalized in intercalated and interstitial cells. In rat renal inner medullary cultured cells, treatment with AngII (100 nmol/L) increased COX-2 expression via AT_1R. In addition, AngII and rat recombinant prorenin (100 nmol/L) treatments increased extracellular regulated kinases 1/2 phosphorylation, independently. Importantly, rat recombinant prorenin upregulated COX-2 expression in the presence of AT_1R blockade. Inhibition of mitogen-activated kinases/extracellular regulated kinases 1/2 suppressed COX-2 upregulation mediated by either AngII or rat recombinant prorenin. Furthermore, PRR knockdown using PRR-short hairpin RNA blunted the rat recombinant prorenin-mediated upregulation of COX-2. These results indicate that COX-2 expression is upregulated by activation of either PRR or AT_1R via mitogen-activated kinases/extracellular regulated kinases 1/2 in rat renal inner medullary cells. (***Hypertension*****. 2013;61:443-449.)** ● **Online Data Supplement**

Key Words: cyclooxygenase-2 ■ (pro)renin receptor ■ collecting duct ■ MAPK ■ ERK1/2

The (pro)renin receptor (PRR) is a member of the renin–angiotensin system initially identified as an accessory protein that coprecipitates with the vacuolar H^+-proton ATPase.[1,2] PRR is expressed in the kidneys, particularly in mesangial cells,[3] podocytes,[4–6] and intercalated type-A cells of distal nephron segments.[2,7] PRR binds renin and prorenin with affinity in the nanomolar range,[8] which increases the catalytic activity of renin and activates prorenin by exposing its active site.[5] Cell surface binding of prorenin to PRR not only increases renin enzymatic activity but also triggers the intracellular phosphorylation of mitogen activated protein kinases/extracellular regulated kinases 1/2 (MAPK/ERK1/2).[5,9]

Activation of MAPK/ERK1/2 pathway upregulates cyclooxygenase-2 (COX-2) expression in cardiac tissues[10] and renal cortex.[11,12] In angiotensin II (AngII)-infused rats, augmentation of COX-2 expression and activity in isolated glomeruli is associated with the activation of MAPK/ERK1/2 pathways.[13] Transgenic rats overexpressing PRR have increased expression of COX-2 and phosphorylated-ERK1/2 (p-ERK1/2) in macula densa cells with normal renal AngII content, suggesting that PRR-mediated COX-2 upregulation may be independent of AngII.[12,14] In the renal inner medulla, prostaglandin E_2 (PGE_2) levels increase in response to AngII infusions which counteract the vasoconstrictor and antinatriuretic effects of AngII.[15] In COX-2 knockout mice chronically infused with AngII, the increases in PGE_2 levels in response to AngII are blunted, indicating that the response is mediated by COX-2.[15]

We recently reported that chronic AngII-infused Sprague-Dawley rats showed increased levels of PRR mRNA and its soluble form in the renal medulla and urine.[7] This model of experimental hypertension exhibits increased synthesis and secretion of renin and most importantly of prorenin by the principal cells of the collecting duct (CD), which is the natural agonist of PRR.[16–18] During AngII-dependent hypertension, enhanced renin enzymatic activity in renal

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inner medulla and urine supports a functional role of the PRR and soluble PRR for local generation of AngII.[16,17,18] However, the effects of PRR activation on COX-2 expression in the renal medulla remain unclear. To test the hypothesis that PRR activation enhances COX-2 expression independently of angiotensin II type 1 receptor (AT_1R) in primary cultured inner medullary (IM) cells, we examined the effects of AT_1R or PRR activation on the stimulation of ERK1/2 pathway and COX-2 expression. By knocking-down PRR using short hairpin RNA (shRNA) technology, we examined the direct role of PRR activation on COX-2 expression in rat renal IM cells.

Materials and Methods

Primary Cultures of Rat IM Cells

Long-term primary cultures of rat renal IM cells were prepared as described previously[17] (see expanded Methods in the online-only Data Supplement).

Immunocolocalization of PRR and COX-2

Methanol-fixed cultured rat IM cells were stained with specific antibodies (AT_1R, aquaporin-2 [AQP-2], COX-2, PRR, Anion exchanger-2, a marker for intercalated cells, and Tenascin C, a marker for interstitial cells) to evaluate cell type population. Non-specific cross-reaction between COX-2 and COX-1 antibody was ruled out by Western blot using a COX-2 protein standard. To further confirm our findings in the cultured cells, we performed immunofluorescence studies in rat kidney sections (3 μm) using anti-PRR and anti–COX-2 antibodies (see expanded Methods in the online-only Data Supplement).

