Renal localization and regulation of 15-hydroxyprostaglandin dehydrogenase

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Yao B, Xu J, Harris RC, Zhang M-Z. Renal localization and regulation of 15-hydroxyprostaglandin dehydrogenase. Am J Physiol Renal Physiol 294: F433–F439, 2008. First published December 5, 2007; doi:10.1152/ajprenal.00436.2007.—Tissue prostaglandin levels are determined by both biosynthesis and catabolism. The current studies report the expression and localization of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), a key enzyme in prostaglandin catabolism in the kidneys. We also investigated potential interactions between 15-PGDH and cyclooxygenase (COX), a key enzyme in prostaglandin biosynthesis. Both 15-PGDH mRNA and protein levels were significantly higher in kidney cortex than in papilla, which is opposite to the expression pattern of COX-2. In situ hybridization indicated that 15-PGDH mRNA was mainly localized to the tubular epithelial cells in kidney cortex and outer medulla but not in the glomerulus or papilla. Dual immunofluorescent staining indicated that 15-PGDH was expressed in the proximal tubule, cortical, and outer medullary thick ascending limb and collecting duct but not in the macula densa or papilla. 15-PGDH levels were significantly lower in a macula densa cell line (MMDD1) than in a proximal tubule cell line. Although a high-salt diet decreased COX-2 expression in macula densa, it increased macula densa 15-PGDH expression in both mouse and rat kidneys. In MMDD1 cells, a COX-2 inhibitor increased 15-PGDH, whereas a COX-1 inhibitor had no effect. Furthermore, intense 15-PGDH immunofluorescent staining was found in both macula densa and glomerulus in COX-2 knockout mice. The intrarenal distribution of 15-PGDH and its interactions with COX-2 suggest that differential regulation of COX-2 and 15-PGDH may play an important role in determining levels of prostaglandins involved in regulation of salt, volume, and blood pressure homeostasis.

Materials and methods

Cell culture. LLCPKc14 is an established proximal tubule epithelial cell line derived from pig kidney (1). MMDD1 is a renal epithelial cell line with properties of macula densa cells (kindly provided by J. Schnermann, National Institutes of Health, Bethesda, MD) (30). LLCPKc14 and MMDD1 cells were cultured in DMEM nutrient mixture/Ham’s F-12 (DMEM/F-12; Invitrogen) supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml) and incubated at 37°C in a humidified atmosphere of 95% air-5% CO2.

Animals. Male Sprague-Dawley rats were purchased from Harlan (150–200 g; Indianapolis, IN). Heterozygous breeding pairs with genetic deletion of the COX-2 gene maintained on a mixed B6/129 background were originally obtained from Jackson Laboratories (stock no. 002476; Bar Harbor, ME). Male rats and B6/129 mice (3 mo old) were maintained on normal rat chow, rat chow deficient in sodium (LS; 0.02–0.03% Na+), or rat chow with high salt (HS; 8% NaCl) for 2 wk (ICN Biochemicals, Costa Mesa, CA). Low salt-treated rats were given a single intraperitoneal injection of furosemide (1 mg/kg) before being placed on the low-salt diet. At the end of the experiments, one kidney was fixed in buffered formalin (10%) and then embedded in paraffin for light microscopy. The other kidney was separated into cortex and papilla for protein and RNA isolation. All animal study protocols were reviewed and approved by the Vanderbilt University Institutional Animal Care and Use Committee. All experiments were conducted according to National Institutes of Health guidelines.

Northern analysis. 15-PGDH mRNA expression in kidney cortex and papilla was determined by Northern analysis. Total RNA from male adult B6/129 mouse kidney cortex and papilla was isolated using TriReagent (Molecular Research Center), and concentrations were quantitated by absorbance at 260 nm with a Bio-Rad spectrophotom-
eter. Total RNA (15 μg) was electrophoresed on 1% formaldehyde-agarose gels, transferred to nylon membranes (Schleicher & Schuell), and probed with a 1.3-kb 32P-labeled cDNA Kpm/XhoI fragment of the 3'-untranslated region of rat 15-PGDH. Northern analysis was repeated three times from independent RNA isolations.

