Cyclooxygenase-2 in the Kidney

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Prostaglandins are produced constitutively by many tissues in the body, including brain, gut, and kidney, and their synthesis increases at sites of inflammation. Cyclooxygenase (COX), the enzyme responsible for the initial rate-limiting metabolism of arachidonic acid to prostaglandin G2 and subsequently to prostaglandin H2, was first purified in ram seminal vesicles and was cloned in 1988 by DeWitt and Smith (1). This enzyme has been renamed cyclooxygenase-1 (COX-1) to indicate that it encodes the constitutive enzyme. Prostaglandins play myriad roles as local mediators of inflammation and as modulators of physiologic functions, such as maintenance of gastric mucosal integrity and modulation of renal microvascular hemodynamics, renin release, and tubular salt and water reabsorption (2). After the recognition that COX was the target of aspirin (3), the pharmaceutical industry developed a substantial number of nonsteroidal anti-inflammatory drugs (NSAID), whose mechanism of action involves competitive or noncompetitive inhibition of COX activity. However, by the early 1990s, experimental studies began to suggest that COX-1 might not be the enzyme responsible for increased prostanoid production in inflammatory states. In cultured cells and tissue, COX activity increased rapidly in response to mitogens and cytokines (4–6), although COX-1 mRNA and immunoreactive protein were not altered. Furthermore, glucocorticoid administration was shown to decrease COX activity in peritoneal macrophages after lipopolysaccharides (7) but not to affect constitutive renal medullary COX activity. These results led Needleman and co-workers (7) to postulate the existence of a second, inflammatory-mediated COX isof orm.

Shortly after the Needleman hypothesis was formulated, Xie and co-workers (8) detected the presence of a second avian COX mRNA species, and Kujubu and co-workers (9) identified a phorbol ester-activated immediate early murine gene (TIS10) that possesses similar COX activity but shares only approximately 66% homology in amino acid sequence. This second COX isof orm has subsequently been renamed cyclooxygenase-2 (COX-2), and a vast amount of research has identified it to be an inducible and glucocorticoid-sensitive gene that is highly expressed in many tissues in response to inflammation. Expression of recombinant enzymes and determination of the crystal structure of COX-2 have provided insights into the observed physiologic and pharmacologic similarities to and differences from COX-1 (10,11). The identification of COX-2 has also led to the development and marketing of both relatively and highly selective COX-2 inhibitors for use as analgesics, antipyretics, and anti-inflammatory agents. In addition to its central role in inflammation, aberrantly up-regulated COX-2 expression is increasingly implicated in the pathogenesis of a number of epithelial cell carcinomas, including colon, esophagus, breast, and skin, and in Alzheimer’s disease and possibly other neurologic conditions (12–14).

Expression of COX-2 in Kidney Cortex

It was initially postulated that the major gastrointestinal and renal side effects of the original NSAID, termed “nonselective” because of their inhibition of both COX-1 and COX-2, would be avoided by the development of COX-2 selective NSAID, because COX-1 was recognized to be abundantly expressed in gastric mucosa and in kidney. However, although COX-2 is not expressed in the normal gastric mucosa, there has now been definitive indication for localized and regulated COX-2 expression in the mammalian kidney. Identification of the pattern of distribution of COX-2 in the kidney has also begun to resolve previous conundrums concerning renal prostanoid physiology. Harris et al. (15) initially determined that COX-2 mRNA was present at low but detectable levels in normal adult rat kidney and that immunoreactive COX-2 could be detected in mesosomes from cortex and papilla. In situ hybridization and immunolocalization demonstrated localized expression of COX-2 mRNA and immunoreactivity in the cells of the macula densa and adjacent cortical thick ascending limb in adult rat kidney cortex. The immunoreactivity of stained cells was intense, but only one (and rarely two) COX-positive cell was observed per site (Figure 1A). The majority of identified glomeruli sectioned through the juxtaglomerular apparatus (JGA) did not have COX-2–positive cells in the macula densa. No COX-2 immunoreactivity was detected in arterioles, glomeruli, or cortical or medullary collecting ducts.