Quantitative Real-Time Polymerase Chain Reaction

COX-2 mRNA expression levels in primary cultured IM cells were measured using TaqMan PCR system using the following primers: 5′- TCCGTAGAAGAACCTTTTCC -3′ (sense); 5′- GGAGTCTGGAACATTGTGAA -3′ (antisense), and 5′-6-FAM- GGAAATAAGGAGCTTCCTGA -BHQ1-3′ (fluorogenic probe). Data were normalized against β-actin mRNA as previously described.[17]

Western Blot Analysis

Protein quantification was performed using a rabbit COX-2 antibody (Cayman, Ann Arbor, MI), a mouse anti-phospho-p44/42 ERK1/2 antibody (Thr202/Tyr204), and a rabbit anti-total ERK antibody (Cell Signaling Technology, Beverly, MA). Densitometric analyses were performed by normalization against β-actin.

PRR Knockdown in Primary Cultures of Rat Renal IM Cells Using shRNA

IM cells were transfected with 1 μg of plasmids (pGeneClip hMGFP Vector) using Lipofectamine LTX (Invitrogen, Carlsbad, CA) for 36 hours. Efficiency of transfection was confirmed by green fluorescent protein detection (see expanded Methods in the online-only Data Supplement). Scramble shRNA sequence was used as a negative control.

Statistical Analyses

An average number of 6 to 8 independent observations were performed for each treatment. Data were evaluated by the Grubb test followed when appropriate by paired and unpaired Student *t* test or by 1-way ANOVA with Tukey post test. For mRNA and protein data, control levels were defined as 100%. Significance was defined as $P<0.05$. Results are expressed as mean±SEM. Nonsignificant difference is represent as NS.

Results

PRR and COX-2 are Coexpressed in Interstitial and Intercalated Cells

To examine the cell type specific localization of PRR and COX-2, primary cultured rat IM CD cells and paraffin embedded rat kidney sections (3 μm) were assessed by immunofluorescence using specific antibodies. As shown in Figure 1A–1I, in long-term primary rat IM CD culture cells (5 days), a subpopulation of cells was positive for AQP-2 a marker of principal cells (red; 1A) whereas they all expressed AT_1R on the cell membrane (red; 1B). In addition, few cells resulted positive for COX-2 (green; 1C) and PRR (green, 1D). Cells expressing COX-2 (green; 1E) were positive for AT_1R (red; 1E) but negative for AQP-2 (red; 1F), indicating that AT_1R colocalizes with COX-2 in interstitial cells (1E), as previously described.[19,20] AQP-2 positive cells (red) did not express Tenascin C (green; 1G), a well-known marker of interstitial cells; nor anion exchanger type 1 (red and AQP-2 in green; 1H), a marker of intercalated type-A cells. Interestingly, PRR (red) and COX-2 (green) were coexpressed by similar cell types (1I). In addition, in the rat kidney sections, PRR was found coexpressed in tubular intercalated type-A cells with anion exchanger type 1 (PRR in green and anion exchanger type 1 in red; 1J) and with COX-2 in the CD (PRR in red and COX-2 in green; 1N) indicating that COX-2 is immunoexpressed by the intercalated cells of the CD. Peroxidase reaction using a COX-2 antibody confirms this observation in inner medullary tissues (1K). Furthermore, COX-2 (red, 1O) and PRR (green, 1P) were colocalized in the interstitial cells of the rat kidney inner medulla (1Q, merged colors). In true consecutive rat kidney sections (1L, 1M) it was observed that PRR-positive expressing cells (green, 1L) were AQP-2–negative (red, 1M), indicating that PRR is not expressed by the principal cells. As described previously,[21] COX-2 and COX-1 colocalize in intercalated cells. Cells stained only for COX-1 were also observed, corresponding to principal cells as described[22] (see online-only Data Supplement). PRR and COX-2 were coexpressed in tubular (Figure 1M, arrowheads) and interstitial cells (Figure 1M, arrows) in rat kidney sections.

