Physiological regulation of cyclooxygenase-2 in the kidney

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Harris, Raymond C., and Matthew D. Breyer. Physiological regulation of cyclooxygenase-2 in the kidney. Am J Physiol Renal Physiol 281: F1–F11, 2001.—In adult mammalian kidney, cyclooxygenase-2 (COX-2) expression is found in a restricted subpopulation of cells. The two sites of renal COX-2 localization detected in all species to date are the macula densa (MD) and associated cortical thick ascending limb (cTALH) and medullary interstitial cells (MICs). Physiological regulation of COX-2 in these cellular compartments suggests functional roles for eicosanoid products of the enzyme. COX-2 expression increases in high-renin states (salt restriction, angiotensin-converting enzyme inhibition, renovascular hypertension), and selective COX-2 inhibitors significantly decrease plasma renin levels, renal renin activity, and mRNA expression. There is evidence for negative regulation of MD/cTALH COX-2 by angiotensin II and by glucocorticoids and mineralocorticoids. Conversely, nitric oxide generated by neuronal nitric oxide synthase is a positive modulator of COX-2 expression. Decreased extracellular chloride increases COX-2 expression in cultured cTALH, an effect mediated by increased p38 mitogen-activated protein kinase activity, and, in vivo, a sodium-deficient diet increases expression of activated p38 in MD/cTALH. In contrast to COX-2 in MD/cTALH, COX-2 expression increases in MICs in response to a high-salt diet as well as water deprivation. Studies in cultured MICs have confirmed that expression is increased in response to hypertonicity and is mediated, at least in part, by nuclear factor-κB activation. COX-2 inhibition leads to apoptosis of MICs in response to hypertonicity in vitro and after water deprivation in vivo. In addition, COX-2 metabolites appear to be important mediators of medullary blood flow and renal salt handling. Therefore, there is increasing evidence that COX-2 is an important physiological mediator of kidney function.

prostaglandin; macula densa; medullary interstitial cell; renin; hypertonicity

PROSTAGLANDINS REGULATE VASCULAR tone and salt and water homeostasis in the mammalian kidney and are involved in the mediation and/or modulation of hormonal action. Cyclooxygenase (prostaglandin synthase; \( \text{G}_{2}/\text{H}_{2} \)), the enzyme responsible for the initial rate-limiting metabolism of arachidonic acid to prosta-

glandin \( \text{G}_{2} \) and subsequently to prostaglandin \( \text{H}_{2} \), was first purified in ram seminal vesicles and was cloned in 1988 by DeWitt and Smith (15). This isoform, which is widely expressed constitutively, has been renamed cy-

clooxygenase-1 (COX-1). Biochemical and physiological studies indicated that COX-1 was not the enzyme pri-

marily responsible for increased prostanoid production in inflammatory states nor was it the glucocorticoid-
sensitive isoform (62). A second, inflammatory-medi-

ated cyclooxygenase isoform was subsequently identi-

fied and designated cyclooxygenase-2 (COX-2) (52, 71,
Expression of recombinant enzymes and determination of the crystal structure of COX-2 have provided insights into the observed physiological and pharmacological similarities to, and differences from, COX-1 (59, 60). 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 upregulated COX-2 expression is increasingly implicated in the pathogenesis of a number of epithelial cell carcinomas and in Alzheimer’s disease and possibly other neurological conditions (19, 56, 85).

In addition to the well-recognized expression of COX-1 in renal vasculature, glomerular mesangial cells, and collecting duct (86), there is now definitive evidence for localized and regulated COX-2 expression in the mammalian kidney. COX-2 mRNA is present at low but detectable levels in normal adult mammalian kidney, and immunoreactive COX-2 is found in microsomes from cortex and papilla (32). In rat kidney, in situ hybridization and immunolocalization demonstrated localized expression of COX-2 mRNA and immunoreactive protein in the cells of the macula densa and adjacent cortical thick ascending limb (cTALH). The immunoreactivity of stained cells was intense, but few COX-positive cells were observed per site. Localized COX-2 expression was also detected in the lipid-laden medullary interstitial cells in the tip of the papilla. Certain investigators have also suggested that COX-2 may be expressed in inner medullary collecting duct (IMCD) cells and intercalated cells in the renal cortex (18, 103–105). Because COX-1 is abundantly and constitutively expressed in both cortical and medullary collecting duct, the physiological consequences of possible COX-2 coexpression remains to be determined. It should also be noted that expression in collecting duct in vivo has not been confirmed by all investigators, so expression and regulation of COX-2 in this segment remain controversial and an area of active investigation.