Regulation of COX-2 Expression in Kidney Cortex

In the mammalian kidney, the macula densa is involved in regulating renin release (16) by sensing alterations in luminal chloride via changes in the rate of Na⁺/K⁺/2Cl⁻ cotransport (17). Inhibition of Na⁺/K⁺/2Cl⁻ cotransport with loop diuret-
ics results in a decrease in chloride reabsorption by the macula densa and an increase in renin secretion (18). It has long been recognized that NSAID administration can elicit a hyporeninemic state, and studies using an isolated perfused JG preparation indicated that NSAID administration prevented the increases in renin release mediated by macula densa sensing of decreases in luminal NaCl (19). Immunoreactive COX-1 cannot be detected in cortical thick limb or macula densa (15,20). In initial studies, Harris et al. (15) examined a high renin state, induced by imposition of a salt-deficient diet, and determined that macula densa/cortical thick ascending limb of Henle COX-2 mRNA and immunoreactive protein increased significantly.

Harding et al. (21) first demonstrated a direct role for macula densa COX-2 activity in mediating renin production and release by showing that NS398, a selective COX-2 inhibitor, inhibited increases in renal renin expression in response to a low-salt diet. Cheng et al. (22) subsequently demonstrated that increases in renin mRNA expression and renal renin activity in response to angiotensin-converting enzyme (ACE) inhibition were also blunted by the highly selective COX-2 inhibitor SC59236. It was further shown that in experimental renovascular hypertension, in which macula densa COX-2 expression is increased (Figure 2) (23,24), COX-2 inhibition blunted increases in renin expression and lowered BP (24). In addition, preliminary results have indicated that in COX-2 knockout mice, renal renin activity did not increase in response to ACE inhibition (25). Direct evidence for a role for COX-2 has been provided recently by Traynor et al. (26), who determined that in an isolated perfused JG preparation, increased renin release in response to lowering the perfusate NaCl concentration was blocked by NS398.

Because COX-2 is involved in regulation of renin production and release, investigators have examined whether components of the renin-angiotensin system might be involved in mediating expression of macula densa/cTALH COX-2 expression. Administration of either an ACE inhibitor or an angio-

**Figure 1.** Localization of renal cortical immunoreactive cyclooxygenase-2 (COX-2) expression. (A) Control: In normal adult rat cortex, occasional cortical thick ascending limb of Henle (cTALH) COX-2 immunoreactivity is noted. (B) Low salt and captopril. Intense immunoreactive COX-2 is apparent in macula densa of most glomeruli (arrows) as well as in isolated cTAL cells. (C) Low salt and captopril + 7-NI. Markedly decreased macula densa COX-2 expression compared with rats that were not treated with the neuronal nitric oxide synthase inhibitor.

**Figure 2.** COX-2 expression in renal cortex after 1 wk of aortic coarctation. Representative experiments of COX-2 mRNA (A) and COX-2 immunoreactive protein (B) are presented. Lanes: 1, sham-operated control; 2, aortic coarctation, right kidney; 3, aortic coarctation, left kidney; 4, aortic coarctation + SC58236, right kidney; 5, aortic coarctation + SC58236, left kidney. In (A), relative expression of glyceraldehyde phosphate dehydrogenase mRNA expression is provided for comparison. Reprinted from reference 24 with permission.
tensin type 1 (AT1) receptor antagonist to rats led to increases in cortical COX-2 expression in vivo (Figure 1B), and mice that are double nullizygotes for both AT1a and AT1b also expressed high levels of COX-2 in the macula densa (25). In addition, it was determined that adrenalectomy increased macula densa/cTALH expression, which was reversed by administration of either glucocorticoids or mineralocorticoids (27). Furthermore, not only the glucocorticoid receptor antagonist RU486 but also the mineralocorticoid antagonist spironolactone increased macula densa/cTALH COX-2, suggesting that mineralocorticoid receptor as well as glucocorticoid receptor may inhibit basal COX-2 expression (27).