In situ hybridization. Digoxigenin (DIG)-labeled sense and antisense probes of rat 15-PGDH were synthesized with the DIG RNA labeling kit (Roche) from linearized plasmids containing a rat 15-PGDH fragment (600 bp in the coding region of 15-PGDH mRNA). After fixation in freshly prepared buffered formalin (10%), hemisectioned kidneys were cut into 5-μm sections and subjected to in situ hybridization. The DIG nucleic acid detection kit (Roche) was used to visualize hybridized probes according to the manufacturer's directions. Eosin was used for counter staining. The slides were examined via a microscope equipped with differential interference contrast optics.

Immunohistochemical/immunofluorescent staining. The animals were anesthetized with Nembutal (70 mg/kg ip), given heparin (1,000 U/kg ip) to minimize coagulation, and perfused with FPAS (3.7% formaldehyde, 10 mM sodium periodate, 40 mM phosphate buffer, and 1% acetic acid) through the aortic trunk, cannulated by means of the left ventricle. FPAS is an acidified aldehyde, which provides excellent preservation of tissue structure, antigenicity, and mRNA and is suitable for immunofluorescent staining with low background (34). The fixed kidney was dehydrated through a graded series of ethanol, embedded in paraffin, sectioned (4 μm), and mounted on glass slides. Immunostaining was carried out as described in previous reports (33). For immunofluorescent staining, deparaffinized sections were blocked with 10% normal donkey serum for 1 h and then incubated with primary antibodies overnight at 4°C. After being washed with PBS, the sections were incubated with Rhodamine red-X-conjugated donkey anti-rabbit IgG (red; Jackson ImmunoResearch Laboratories), washed with PBS, and then incubated with fluorescein Dolichos biflorus agglutinin (green; FITC-labeled DBA; a marker for collecting duct epithelial cells), fluorescein Lotus tetragonolobus agglutinin (green; FITC-labeled LTA; a marker for proximal tubule epithelial cells) (Vector Laboratories, Burlingame, CA), or FITC-conjugated donkey anti-sheep IgG (green; detecting Tamm-Horsfall protein (THP), a marker for thick ascending limb) (Santa Cruz Biotechnology). Sections were viewed and imaged with a Nikon TE300 fluorescence microscope and spot-cam digital camera (Diagnostic Instruments). The primary antibodies that were used for immunofluorescence studies included rabbit anti-rat 15-PGDH antibody (1:150, no. 160615; Cayman Chemicals), rabbit polyclonal anti-murine COX-2 antibody (1:2,000, no. 160106; Cayman Chemicals), and sheep anti-human THP (1:5,000, no. 8595-0054; Biogenesis).

Immunoblotting. Cultured cells or kidney samples were homogenized with RIPA buffer and centrifuged, and an aliquot was taken for protein measurement. When Western blot analysis was performed, each lane was loaded with the same amount of protein. The proteins were separated on SDS-PAGE under reducing conditions and transferred to Immobilon-P transfer membranes (Millipore). After blocking with 20 mM Tris-HCl, pH 7.4, 500 mM NaCl, 5% nonfat milk, and 0.1% Tween 20 for 3 h at room temperature, the blots were incubated overnight at 4°C with affinity-purified rabbit polyclonal anti-murine PGDH antibody (1 μg/ml), rabbit polyclonal anti-murine COX-2 (0.05 μg/ml), or monoclonal anti-β-actin antibody (0.25 μg/ml). The primary antibodies were detected with peroxidase-labeled goat anti-rabbit IgG or peroxidase-labeled sheep anti-mouse IgG (Santa Cruz Biotechnology) and exposed on film by using enhanced chemiluminescence (Amersham International).