COX-2 EXPRESSION IN THE RENAL CORTEX

Published studies have documented COX-2 expression in macula densa/cTALH and medullary interstitial cells in the kidney of mouse, rat, rabbit, and dog (27, 32, 48, 51). In contrast, there has been some controversy about COX-2 localization in human kidney. Initial studies of COX-2 localization in human kidney failed to detect COX-2 in either macula densa or medullary interstitial cells and instead reported expression in podocytes and arteriolar smooth muscle cells (49). However, a more recent study in humans >60 yr of age detected COX-2 in both macula densa and medullary interstitial cells (70) and increased macula densa COX-2 in patients with Bartter’s syndrome (50). It has been suggested that the increased macula densa COX-2 that has been detected in elderly humans may be secondary to decreased basal renin production associated with aging (70).

In the mammalian kidney, the macula densa is involved in regulating renin release by sensing alterations in luminal chloride via changes in the rate of Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransport (72, 78, 80). Measurements in vivo in isolated perfused kidney and isolated perfused juxtaglomerular preparations all indicated that administration of nonspecific cyclooxygenase inhibitors prevented the increases in renin release mediated by macula densa sensing of decreases in luminal NaCl (20, 21, 25, 44, 46, 55). Induction of a high-renin state by imposition of a salt-deficient diet, angiotensin-converting enzyme (ACE) inhibition, diuretic administration, or experimental renovascular hypertension all significantly increase macula densa/cTALH COX-2 mRNA and immunoreactive protein (32, 34, 45, 93, 96, 104).

When rats were fed either a sodium-deficient diet for 7 days or the ACE inhibitor captopril, cortical COX-2 mRNA and immunoreactive protein increased, and there were additive effects when animals on a low-sodium diet were also administered an ACE inhibitor (9). Rats receiving both a low-salt diet and captopril expressed COX-2 in the macula densa of virtually every glomerulus. Individual COX-2-positive cells were also present in the preglomerular segment of the cTAL but were absent from the postglomerular epithelium (initial segment of the distal convoluted tubule). Subtype-specific angiotensin II receptor antagonists were also tested. Administration of the type 2 angiotensin II receptor (AT\(_2\)) antagonist PD-123319 did not alter cortical COX-2 mRNA or immunoreactive protein levels in either control or sodium-restricted rats whereas the AT\(_1\) receptor antagonist losartan significantly increased COX-2 mRNA and immunoreactive protein expression, similar to what was observed with ACE inhibition (9).

To examine further the role of AT\(_1\) receptor in regulation of cTALH/macula densa COX-2 expression, COX-2 immunoreactivity was determined in mutant mice with homozygous deletions of both Agtr1 subtypes. In adult wild-type mice on a control diet, minimal renal cortical COX-2 immunoreactive protein was detected, whereas in Agtr1\(^+/−\) mice, abundant COX-2 immunoreactivity was observed in the macula densa/cTALH region (9). Similar results have been obtained in Agtr1a\(^−/−\) mice (100).

To examine whether angiotensin II directly modulated cTALH COX-2 expression, primary cultures of rabbit cTALH were isolated by immunodissection with an anti-Tamm-Horsfall antibody. When grown to confluence and made quiescent for 24 h by removal of serum, these cells, as indicated by Northern and Western analysis, expressed detectable basal levels of COX-2 immunoreactivity. In quiescent cells, administration of angiotensin II did not alter basal levels of COX-2 expression. However, when COX-2 immunoreactivity expression was stimulated by phorbol dibutyrate administration, preincubation with angiotensin II inhibited phorbol ester-mediated induction of COX-2 expression, which was prevented by preincubation with losartan but not PD-123319 (8).
To determine whether increased COX-2 expression mediated increases in renin production in response to ACE inhibition, rats were treated with captopril for 1 wk with or without the COX-2-specific inhibitor SC-58236. Plasma renin activity, renal renin activity, and renal cortical renin mRNA increased significantly in the captopril-treated group, and these increases were significantly blunted by simultaneous treatment with SC-58236 (8). In similar studies, the selective COX-2 inhibitor NS-398 inhibited increases in renal renin expression in response to a low-salt diet (31). Increases in renin mRNA expression and renal renin activity in an experimental model of renovascular hypertension were also blunted by SC-58236 (93). Furthermore, Traynor et al. (92) determined that, in an isolated perfused juxtaglomerular preparation, increased renin release in response to lowering the perfusate NaCl concentration was blocked by NS-398 (92).