It is known that renal renin production is modulated by angiotensin II (28,29). Increased renal tubule reabsorption, mediated directly by angiotensin II and indirectly by aldosterone, will reestablish intravascular volume homeostasis and thereby decrease the stimulus for renin release. In addition, angiotensin II directly inhibits renal renin production and release by a so-called “short loop feedback inhibition” (29). Administration of either ACE inhibitors or AT1 receptor antagonists results in increases in JG renin expression, even in the absence of any detectable alteration in intravascular volume or renal hemodynamics (28,30,31).

It has traditionally been assumed that angiotensin II inhibits renin production by direct action on the JG cells (32,33). However, a recent study by Matsusaka et al. (34) in chimeric mice carrying “regional” null mutation of the AT1a receptor, the AT1 receptor subtype exclusively present in mouse JG cells, has questioned whether angiotensin II does act directly on JG cells. In these studies, the JGA of AT1a receptor−/− mice were markedly enlarged, with intense expression of renin mRNA and protein. In the chimeric mice, the changes in the JGA were proportional to the degree of chimerism, but the degree of JGA hypertrophy/hyperplasia and the expression of renin mRNA and protein were not different in AT1a receptor+/+ and AT1a receptor−/− JG cells. Therefore, the presence or absence of AT1 receptors on JG cells did not seem to be the determining factor of whether angiotensin II could regulate JGA renin synthesis.

The results of studies of COX-2 expression suggest an alternative or additional mechanism by which angiotensin II may inhibit renin release (22). Angiotensin II may act to inhibit cTALH/macula densa COX-2 expression by direct action and indirectly by increasing aldosterone production, thereby limiting the relative increases in COX-2 expression in response to volume depletion and thus the macula densa’s ability to signal renin release.

Because of the localization of neuronal nitric oxide synthase (nNOS) to the same regions of the kidney as COX-2 (macula densa and inner medulla), Cheng et al. (35) examined whether nNOS activity might be involved in mediating increased renal COX-2 expression. Increases in cTALH/macula densa COX-2 expression induced by a low-salt diet or low-salt diet+ACE inhibition were significantly inhibited by simultaneous administration of the selective nNOS inhibitors 7-NI or SMTC (Figure 1C). In addition, increased papillary COX-2 expression induced by a high-salt diet was significantly inhibited by simultaneous 7-NI administration.

In primary cultures of immunodissected rabbit cTALH cells, which express immunoreactive nNOS, administration of either cyclic guanosine monophosphate or an NO donor increased COX-2 expression, whereas administration of either nonselective (NG-nitro-L-arginine methyl ester) or nNOS-selective (7-NI) NOS inhibitors decreased basal COX-2 expression. Basal renal cortical COX-2 was also decreased in response to nNOS inhibition (35). Therefore, it is plausible to suggest that NO may be a positive regulator of COX-2 expression under both basal and stimulated conditions.

Although there is experimental evidence that macula densa-derived NO may counteract the vasoconstriction of tubulolomerular feedback and also mediate renin release signaled by the macula densa, it has been argued that the short half-life of NO and the long (for NO) potential distance between macula densa cells and the glomerular vascular pole make it less likely that macula densa-derived NO acts directly on renin-producing cells, especially in conditions that lead to recruitment of renin-producing cells. Rather, direct effects of NO on JG cells might be expected to be mediated by NO derived from vascular endothelial NOS (36). Recent studies by Ichihara et al. (37) suggested that NO-mediated counteraction of TG feedback vasoconstriction is blocked by COX-2–specific inhibitors.