RESULTS

15-PGDH expression in mouse kidney. Northern analysis and immunoblotting were used to investigate 15-PGDH mRNA and protein expression in mouse kidney. As indicated in Fig. 1A, 15-PGDH mRNA expression was significantly higher in cortex than in papilla. In immunoblots with an anti-15-PGDH antibody (Fig. 1B), loading 30 μg of protein extract from papilla produced a barely detectable band compared with the easily detectable band with 10 μg of protein from cortex, indicating substantially higher 15-PGDH protein expression in cortex than in papilla.

Localization of 15-PGDH mRNA and protein in rodent kidneys. In situ hybridization was carried out to determine the localization of 15-PGDH mRNA expression in mouse and rat kidneys. In mouse kidney cortex, 15-PGDH mRNA expression was primarily detected in the tubular epithelia but not in the macula densa or glomerulus (Fig. 2A). In normal adult rat kidney, 15-PGDH mRNA was also detected in the tubular epithelia in the cortex and outer medulla but was undetectable in glomerulus or papilla (Fig. 2, B–D).

Immunostaining indicated that in normal adult mouse kidney, 15-PGDH protein was primarily present in the tubular epithelia in the cortex and outer medulla and was minimally

![Fig. 1. 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) expression in the kidney. Both Northern blot analysis (A) and immunoblotting (B) demonstrated higher 15-PGDH expression in mouse kidney cortex than in inner medulla/papilla. Western blot analysis (C) indicated higher 15-PGDH expression in a cultured proximal tubule-like cell line (LLCPK1) than in a macula densa cell line (MMDD1). Each blot is representative of 3 separate experiments.](http://ajprenal.physiology.org/)

**A**

**Cortex**

**Papilla**

15-PGDH

18S

28S

**B**

**Cortex**

**Papilla**

15-PGDH

β-Actin

**C**

**Cortex**

**Papilla**

15-PGDH

β-Actin

100 30 10 30 (μg)

Protein:

15-PGDH

β-Actin

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detectable in the glomerulus and papilla, similar to the expression pattern of mRNA 15-PGDH protein expression (Fig. 3).

To characterize the tubule segments that express 15-PGDH, we performed double immunofluorescent staining. In the cortex, most 15-PGDH-positive cells were localized to LTA-positive cells, indicating 15-PGDH expression in the proximal tubules (Fig. 4A). Some 15-PGDH-positive tubular epithelial cells were also DBA positive in cortex (data not shown) and outer medulla (Fig. 4B), indicating 15-PGDH expression in the collecting ducts. In the cortex, thick ascending limbs (THP

Fig. 3. Localization of 15-PGDH protein in mouse kidney. A and B: in normal adult mouse kidney cortex, 15-PGDH was present in the tubular epithelia, including the PT, but not in G. C and D: intense 15-PGDH immunoreactivity was found in the tubular epithelial cells in outer medulla. E: 15-PGDH signal was found in outer medulla but not in inner medulla. F: no 15-PGDH signal was found in inner medulla. Original magnifications are as indicated.
positive) adjacent to macula densa had weak 15-PGDH signal, whereas thick ascending limbs far from macula densa had strong 15-PGDH signal (Fig. 4C). In the outer medulla, some tubules were positive for 15-PGDH and FITC-labeled Dolichos biflorus agglutinin (DBA; green), an indication of 15-PGDH expression in collecting duct (CD). Some 15-PGDH-positive cells were THP negative in the outer medulla (arrows). Therefore, 15-PGDH was expressed in the thick ascending limbs in cortex and outer medulla.

15-PGDH expression in cultured proximal tubule and macula densa cell lines. 15-PGDH expression was determined in the macula densa cell line MMDD1 (30). For comparison, expression was also determined in the cultured proximal tubule cell line LLCPKc14 (1). As indicated in Fig. 1C, 30 µg of MMDD1 protein produced a barely detectable immunoreactive band, whereas 7.5 µg of LLCPKc14 protein produced a substantial band. This result suggests that 15-PGDH expression may be higher in proximal tubule than in macula densa.