A further confirmation of the role of COX-2 in the regulation of renin release is demonstrated by recent studies utilizing mice with genetic deletion of COX-2 (16, 68). In control +/+ mice, there was sparse cortical immunoreactive COX-2 expression in macula densa cells and surrounding macula densa. Treatment with the ACE inhibitor captopril increased expression of macula densa COX-2 expression, similar to that seen in rats (12). As expected, no immunoreactive COX-2 was detected in −/− mice under control conditions or in response to captopril treatment. In response to treatment with captopril, renal renin mRNA and immunoreactive renin expression were significantly increased in +/+ mice but were minimally altered in −/− mice. In the absence of captopril treatment, immunoreactive renin was localized to juxtaglomerular (JG) cells in both control and COX-2-knockout mice. The number of renin-expressing JG cells, as determined by the area of renin immunoreactivity normalized to the area of renal cortex, was higher but not statistically significant in +/+ mice compared with −/− mice. Captopril increased immunoreactive renin expression significantly in +/+ mice. The increases in the number of cells expressing immunoreactive renin in the +/+ mice with captopril treatment were the result of both more JG cells expressing renin and the recruitment of additional renin-expressing cells in the more proximal afferent arteriole. In contrast, there was minimal, if any, recruitment of renin-expressing cells in the more proximal afferent arteriole of the −/− mice. Previous studies have documented that increases in expression of renin by ACE inhibition, as well as in immature kidney (24), in renovascular hypertension (90), and after adrenalectomy (89), have been shown to occur not only by an increase in the content of renin in individual JG cells but also by recruitment of both additional juxtaglomerular cells and more proximal afferent arteriolar cells that do not normally produce renin (22, 23). It is noteworthy that increased macula densa/cTALH COX-2 expression is also seen in all of these conditions (11, 34, 93, 106, 107) and that ACE inhibitor-stimulated recruitment of renin-producing cells was not apparent in the COX-2-knockout mice. Yang et al. (100) have also recently reported that COX-2-knockout mice fail to increase renal renin content in response to dietary salt deficiency (100).

Renal renin production is modulated by angiotensin II (28, 83). 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 so-called “short-loop feedback inhibition” (83). Administration of either ACE inhibitors or AT1 receptor antagonists results in increases in both renin mRNA and immunoreactive protein in the kidney, in the absence of any detectable alteration in intravascular volume or renal

Fig. 1. A: possible mechanism by which macula densa cyclooxygenase-2 (COX-2) may stimulate renin production and release. Although not yet proven, it is postulated that Gs-coupled prostaglandins (PGEs2, acting via EP2, or EP4 receptor subtypes and/or PGI2) activate adenylyl cyclase (AC) to increase cAMP in juxtaglomerular cells, leading to renin release. As discussed herein, our results indicate that decreased extracellular Cl−K+Cl−K+Cl−Cl−-mediated arachidonic acid (AA) release has not yet been studied. PKA, protein kinase A. B: possible interactions of neuronal nitric oxide synthase (nNOS) and renin-angiotensin system to regulate COX-2 expression in macula densa and cortical thick ascending limb of Henley (cTALH).
hemodynamics (7, 22, 28). It has traditionally been assumed that angiotensin II inhibits renin production by a direct action on JG cells (41, 47). However, studies in chimeric mice carrying “regional” null mutation of the angiotensin type 1a (AT1a) receptor, the AT1 receptor subtype exclusively present in mouse JG cells, have questioned whether angiotensin II does act directly on JG cells (63). In these studies, the JG cells of AT1a receptor −/− mice were markedly enlarged, with intense expression of renin mRNA and protein. In the chimeric mice, the changes in the JG cells were proportional to the degree of chimerism, but the degree of JG hypertrophy and/or 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 was not the only factor determining whether angiotensin II could regulate JG renin synthesis. It is therefore possible that angiotensin II-mediated inhibition of COX-2 expression may also contribute to feedback inhibition of renin secretion (Fig. 1).