AngII Increases COX-2 Expression in Cultured Rat IM Cells Via AT_1R

The maximum response for COX-2 protein expression was obtained using a single dose of AngII (100 nmol/L) after 6 hours, whereas AngII content in the media was reduced ≈20% after 3 to 6 hours. No effects were observed on COX-1 protein levels (see online-only Data Supplement). AngII (100 nmol/L) increased COX-2 mRNA (AngII: 191±3 % versus vehicle: 100±4 %; $P<0.05$; n=8, Figure 2A) and protein levels (AngII: 170±9 % versus vehicle: 100±10 %; $P<0.05$; n=8, Figure 2B). These effects were blocked by 1 μmol/L candesartan (AngII+Cand: 103±5 % versus vehicle: 100±10 %; n=6, *P*=NS), indicating that AngII-dependent stimulation of COX-2 in IM cells is mediated by AT_1R activation.

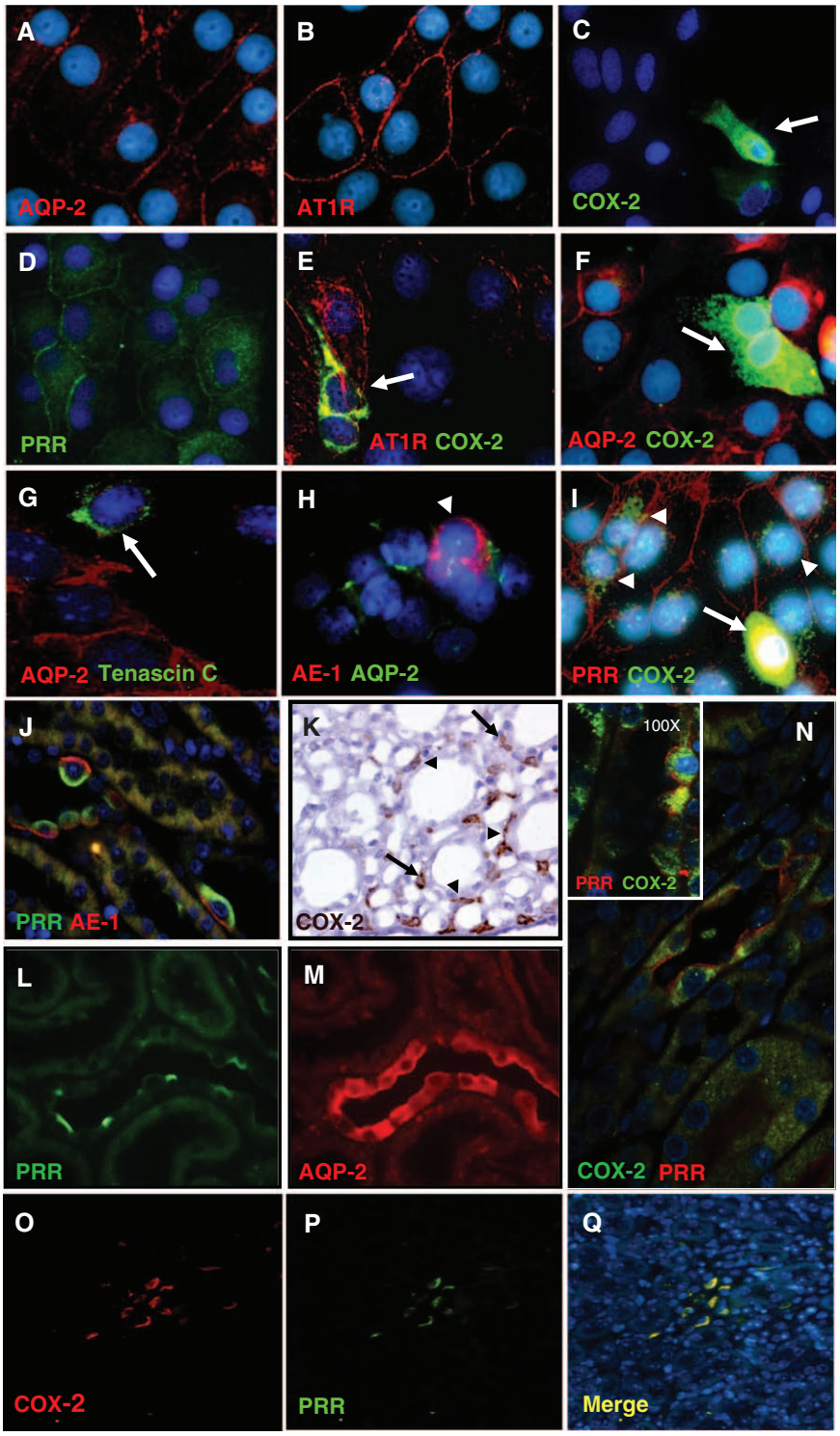

Figure 1. Characterization of long-term rat inner medullary (IM) cells (**A–I**). IM cells show specific immunoexpression of AQP-2 (red; **A**), AT_1R (red; **B**), COX-2 (green; **C**), and PRR (green; **D**). AT_1R (red) colocalizes with COX-2 (green) in the same type of cells (**E**), indicating that interstitial cells (**arrows**) strongly stained for COX-2 (green) also coexpress AT_1R (red) in the plasma membrane, as previously described (**E**). Cells expressing AQP-2 (red; **F**), the principal cells, do not coexpress COX-2 (**arrow**, green; **F**). Tenascin C (**arrow**, green; **G**), a marker for interstitial cells, does not colocalize with AQP-2 (red; **E**). Likewise, anion exchanger type 1 (AE-1; red; **H**), a known immunomarker for intercalated type-A cells (**arrowheads**), does not colocalizes with AQP-2 (green; **H**). Evidence for the presence of PRR (red; **I**) colocalizing with COX-2 (**arrowheads**, green; **I**) is shown in **I**. Nuclei were counterstained with DAPI (4′,6-diamidino-2-phenylindole; blue). In addition, in normal rat kidney sections (3 μm) immunofluorescence studies demonstrate that PRR (apical green immunoreactivity) and AE-1 (basolateral red immunoreactivity) colocalize in tubular intercalated type-A cells (**J**). **K**, Specific immunoreactivity for COX-2 in the