15-PGDH expression in the macula densa was regulated by dietary salt intake. In kidney cortex, prostaglandins derived from macula densa COX-2 play an important role in regulation of vascular tone and renin biosynthesis and excretion (11). In normal adult rat cortex, only isolated macula densa cells are COX-2 positive (12). Using immunofluorescent staining, we confirmed previous reports that after salt restriction, the number of COX-2-positive cells in the renal cortex increases significantly (Fig. 5A) (12). These COX-2-positive cells were localized to macula densa and adjacent cortical thick ascending limbs (arrows). Interestingly, all COX-2-positive cells in macula densa and adjacent cortical thick ascending limbs (arrows) were 15-PGDH negative (Fig. 5A, arrows). In the high salt-treated rat, macula densa COX-2 was undetectable (data not shown), whereas macula densa 15-PGDH expression was now evident (Fig. 5A). Macula densa 15-PGDH was not detectable in control wild-type mice but was evident after high-salt treat-
ment (Fig. 5B). Glomerulus 15-PGDH was minimal in rat and mouse kidneys and was not affected by alteration of dietary salt intake. Immunoblotting indicated that whole cortical 15-PGDH expression was similar in control, low salt-, and high salt-treated rat kidneys (Fig. 6A). Therefore, alterations of dietary salt intake may selectively regulate 15-PGDH expression in the macula densa and adjacent cortical thick ascending limbs, and an inverse relationship may exist between the expression of COX-2 and 15-PGDH in the macula densa.

15-PGDH was highly expressed in the macula densa and glomerulus in COX-2 knockout mouse kidney. To investigate further the interactions between kidney COX-2 and 15-PGDH in vivo, we studied COX-2 knockout mice. In contrast to absent 15-PGDH expression in macula densa and glomerulus in normal wild-type mice, an intense 15-PGDH signal was found in these sites in COX-2 knockout mice, whereas 15-PGDH immunoreactivity was similar in tubular epithelial cells in the cortex and outer medulla in COX-2 knockout mice and littermate controls (Fig. 5B). Immunoblotting showed that 15-PGDH expression in whole cortex was comparable between wild-type and COX-2 knockout mouse kidneys (Fig. 6B). Therefore, increased 15-PGDH expression in COX-2 knockout mice was restricted to macula densa and glomerulus. These results suggest that 15-PGDH expression in macula densa and glomerulus is tonically suppressed by COX-2 activity. In COX-2 knockout mice, 15-PGDH immunoreactivity was still undetectable in inner medulla/papilla (data not shown). We treated MMDD1 cells with either a selective COX-2 inhibitor, SC-58236 (100 nM), or a selective COX-1 inhibitor, SC-58560 (100 nM), for 24 h. As indicated in Fig. 6C, COX-2 inhibition led to increased 15-PGDH in MMDD1 cells. In contrast, COX-1 inhibition had no apparent effect on MMDD1 15-PGDH expression.