Adrenalectomy also increased macula densa/cTALH COX-2 expression, which was reversed by administration of either glucocorticoids or mineralocorticoids (106). In addition, administration of the mineralocorticoid inhibitor spironolactone also stimulated macula densa COX-2 expression, suggesting that mineralocorticoids and/or glucocorticoids may tonically inhibit macula densa/cTALH COX-2 expression (106).

Neuronal nitric oxide synthase (nNOS) is localized to the same regions of the kidney as COX-2 (macula densa and inner medulla), and it has been suggested that nitric oxide (NO) may be involved in modulation of renin production and release and/or tubuloglomerular feedback (3, 4, 81, 94). However, the short half-life of NO and the long distance (for NO) 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 (53). However, recent studies by Ichihara et al. (39, 40) have suggested that NOS-mediated modulation of tubuloglomerular feedback vasoconstriction is blocked by COX-2-specific inhibitors. It is known that NO can enhance cyclooxygenase activity (77). To determine whether nNOS activity might be involved in mediating increased cTALH/macula densa COX-2 expression, rats on either control or low-salt diets were administered the specific nNOS inhibitors 7-nitroindazole or S-methyl-i.-thiocitrulline (1, 67). Both inhibitors significantly decreased increased macula densa COX-2 mRNA and immunoreactive protein expression in response to low salt with or without ACE inhibitors. In cultured cTALH, both nonspecific and nNOS-specific NOS inhibitors significantly inhibited basal immunoreactive COX-2 expression, and administration of the NO donor S-nitro-N-acetyl-penicillamine or cGMP significantly stimulated COX-2 expression (9).

Previous studies have documented that decreased intraluminal chloride concentration is the signal for macula densa stimulation of renin secretion (57, 80). Ion substitution experiments of tubular perfusate demonstrated that substitution of other cations for sodium did not affect renin secretion, whereas substitution of other anions for chloride led to increased renin secretion (58). Macula densa sensing of luminal chloride concentration is dependent on net apical transport, mediated by luminal Na⁺-K⁺-2Cl⁻ cotransport (76, 78). The Na⁺-K⁺-2Cl⁻ cotransporter possesses a high affinity for Na⁺ and K⁺, such that minimal alterations in transport occur with physiological changes in Na⁺ or K⁺ concentrations; however, the affinity for chloride is lower and falls within the range of loop chloride values, thereby resulting in an uptake mechanism that is very sensitive to any change in luminal chloride (26). The role of Na⁺-K⁺-2Cl⁻ cotransport in this macula densa sensing is further supported by the observation that loop diuretics, which inhibit Na⁺-K⁺-2Cl⁻ cotransport, increase renin activity, even in the absence of volume depletion (35, 61, 65).

To investigate the role of alterations in extracellular ion composition on COX-2 expression, cultured cTALH were incubated in a medium containing 26 mM Na⁺ and 7 mM Cl⁻ (low-salt medium). Under these conditions, immunoreactive COX-2 expression increased significantly, whereas when the cells were incubated in the same solution in which 100 mM NaCl had been added (“NaCl solution”), COX-2 expression was not altered (10). Similarly, when 100 mM choline chloride was added (final Cl⁻ concentration = 107 mM), COX-2 expression was not increased above baseline, but when additional sodium was added with sodium gluconate, NaNO₃, or sodium isethionate, COX-2 expression was significantly increased (Fig. 2A). Decreased extracellular NaCl increased COX-2 expression within 1 h, and it remained elevated for up to 16 h. Addition of 200 mM mannitol to the low-salt medium did not prevent increased COX-2 expression, indicating that extracellular osmolality per se was not the inciting factor. The fact that the Na⁺-K⁺-2Cl⁻ cotransport inhibitor bumetanide also significantly increased immunoreactive COX-2 expression suggests that alter-

**Fig. 2. Regulation of COX-2 in cTALH/macula densa by decreased extracellular Cl⁻ concentration ([Cl⁻]). A: assay of immunoreactive COX-2 expression in quiescent primary cultured rabbit cTAL cells incubated with various media for 6 h. *P < 0.01 compared with control. B: p38-specific inhibitor PD-169316-inhibited increases in COX-2 expression in cultured cTAL cells exposed to decreased extracellular NaCl. *P < 0.01. C: increased phosphorylated p38 (p38) expression in cultured cTAL cells by decreased extracellular [Cl⁻]. Cultured cTAL cells were incubated for 6 h. **P < 0.01 compared with control. D: localization of pp38 in rat renal cortex in response to a salt-deficient diet for 14 days. Low salt increased pp38 expression in macula densa and surrounding cTALH (figure modified from Ref. 11).
ations in intracellular ionic content initiate increased COX-2 expression. In a recent report by Yang et al. (102), cultured immortalized mouse macula densa cells derived from an SV40 transgenic mouse were also shown to increase COX-2 expression as well as prosta-
glandin production in response to decreased extracellular chloride or loop diuretics.