interstitial cells (**arrows**) and some tubular cells (**arrowheads**) in the rat inner renal medulla (brown chromogen, 3,3′-Diaminobenzidine DAB). In consecutive kidney sections (**L** and **M**), is evident that PRR (green; **L**) is immunoexpressed by negative AQP-2–expressing cells (red; **M**). **N**, Examples of the colocalization of PRR (red; cell membrane) with COX-2 (green; intracellular localization) in the intercalated cells of the collecting ducts. In addition, this panel contains a high resolution microphotograph (left upper corner, 100×, oil immersion) for clear details. The lower panels **O** to **Q** show microphotographs (20×magnification) of a rat kidney section stained using dual immunofluorescence for COX-2 (red; **O**) and PRR (green; **P**) counterstained with DAPI (blue fluorochrome) demonstrate that COX-2 and PRR also colocalized in interstitial cells (merge, **Q**). Images were visualized using a Nikon Eclipse 50i immunofluorescence microscope and microphotographs were captured using a digital camera Nikon DS-U2/L2. AT_1R indicates angiotensin II type 1 receptor; AQP-2, aquaporin; COX, cyclooxygenase-2; PRR, (pro)renin receptor.

COX-2 Expression Is Stimulated Independently by PRR and AT_1R via ERK1/2 Pathway in Rat IM Cells

To stimulate PRR in IM cells, we used a nanomolar dose of a rat recombinant prorenin (rrPR).[8] As shown in Figure 3A, AngII (100 nmol/L) and rrPR (100 nmol/L) treatments both independently increased p-ERK1/2 at 15 minutes (AngII: 185±12 %; rrPR 156±12 % versus vehicle: 100±13 %; n=6, $P<0.05$). Combined AngII and rrPR treatments caused a similar response at 15 minutes, without an additive effect (AngII+rrPR: 180±15 %; n=6, $P<0.05$). To test whether COX-2 expression was upregulated by PRR or AT_1R activation via ERK1/2 pathway, IM cells were treated with the ERK1/2 inhibitor UO126 (10 μmol/L). Treatment with rrPR plus candesartan at 1 μmol/L (AT_1R blocker) increased COX-2 protein levels (rrPR: 154±8 % versus vehicle: 100±5 %; n=6, $P<0.05$) to a similar extent observed in IM cells treated with AngII (AngII: 167±9 % versus vehicle: 100±5 %;