Expression and Regulation of COX-2 in Kidney Medulla

In addition to cortical COX-2 expression, localized COX-2 expression was detected in the lipid-laden medullary interstitial cells in the tip of the papilla (Figure 3). The medullary collecting duct expresses abundant COX-1 (15,20), and it has been proposed that papillary prostaglandins mediate vasa recta dilation to maintain medullary blood flow and antagonize vasopressin-mediated water and solute reabsorption (38). However, in addition to collecting duct, a subset of medullary interstitial cells have long been recognized to contain abundant

![Figure 3. Expression of COX-2 in medullary interstitial cells. In the tip of the papilla, COX-2 is absent from collecting ducts (c) but is localized to cytoplasmic granules and the perinuclear cisternae of interstitial cells. Reprinted from reference 15 with permission.](image-url)
arachidonic acid and to be a rich source of prostaglandins (39), although COX-1 expression in these cells was sparse. Medul-
lary COX-2 expression decreases significantly with salt deple-
tion and increases with a high-salt diet (40,41) and with water
depprivation (42). COX-2 in cultured medullary interstitial cells
increases in response to extracellular hyperosmolarity and
seems to confer protection against hyperosmolality-induced
apoptosis (42,43).

Recent studies have also localized COX-2 to inner medul-
lary collecting duct cells and to intercalated cells in the renal
cortex. COX-1 is abundantly and constitutively expressed in
both cortical and medullary collecting duct; therefore, the
physiologic consequences of this observed COX-2 coexpres-
sion remains to be determined. In the inner medulla, it is
possible that it may serve a cytoprotective role (vide supra),
but its localization to intercalated cells suggests possible addi-
tional roles in acid-base regulation (41,42,44,45).

Expression of COX-2 in Development
Fetal and early postnatal kidneys possess functional COX
activity and are a rich source of prostaglandins (46,47). Our
studies localized COX-2 mRNA and immunoreactive protein
in the kidneys of normal rats beginning at E16 and determined
that renal COX-2 expression is highly developmentally regu-
lated (48). During kidney development, immunoreactive
COX-2 is first observed in mid-gestation embryonic stages,
notably in cells undergoing induction and/or morphogenesis;
this form is found in subcapsular epithelial structures in the

![Figure 4. Expression of COX-2 in developing rat kidney.](image-url)

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kidney for the duration of nephrogenesis (through postnatal wk 2 in the rat; Figure 4). The mature intense form of immunoreactive COX-2 appears primarily in functional nephrons as they mature (48). A similar pattern of COX-2 expression is seen in the developing mouse kidney (49). In the postnatal kidney, COX-2 expression is relatively low at birth, increases in the first two postnatal weeks, and gradually declines to low levels in normal adult rats (48). Increased postnatal COX-2 expression was the result of expression at high levels in specific epithelial cells in the cortical segment of Henle’s TAL.

This expression pattern of COX-2 in the developing kidney is of interest because of the evidence that COX metabolites play important functional and developmental roles in the fetal kidney. Matson et al. (50) administered the nonspecific COX inhibitor indomethacin to fetal lambs during the third trimester of gestation. These lambs developed increased renal vascular resistance and decreased fetal renal blood flow, as well as increases in urinary sodium and chloride excretion and decreases in plasma renin activity. In humans, an increased incidence of oligohydramnios has been observed in women who chronically consumed significant amounts of aspirin or other COX inhibitors during the third trimester of pregnancy (51). Because the fetal urine is the source of a significant amount of the amniotic fluid, these studies suggested that inhibition of COX led to the suppression of fetal renal function.

There also is evidence that COX metabolites may mediate normal renal development. Chronic administration of indomethacin to pregnant Rhesus monkeys led to renal hypoplasia in the neonates, with kidney mass reduced by 15% compared with control animals (52). The observed defect was specific for the kidney, because in the treated animals, development of other organs was not affected, except for hepatic hypertrophy. Chronic use of COX inhibitors during human pregnancy has also been related to fetal renal maldevelopment; kidneys from infants who came to term or died in the early postnatal period had few differentiated proximal tubules in the inner cortex and crowding of the glomeruli (53,54). The outer cortex was more severely affected, with evidence of poorly differentiated glomeruli, undifferentiated tubule epithelia, and tubular dilation. In addition, the medullary pyramids were crowded with small immature tubules.