In cultured cTALH cells, the p38 mitogen-activated protein (MAP) kinase-specific inhibitor PD-169316 significantly blocked COX-2 upregulation induced by the low-salt medium whereas the MAP kinase kinase (MEK1) inhibitor PD-98059 produced numerically but not statistically significant decreases in COX-2 expres-
sion (Fig. 2B) (10). Activation of p38 occurred in cul-
tured cTALH in either low-salt or low-chloride medium preceding increases in COX-2 expression (Fig. 2C), and p38 activation was sustained in a pattern similar to that for COX-2. Smaller transient increases in phos-
phorylated c-Jun-NH$_2$-terminal kinase (pJNK) and extracellular signal-regulated kinase (pERK) were also detected. A similar increase in MAP kinase expression in response to low chloride and inhibition of COX-2 expression by a p38 inhibitor was also observed in mouse macula densa cells, although in these cells PD-98059 did produce significant inhibition (102).

To determine whether alterations in p38 activity can be observed in vivo under conditions in which macula densa/cTALH COX-2 expression is increased, rats were placed on a salt-deficient diet for 14 days. In renal cortex, a low-salt diet induced a significant increase in immunoreactive expression of the active, phosphory-
lated form of p38 (pp38). In control rat cortex, immu-
noreactive pp38 expression was predominantly local-
ized to the macula densa and cTALH. The localization of pp38 expression was similar in the animals on a low-salt diet, but the intensity of expression was in-
creased, consistent with the increases detected by im-
umunoblotting and in a distribution similar to that for renal cortical COX-2 expression (Fig. 2D) (10). There-
fore, these results suggest that alterations in extracel-
lular chloride may increase COX-2 expression in cTALH, at least in part, by increasing p38 activity. Increases in COX-2 expression that occur with intracellular volume depletion in this nephron segment may be the result of decreases in luminal chloride.

**COX-2 EXPRESSION IN THE RENAL MEDULLA**

The renal medulla is a major site of prostaglandin synthesis and abundant COX-1 and COX-2 expression (27, 53a, 104). The two cyclooxygenase isoforms exhibit differential compartmentalization within the medulla, with COX-1 predominating in the medullary collecting ducts and COX-2 predominating in medullary intersti-
tial cells (27, 30, 32, 49). COX-2 may also be expressed in endothelial cells of the vasa recta supplying the inner medulla (49, 69). In primary culture, collecting duct cells synthesize prostaglandins exclusively through COX-1 (29) whereas interstitial cells primarily synthesize prostaglandins via COX-2 (30, 108). Inter-
estingly, COX-2 expression cannot even be induced in primary cultures of cortical collecting ducts after treatment with tumor-promoting phorbol esters (29). Methylation of COX-2 DNA has been associated with silenc-
ing of COX-2 expression in some colon cancers (91), but whether differential methylation contributes to the cellular compartmentalization of COX-2 in the normal kidney is unknown. In contrast, transformed collecting duct cell lines (including M-1 and mIMCD3) appear to express COX-2 (18, 101, 103). It remains unclear whether the expression of COX-2 in the M-1 and mIMCD3 collecting duct cell lines represents an effect of SV40 transformation and/or culture conditions. In the human ureter and bladder epithelium, which like the collecting duct are also derived from ureteric bud, COX-2 expression is only detected in the setting of malignant transformation (51). Because of the potential chemopreventive and therapeutic effects of nonste-
roidal anti-inflammatory drugs (NSAIDs) in epithelial cancers (88), the factors contributing to the aberrant expression of COX-2 in malignant epithelia is an area of intense investigation (91).