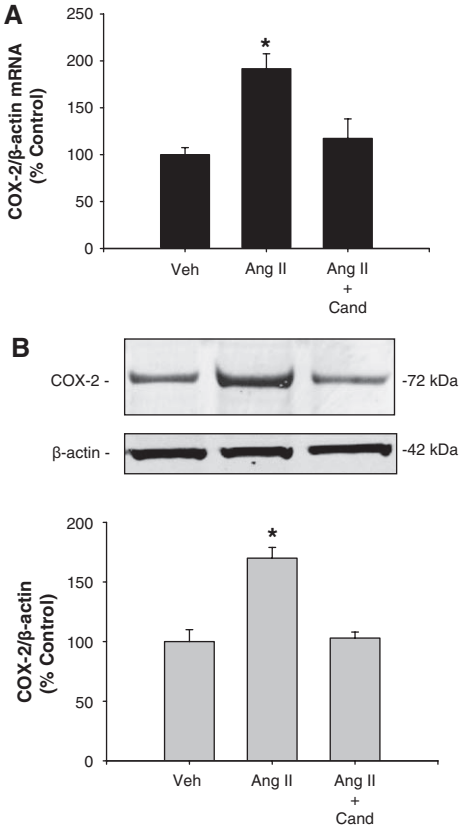

Figure 2. Cyclooxygenase-2 (COX-2) mRNA (**A**) and protein levels (**B**) levels were augmented after 6 h of angiotensin II (AngII) treatment; candesartan (Cand; 1 μmol/L) blocks this effect. n=6 to 8; **P*<0.05. Veh indicates vehicle.

n=6, *P*<0.05). Importantly, upregulation of COX-2 by rrPR or AngII treatments was prevented by ERK1/2 inhibitor UO126 (rrPR+UO126: 105±8 %; AngII+UO126: 112±3 %; n=6, *P*=NS; Figure 3B).

PRR Knockdown Suppresses the PRR-Mediated Increases in COX-2 Expression in Rat IM Cells

Target cells for transfections were interstitial and epithelial tubular cells, and PRR-shRNA reduced PRR protein expression compared with cells transfected with green fluorescent protein-scramble shRNA (47±4 % versus vehicle: 100±7 %; n=6, *P*<0.05; see online-only Data Supplement). Additional sets of cells were treated using the same dose of rrPR or AngII as in previous experiments and compared with cells transfected with PRR-shRNA. As shown in Figure 4A and 4B, rrPR treatment in the presence of candesartan increased COX-2 mRNA (160±7% versus vehicle: 100±4%; n=6, *P*<0.05) and protein (165±5% versus vehicle: 100±10%; n=6, *P*<0.05) levels. No additive effects were observed by combining AngII+rrPR (202±24% versus vehicle: 100±4%; n=6, *P*<0.05). AngII stimulation of COX-2 mRNA (191±3% versus vehicle: 100±4%; n=6, *P*<005) and protein (170±9% versus vehicle: 100±10%; n=6, *P*<0.05) levels were prevented by candesartan. Importantly, PRR-shRNA transfection of IM cells avoids the stimulatory effect of rrPR on COX-2 mRNA (84±10% versus vehicle: 100±4%; n=6, *P*=NS) and protein (108±5% versus vehicle: 100±10%; n=6, *P*<0.05) levels. PRR-shRNA did not affect the upregulation of COX-2 mediated by AngII (mRNA: 165±10%; n=6, *P*<0.05, protein: 158±10%; n=6, *P*<0.05).

Discussion

The present study demonstrates that COX-2 expression is upregulated in rat kidney IM cells by activation of either PRR or AT_1R via MAPK/ERK1/2 pathway. This study also shows that long-term primary cultured rat IM cells are composed of CD epithelial cells and interstitial cells, and most importantly the novel finding that COX-2 and PRR colocalize in interstitial and CD type-A intercalated cells.

Previous studies in rats and mice have shown that AngII stimulates renin and prorenin synthesis and secretion by the principal cells of the CD[16,17,23,24] despite the suppression exerted on juxtaglomerular renin.[18,25] In diabetic rats, the major source of prorenin synthesis and secretion are the principal cells of the CD.[25] We demonstrated that AngII treatment is able to stimulate mainly prorenin synthesis in freshly isolated rat IM cells,[17] and that the urines of AngII-dependent hypertensive rats possess abundant renin and prorenin.[18] PRR can be found in 3 different molecular forms: (1) the M8.9, which is complexed with the proton vacuolar H^+-proton ATPase[2,26]; (2) the 28-kDa soluble form of PRR, which can be detected in plasma[27] and urine[7]; and (3) the full-length form (37 kDa) located in the cell plasma membrane, which is able to trigger the phosphorylation of ERK1/2 after binding of renin or prorenin.[12,14,28]