DISCUSSION

These studies examined the renal expression of 15-PGDH, a key enzyme in prostaglandin catabolism, and its potential interactions with COX, the key enzymatic step in prostaglandin biosynthesis. In the kidney cortex, COX-1 has been localized to arteries and arterioles, glomeruli, and collecting ducts but not to the proximal or distal convoluted tubules, Henle’s loop, or macula densa (22). In the outer medulla, COX-1 is expressed in the collecting duct epithelia. In the inner medulla/papilla, COX-1 is highly expressed in the collecting duct epithelial cells as well as medullary interstitial cells (12, 32). In the cortex, COX-2 has been reported to be expressed in the macula densa, cortical thick ascending limbs (12), podocytes (6, 15), mesangial cells (13, 25), and vasculature (26). In the medulla, COX-2 has been localized to medullary interstitial cells and collecting ducts of inner medulla/papilla (12, 31, 32). In the present studies, 15-PGDH was primarily localized to the proximal tubules (LTA positive) and thick ascending limbs (THP positive) and collecting duct (DBA positive) in the cortex and outer medulla. 15-PGDH expression was minimal in glomerulus, macula densa, and inner medulla/papilla in normal rodent kidney. The localization of 15-PGDH mRNA and protein in the current studies agrees with previous activity studies indi-
The mammalian kidney is a rich source of prostaglandins (9, 23). Although tissue prostaglandin levels are determined by both biosynthesis and degradation, the potential interaction of cyclooxygenase and 15-PGDH in the kidney has not been investigated to date. The role of 15-PGDH in tumorigenesis has recently received attention. Elevated COX-2 and COX-2-derived PGE2 levels are involved in tumorigenesis in a variety of tissues, and inhibition of COX-2 activity can suppress tumorigenesis (3). Recently, it has been reported that increased COX-2 activity and decreased 15-PGDH activity both contribute to increased COX-2-derived PGE2 levels in human breast cancer, colon cancer, and lung cancer, among others, and 15-PGDH has been implicated as an in vivo suppressor of tumorigenesis in these tissues (3, 17, 28, 29). In A549 human lung adenocarcinoma cells, cytokines induced COX-2 expression while simultaneously inhibiting 15-PGDH expression, and overexpression of COX-2, but not COX-1, led to decreased 15-PGDH expression in A549 cells. Conversely, overexpression of 15-PGDH attenuated interleukin-1β-induced COX-2 expression in A549 cells (24). Glucocorticoids are potent endogenous COX-2 inhibitors, and in A549 cells, dexamethasone induced 15-PGDH expression (24). Together, these data suggest that the expression of COX-2 and 15-PGDH may be reciprocally regulated.

Interactions between COX-2 and 15-PGDH are of potential importance in kidney function. In kidney cortex, COX-2-derived prostaglandins from macula densa and/or vasculature have been implicated in vasodilation and stimulation of renin biosynthesis and release. In low salt-treated animals, COX-2 expression increased in the macula densa and adjacent cortical thick ascending limbs (12), whereas 15-PGDH expression in these sites was minimal. Therefore, increased COX-2 expression and minimal 15-PGDH may both contribute to increased COX-2-derived prostaglandin levels in the macula densa and subsequent increases in renin biosynthesis and release. In high salt-treated animals, COX-2 expression in the macula densa/cortical thick ascending limbs decreased, whereas 15-PGDH expression in the macula densa increased. Therefore, decreased COX-2 expression and increased 15-PGDH may both contribute to reduced COX-2-derived prostaglandin levels in the macula densa and subsequent inhibition of renin biosynthesis and release.

Outer medullary thick ascending limb and collecting duct are important contributors to the medullary interstitial osmoregulatory function, which allows effective distal nephron salt and water reabsorption (23), and PGE2 has been reported to inhibit sodium reabsorption in these sites (2). 15-PGDH expressed in the thick ascending limb and collecting duct in the outer medulla may contribute to Na+ reabsorption through rapid degradation of prostaglandins arising from local generation, diffusion from vasculature, or filtration. Indeed, Nomura et al. (20) recently reported that both prostaglandin uptake carrier prostaglandin transporter and 15-PGDH are expressed in renal collecting ducts. Lack of 15-PGDH in inner medulla/papilla may contribute to the constitutive activity of COX-2-derived prostaglandins in medullary interstitial cells (9, 12, 32).

In summary, we have delineated for the first time the expression and localization of 15-PGDH, the key enzyme in prostaglandin degradation in the kidney. We also found that 15-PGDH was downregulated by COX-2 activity. Further studies are required to elucidate the identity of the COX-2-dependent 15-PGDH activity.
metabolites that downregulate 15-PGDH expression and to investigate possible physiological and/or pathophysiological roles for 15-PGDH in the kidney.

REFERENCES