Dynamic regulation of medullary COX-2 expression is an important component of the renal response to physiological stress, including water deprivation and lipopolysaccharide (30, 103, 105). After dehydration, renal medullary COX-2 mRNA and protein expression are significantly induced (30, 103), primarily in med-
ullary interstitial cells (30) (Fig. 3). In contrast, COX-1 expression in the mouse kidney is unaffected by water deprivation. Although hormonal factors may also con-
tribute to COX-2 induction, shifting cultured renal medullary interstitial cells to hypertonic media (using either NaCl or mannitol) is sufficient to directly induce COX-2 expression. In contrast to cultured MDCK renal epithelial cells (103), where increased osmolality achieved by using the cell-permeable solute urea in-
creased COX-2, urea did not increase COX-2 expres-
sion in interstitial cells (30), suggesting that changes in cell volume are critical to the effects of toxicity on COX-2 expression. Finally, because prostaglandins are known to play an important role in maintaining renal function after water deprivation (79), it may be that hypertonic induction of COX-2 plays an important role in preserving renal function.

At the cellular level, COX-2 expression is highly regulated by several processes that alter its transcrip-
tion rates, message stability, and efficiency of message translation (17, 33, 37). Transcription of COX-2 is exquisitely regulated by a variety of cytokines and growth factors, including tumor necrosis factor (99), interleukin-1β (IL-1β), and platelet-derived growth factor (36, 37). These effects are mediated, in part, by the coordinated activation of several transcription fac-
tors that bind to, and activate consensus sequences in, the 5′-flanking region of the COX-2 gene for nuclear factor-κB (NF-κB), NF-IL-6-C/EBP, and a cAMP-res-
pone element (CRE) (Fig. 4). After stimulation with platelet-derived growth factor, JNK-activated binding of fos/jun heterodimers to the CRE site of COX-2 pro-
motes COX-2 transcription (98). Induction of COX-2 mRNA transcription by endotoxin (lipopolysaccharide)
may also involve CRE sites, (64, 92a) and NF-κB sites (42, 43).

Similar transcriptional elements appear to be involved in regulating COX-2 expression in response to renal medullary tonicity. Water deprivation activates COX-2 expression in medullary interstitial cells by activating the NF-κB pathway (30). After exposure of medullary interstitial cells to hypertonic conditions, nuclear extracts demonstrate increased binding of p65 and p50 to a canonical NF-κB site present in the human COX-2 promoter (30). Furthermore, transduction with a dominant negative inhibitor of NF-κB (IκBmut) markedly blunted the increase in COX-2 mRNA and protein expression observed after hypertonicity. Other studies suggest MAP kinase/JNK contributes to hypertonic induction of COX-2 (101). Water deprivation is associated with activation of several MAP kinases, including JNK, ERK, and p38, in the renal medulla (95); however, the in vivo sites where water deprivation activates these kinases have not yet been identified. In cultured mouse mIMCD-K2 cells, hypertonicity directly induces COX-2 via a mechanism involving p38 and MEK1 kinases (95). Inhibition of COX-2 severely reduced the ability of both cultured mIMCD-K2 and medullary interstitial cells to survive an increase in tonicity, suggesting that increased COX-2 expression may be an important survival factor in the renal medulla (see below).

The observed changes in COX-2 reporter activity in response to hypertonicity are not as robust as one might expect from the changes observed in COX-2 protein expression. Accumulating evidence suggests that COX-2 is modulated at multiple steps in addition to transcription rate, including stabilization of the mRNA and enhanced translation (37, 74, 87). Glucocorticoids, including dexamethasone, downregulate COX-2 mRNA in part by destabilizing the mRNA (74). The 3' untranslated region of COX-2 mRNA contains 22 copies of an AUUUA motif that are important in destabilizing COX-2 message in response to dexamethasone, whereas other 3'-sequences appear important for COX-2 mRNA stabilization in response to interleukin-1β (37, 74, 87). Effects of the 3'-untranslated region as well as other factors regulating efficiency of COX-2 translation have also been suggested (17, 38).