Because of the localization of PRR in the mesangium and its ability to increase COX-2,[12,14] and profibrotic genes such as transforming growth factor β1, plasminogen activator inhibitor-1, collagen, and fibronectin,[3,28] PRR has been implicated in the pathogenesis of chronic kidney disease.[12,14,29] PRR has been implicated in the pathogenesis of chronic kidney disease.[12,14,29] PRR activation promotes inflammation, through the activation of MAPK/ERK1/2 signaling pathways.[12,14,30] Transgenic rats overexpressing PRR develop proteinuria and slowly progressive nephropathy.[12,14] This model also exhibits increased levels of p-ERK1/2 and COX-2 expression in the renal cortex with normal AngII content, suggesting that COX-2 upregulation is independent of AngII.[12,14] Furthermore, it has been shown that PRR overexpression in smooth muscle cells leads to elevated blood pressure and high plasma aldosterone levels, suggesting a role in circulating AngII formation.[30] The pathophysiological AngII-independent effects mediated by PRR may be relevant during conditions in which systemic AngII levels are suppressed or normal, such as occur in diabetic patients, in which high plasma prorenin levels, but not plasma renin activity, predict the onset of microvascular complications, and correlate with high COX-2 expression, cell proliferation (short-term activation), and fibrosis (long-term activation).[29–31]

COX-2 is mainly expressed in the interstitial cells,[32] macula densa,[33] and epithelial cells of the thick ascending limb[34]; however, it has also been described in the CD cells.[21,35] In the present study, we demonstrated that in normal rat kidney, COX-2 is detected in PRR-positive type-A intercalated cells. Importantly, we found that renal interstitial cells, which abundantly express COX-2, also express specific PRR immunoreactivity. Likewise, in rat renal cultured IM cells,