Targeted disruption of murine COX-2 has indicated an important role for this enzyme in renal development (55,56). At maturity in homozygous COX-2 null mice, the kidneys are small, with fewer developed nephrons than in wild-type kidneys. Undeveloped mesenchymal tissue, immature glomeruli, and dysplastic tubules were present in the outer cortex. Hypoplasia or atrophy of the medulla accompanied by microlcystic lesions in the corticomedullary junction was also present in the knockout mice. There is apparent variability in the extent of the structural renal damage and the development of end-stage renal failure among individual mice, which does not necessarily seem to be related to the background upon which the mice are bred. No apparent developmental or functional abnormalities have been described in mice with targeted disruption of COX-1 (57). Of interest, maternal administration of a selective COX-2 inhibitor to wild-type mice and rats during the fetal and/or perinatal period led to renal lesions similar to that observed in the homozygous COX-2 null mice (49).

**Expression of COX-2 in Renal Injury**

COX metabolites have been implicated in functional and structural alterations in glomerular and tubulointerstitial inflammatory diseases (58–60). Studies have suggested that prostanoids may also mediate altered renal function and glomerular damage after subtotal renal ablation, and glomerular prostaglandin production may be altered in such conditions (61–67). After subtotal renal ablation, there were selective increases in renal cortical COX-2 mRNA and immunoreactive protein expression, without significant alterations in COX-1 expression (Figure 5). This increased COX-2 expression was most prominent in the macula densa and surrounding cTALH, the site of expression of cortical COX-2 in the normal rat kidney (68). In addition, there was detectable COX-2 immunoreactivity in some glomeruli from remnant kidneys, with
increased expression in visceral epithelial cells and mesangial cells.

Wang et al. (69) determined that administration of the COX-2 selective inhibitor SC58236 decreased proteinuria and inhibited development of glomerular sclerosis in rats with reduced functioning renal mass. In addition, the COX-2 inhibitor decreased mRNA expression of transforming growth factor-β1 and of types III and IV collagen in the remnant kidney. Whether the effects of COX-2 inhibition were the result of modulation of inflammatory stimuli that may mediate cell injury and alterations in matrix deposition or effects on glomerular hemodynamics will require further study. However, it is of interest that the same degree of hypertension was present in both vehicle-treated and SC58236-treated animals, indicating that the decreases in renal injury were not the result of alterations in systemic BP. Decreases in proteinuria and preservation of renal structural integrity in the passive Heymann nephritis have also been observed with administration of the relatively selective COX-2 inhibitor flosulide (70,71). Because renal COX-2 expression is also increased in glomerulonephritides such as lupus nephritis (72), it is possible that COX-2 inhibitors may also alter the natural history of glomerular inflammatory lesions.

Expression of COX-2 in Human Kidney

Although published studies have documented a similar pattern of COX-2 expression (macula densa/cTALH and medullary interstitial cells) in kidney of mouse, rat, rabbit, and dog (15,49,73,74), it was previously controversial whether human kidney demonstrated the same COX-2 localization. The initial studies of COX-2 localization in human kidney failed to detect COX-2 in either location and instead reported expression in podocytes and arteriolar smooth muscle cells (75). However, a more recent study in humans older than 60 yr was able to detect COX-2 in the macula densa and medullary interstitial cells (76), and a preliminary report also has detected increased macula densa COX-2 in patients with congestive heart failure and with Barter’s syndrome (77). Therefore, it is likely that COX-2 may well play similar physiologic roles in the human kidney as has been noted for other mammals.

Conclusion

In summary, COX-2 mRNA and immunoreactive protein are constitutively expressed at high levels in restricted locations in the mammalian kidney, the macula densa and surrounding cTALH, and the medullary interstitial cells. Regulation of expression during development and in a variety of physiologic and pathophysiologic conditions indicates potentially important roles for COX-2 metabolites in glomerulogenesis, regulation of renal hemodynamics, and the renin-angiotensin system.

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References