Regardless of the mechanisms leading to the expression of COX-2 in the renal medulla, the expression of COX-2 in medullary interstitial cells appears to play an important role in the ability of these cells to survive hypertonic stress after dehydration. Normally, ~100% of cultured medullary interstitial cells survive an abrupt increase in ambient tonicity from 300 to 500 mosmol/kgH2O; however, in the presence of submicro molar concentrations of the COX-2 inhibitor SC-58236, only ~50% of cells survive (30). Similarly, transduction of cultured medullary interstitial cells with a COX-2 anti-sense adenovirus significantly reduced survival from 96% in control adenovirus-transfected cells to ~50%. COX-2 also appears to play an important role in maintaining medullary interstitial cell viability, because, after treatment with a COX-2 inhibitor, water deprivation induced dramatic apoptosis of medullary interstitial cells, whereas simple water deprivation had no effect on their survival (30). Importantly, this effect was selective, and no apoptosis of medullary collecting duct cells was observed. These observations may have important implications for understanding the pathogenesis of NSAID-associated renal medullary injury.
COX-2-derived prostanoids may also play a critical role in maintaining renal medullary blood supply, renal salt excretion, and systemic blood pressure. COX-2-rich medullary interstitial cells span the area between the vasa rectae and medullary tubules, including thick limbs (54). Cultured medullary interstitial cells produce abundant PGE$_2$ (29), which has been shown to directly dilate vasa rectae, counteracting the constrictor effect of angiotensin and endothelin and thereby helping to maintain renal medullary blood flow (84). Measurements of medullary blood flow in intact renal papilla show that prostaglandins play an important role in maintaining the medullary blood supply, particularly in the setting of volume depletion (75). Recent studies in mice suggest that SC-58236, a COX-2-selective NSAID, significantly reduced renal medullary blood flow, whereas SC-58560, a COX-1-selective NSAID, had no acute effect (73).

Regulation of renal medullary blood flow has significant implications for regulating salt excretion and systemic blood pressure (13, 14). Reduced medullary interstitial pressure increases renal salt absorption (14). Medullary interstitial prostaglandins may modulate epithelial solute and water reabsorption not only via hemodynamic effects but also through direct effects on epithelial sodium absorption by the thick ascending limb and collecting duct (6). Loss of the tonic inhibitory effect of COX-2-derived PGE$_2$ on salt absorption by these segments may contribute to sodium retention seen with NSAIDs (5). Taken together, these data suggest that COX-2 inhibition in the renal medulla might not only enhance salt retention but also compromise medullary blood flow, risking hypoxic injury to the cellular elements in the renal medulla as well as directly risking medullary interstitial cell viability.

Long-term use of COX-inhibiting NSAIDs has been associated with papillary necrosis and progressive renal structural and functional deterioration (2, 82). NSAID-induced renal damage is more likely to occur in the setting of dehydration, suggesting a critical dependence of renal function on COX metabolism in this condition (79). Increased COX-2 expression after dehydration is consistent with this finding. Interestingly, in the present experiments, only patches of apoptotic medullary interstitial cells were seen after COX-2 inhibition and water deprivation, and papillary necrosis was not detected. This result, which is consistent with an earlier report that renal medullary interstitial cells are an early target of injury in analgesic nephropathy (66), also suggests that repeated injury to these cells may be required for development of analgesic nephropathy. Other roles for medullary interstitial cell COX-2-derived prostanoids may relate to maintenance of medullary blood flow. Taken together, increased COX-2

**Fig. 4. Transcriptional regulation of COX-2.**

A: Multiple response elements in the COX-2 promoter region. AP-2, activator protein-2; CRE, cAMP-responsive element; IL-6, interleukin-6; MEF-2, myocyte-enhancer factor. B: Activation of COX-2 expression by several of the response elements in response to growth factors, cytokines, serum, and hypertonicity. Activation of COX-2 expression by hypertonicity has been shown to involve nuclear factor (NF)$\kappa$B, c-Jun-NH$_2$-terminal kinase (JNK), and mitogen-activated protein or extracellular signal-regulated kinase (ERK; MEK1). In this sense COX-2 expression may be considered another osmotic response gene, responding to stimuli similar to the betaine transporter (BGT1) and aldose reductase (left). SAPK, stress-activated kinase; SEK, SAPK/ERK kinase; MEK, mitogens-activated protein (MAP) or ERK kinase.
expression could play an important role in maintaining viability and blood supply of the renal medulla.

In summary, COX-2 is expressed in the mammalian kidney in a localized distribution, and expression is physiologically regulated in response to alterations in the animal’s volume status. The regulation of enzyme expression in response to alterations in extracellular ionic composition indicates potentially important roles in the modulation of renal regulation of salt and water homeostasis.

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