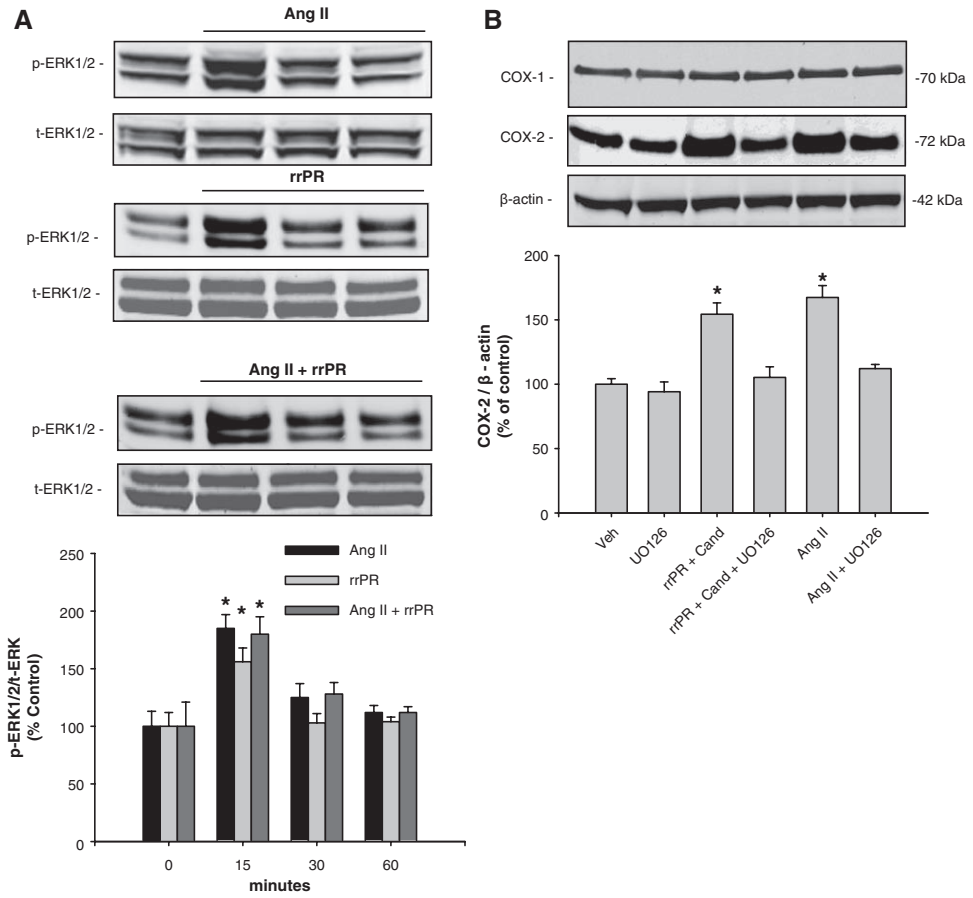

Figure 3. Cyclooxygenase-2 (COX-2) protein expression is upregulated by angiotensin II (AngII) and rat recombinant prorenin (rrPR) via ERK1/2 pathway. **A**, Representative Western blot showing phosphorylated extracellular regulated kinases 1/2 (p-ERK1/2) levels in response to AngII, rrPR, and AngII+rrPR treatment at 0, 15, 30, and 60 min. **P*<0.05 vs control (0 min, n=6). **B**, Representative Western blot showing that COX-2 protein levels are augmented by rrPR treatment in the presence of AT_1R antagonist to avoid the possibility of AngII formation in inner medullary cells. As shown before, AngII also increase COX-2 protein levels. The ERK1/2 inhibitor UO126 (10 μmol/L) blunted the effect of both treatments (**P*=NS vs vehicle; n=6). No effect was observed on COX-1 protein levels. Veh indicates vehicle; Cand, candesartan; NS, nonsignificant.

PRR, COX-2, and AT_1R colocalized in interstitial and type-A intercalated cells.

COX-2 plays a crucial role in regulating salt and water reabsorption and medullary blood flow,[36–38] and its role is known in counterbalancing the effects of AngII through PGE_2 production in medullary tissues.[15] In fact, COX-2 inhibitors cause sodium retention in human subjects with normal kidney function.[39] This effect has also been observed in experimental animals subjected to systemic or selective medullary COX-2 inhibition.[15,40] Salt loading downregulates COX-2 expression in renal cortex but upregulates its expression in the renal medulla;[40] however, the mechanisms implicated in these events remain unclear. Mineralocorticoid receptor agonism can induce COX-2 in vivo but not in cultured cells, suggesting that COX-2 upregulation is mediated by indirect pathways involving induced electrolyte hypertonicity in the interstitial fluid.[41] These data suggest a complex interaction between signaling pathways in the regulation of COX-2 expression in IM cells. This complexity may reflect a cell type–specific response, for example, to hypertonicity or high intrarenal AngII levels as observed in AngII-dependent hypertension.[17]

It has been shown that AngII increases glomerular PGE_2 production and COX-2 expression via ERK1/2 pathway and that these effects are prevented by the AT_1R blockade.[13] Because we previously showed the increased expression of PRR mRNA levels in the CD of AngII-infused rats,[7] we further examined whether PRR activation upregulates COX-2 independently of AngII. Treatment with rrPR in the presence of the AT_1R blocker candesartan to avoid intrinsic activation of AT_1R by possible endogenous AngII increased COX-2 and augmented p-ERK1/2 at 15 minutes. The same effect was observed by AngII, indicating that activation of both PRR and AT_1R contributed to the phosphorylation of ERK1/2. Lack of an additive effect observed with both treatments (AngII and rrPR) may be explained by the fact that rat renal IM cells were composed by a mixed population of cells, thus the COX-2 stimulation in interstitial and intercalated cell in response to AT_1R and PRR activation may differ. Further studies are needed to clarify this issue. This experimental evidence may suggest a key role of PRR in the light of recent evidence showing PRR upregulation by changes in dietary salt[42] implicating that PRR may play a role in renal sodium handling through the activation of intracellular ERK1/2 pathway in renal tubules.

Finally, to test whether PRR downregulation can alter COX-2 expression, we further knocked-down the PRR expression using shRNA technology. A 63% reduction in

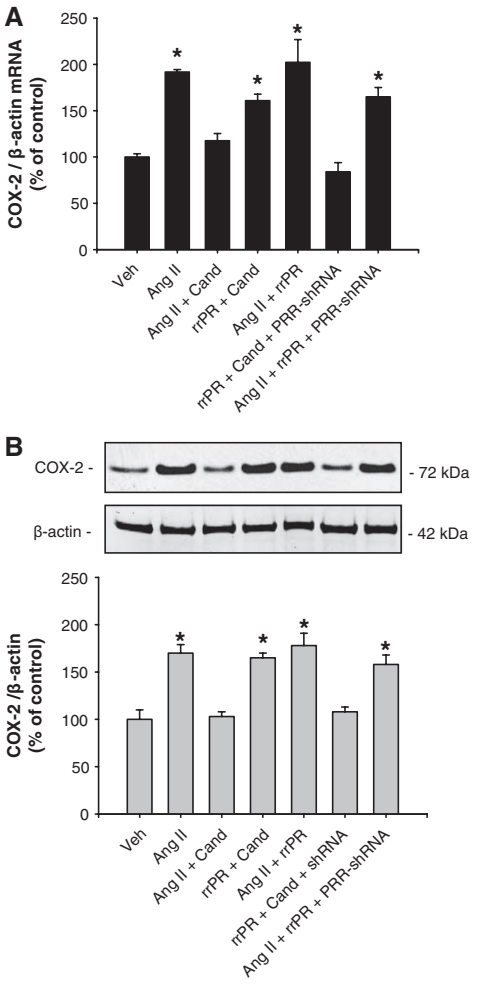

Figure 4. Prorenin receptor (PRR) knockdown suppressed the PRR-mediated upregulation of cyclooxygenase-2 (COX-2) in inner medullary (IM) cells. COX-2 mRNA (**A**) and protein (**B**) levels were augmented by angiotensin II (AngII, 100 nmol/L), and this effect was suppressed by AT_1R blockade with candesartan (Cand; 1 μmol/L). Rat recombinant prorenin (rrPR; 100 nmol/L) plus Cand also upregulates COX-2, demonstrating an independent effect. PRR-mediated upregulation of COX-2 was completely blunted in IM cells previously transfected with PRR-shRNA. **P*<0.05 vs vehicle; n=6. Veh indicates vehicle.

PRR protein expression prevented the upregulation of COX-2 mRNA and protein, supporting our hypothesis of an AngII-independent pathway for COX-2 regulation.

In summary, type-A CD intercalated cells and interstitial cells coexpress COX-2, PRR, and AT_1R. COX-2 expression is upregulated in rat renal IM cells through the independent activation of both PRR and AT_1R. These findings provide basis for the critical contributions of PRR and AT_1R in the regulation of COX-2 in the renal medulla.

Perspectives

Although previous studies in vivo have shown that AT_1R activation in the renal medulla led to COX-2-dependent PGE_2 synthesis, our data demonstrate that COX-2 can also be upregulated by PRR activation independently of AngII in rat IM cells. Most of the pathophysiological effects of PRR have been reported in renal cortical tissues, particularly in mesangial cells using transgenic models that overexpress PRR; however, the present study supports the notion that in the renal inner medulla the activation of PRR contributes to the stimulation of COX-2 via ERK1/2. These findings are of great relevance in the light of recent in vivo evidence demonstrating that during AngII-dependent hypertension there is stimulation of renin and prorenin synthesis and secretion by the CD cells[17,18] and upregulation of PRR transcript. Clearly, more studies are needed to carefully test whether during AngII-dependent hypertension, the activation of PRR in intercalated and interstitial cells by its natural agonists, contribute to buffer the local effects of AngII in the renal medulla by stimulating COX-2 and promoting the synthesis of vasodilator and natriuretic prostanoids.

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Disclosures

None.

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Novelty and Significance

What Is New?

- This study provides evidence for a new role of the prorenin receptor (PRR) in the regulation of cyclooxygenase-2 (COX-2) in the rat renal medulla via mitogen-activated protein kinase/extracellular regulated kinases. In addition, we provide evidence that the PRR and COX-2 are colocalized in the intercalated cells of the collecting duct and in the interstitial cells, which further supports our hypothesis.

What Is Relevant?

- Our findings are of critical importance because they support the notion that activation of PRR by upregulating COX-2 via extracellular regulated kinases 1/2 in the interstitial and intercalated cells may increase prostaglandins synthesis, thus contributing to buffer local vasoconstrictor and antinatriuretic effects of angiotensin II.

Summary

PRR and COX-2 are coexpressed in interstitial cells and intercalated collecting duct cells. Activation of PRR by recombinant prorenin upregulated COX-2 even in the presence of AT1 receptor blockade in rat primary cultured renal inner medullary cells. Upregulation of COX-2 by angiotensin II or prorenin was extracellular regulated kinases 1/2 signaling–dependent. PRR knockdown prevented COX-2 upregulation mediated by prorenin treatment in rat inner medullary cells. Upregulation of COX-2 in inner medullary cells is mediated by angiotensin II and by the angiotensin II–independent activation of PRR.