Mineralocorticoid regulation of cyclooxygenase-2 expression in rat renal medulla

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Mineralocorticoid regulation of cyclooxygenase-2 expression in rat renal medulla. Am J Physiol Renal Physiol 283: F509–F516, 2002. First published April 30, 2002; 10.1152/ajprenal.00236.2001.—The renal inner medulla and its distal one-third, the papilla, are major sites of prostanoid synthesis involved in water and electrolyte homeostasis. These sites contain variable levels of cyclooxygenase (COX)-2, a key prostaglandin synthase enzyme that is sensitive to adrenal steroids. Immunoreactive renal medullary COX-2, restricted to interstitial cells in control adult rats, shows a gradient of intense staining at the tip of the papilla that gradually diminishes to undetectable levels in the proximal inner medulla. We used adrenalectomy (ADX) and steroid replacement to investigate the effects of steroids on papillary COX-2. Immunoblots demonstrate that papillary COX-2 was reduced by one-half after 2 wk ADX; glucocorticoid replacement averted the decline but not to control levels. Mineralocorticoid (deoxycorticosterone acetate; DOCA) replacement stimulated papillary COX-2 more than fivefold over control; both the intensity of immunostaining and the numbers of COX-2-positive cells in the inner medulla increased. Similar stimulation of papillary COX-2 resulted from DOCA treatment of normal control rats, but the response was blunted in rats fed a low-salt diet and absent in Brattleboro rats. DOCA treatment of mouse renal medullary interstitial cells in culture diminishes to undetectable levels in the proximal inner medulla. We used adrenalectomy (ADX) and steroid replacement to investigate the effects of steroids on papillary COX-2. Immunoblots demonstrate that papillary COX-2 was reduced by one-half after 2 wk ADX; glucocorticoid replacement averted the decline but not to control levels. Mineralocorticoid (deoxycorticosterone acetate; DOCA) replacement stimulated papillary COX-2 more than fivefold over control; both the intensity of immunostaining and the numbers of COX-2-positive cells in the inner medulla increased. Similar stimulation of papillary COX-2 resulted from DOCA treatment of normal control rats, but the response was blunted in rats fed a low-salt diet and absent in Brattleboro rats. DOCA treatment of mouse renal medullary interstitial cells in culture had no effect, but increased tonic of the culture medium caused strong upregulation of COX-2. Urea, a permeant molecule, had no effect. Together, these results suggest that mineralocorticoids lead to upregulation of COX-2 in rat renal medulla by indirect pathways, probably involving induced electrolyte hypertonicity in the interstitial fluid.

adrenal steroid; prostaglandin G/H synthase; papilla; deoxycorticosterone acetate

PROSTAGLANDINS, CYCLIC DERIVATIVES of arachidonic acid, mediate and/or modulate hormone action in regulating vascular tone and salt and water homeostasis in the mammalian kidney. The production of prostanoids is regulated at the initial cyclooxygenase (COX) step that converts arachidonic acid to prostaglandin (PG) H2. Aspirin and other nonsteroidal anti-inflammatory drugs specifically inhibit activity of the COX enzyme. In early studies of adult rat kidney, COX activity and immunoreactivity (COX-ir) were localized to the walls of arteries and arterioles, glomeruli, and collecting ducts (23), with highest levels concentrated in the inner medulla/papilla.

Two distinct COX genes have been identified: “constitutive” COX-1 encodes a 2.7- to 2.9-kb transcript, and “inducible” COX-2 encodes a 4.0- to 4.5-kb transcript (14, 20). COX-2 has been characterized as an immediate early gene that mediates inflammation, and many anti-inflammatory effects of adrenal glucocorticoid hormones putatively arise through downregulation of COX-2 expression. Because adrenal steroids and PGs significantly affect renal function, interactions between steroids and COX expression in the kidney are of interest.

More recently, renal expression of COX-2 has been shown to be highly regulated and spatially distinct from COX-1. Under control conditions, strong COX-2-ir was detected in interstitial cells of the inner medulla and in sparse scattered cells of cortical thick ascending limb of Henle’s loop (cTAL) and macula densa (11); cortical COX-2 was amplified in rats fed low-salt diets. Subsequent studies have demonstrated regulation of renal cortical COX-2 expression during development and in response to physiological changes such as volume expansion, inhibition of angiotensin-converting enzyme, and renal ablation (3, 25, 29). A direct role for steroids was suggested by experiments including adrenalectomy, steroid supplementation, and steroid receptor inhibition that demonstrated tonic downregulation of cortical COX-2 by both glucocorticoids and mineralocorticoids (MC) (28).

Both isoforms of COX have been detected at high levels in the inner medulla and papilla of normal mammalian kidneys; however, different laboratories using various detection methods in various species have reported disparate expression patterns. Consistent with the inducible/constitutive dichotomy, COX-2 levels and loci are more variable than COX-1. Significant species/strain variations are apparent; e.g., COX-2 reportedly...
is concentrated in outer medullary interstitial cells in rabbits (7), in papillary interstitial cells in rats and humans (11, 18), or in collecting duct epithelium in water-deprived rats (26) or not at all in monkeys and humans (12).

In light of the profound steroid effects on cortical COX-2 expression (28), the present studies were designed to employ similar experimental regimens of steroid modulation to elucidate factors regulating COX-2 expression in the renal papilla of adult rats.

METHODS

Animals. Male or female rats of Sprague-Dawley and Long-Evans strains as well as F1 hybrids (LE-SD) were used at 200–300 g. Surgical procedures were performed under sterile conditions and Nembutal anesthesia. After bilateral adrenalectomy (ADX) via a single dorsal incision, sham-operated controls and ADX rats received 1% NaCl in tap water ad libitum to prevent volume depletion. Glucocorticoid or MC replacement or supplementation was achieved with subcutaneous pellets of silastic rubber containing (3:1) deoxycorticosterone acetate (DOCA) at 1.5 g/kg body wt or pressed pellets containing (1:1) corticosterone (CS) at 1.5 g/kg body wt to achieve a serum dosage of 15 mg·day⁻¹·kg⁻¹. For dietary experiments, the rats received low-salt food (0.03% NaCl) for 2 wk (ICN Radiochemicals). To confirm the efficacy of these treatments, metabolic cages were used to collect 24-h urine immediately before termination of the experiment; rats on low-salt diet for >6 days had urinary sodium below the levels of detection (<1 meq/l). Water deprivation was carried out by removal of water from the rats for defined periods, with continued free access to food. Combinations of the above protocols are explained in the RESULTS.

Immunohistochemistry. In general, at the termination of an experiment, the rats were deeply anesthetized with Nembutal (70 mg/kg ip); one kidney was removed for Western blot analysis, and the other was preserved in situ for histology by perfusion through a transcardial aortic cannula. Exsanguination with 500 ml/kg heparinized saline (0.9% NaCl, 2 units/ml heparin, and 0.02% sodium nitrite) was followed by 1,500 ml/kg of fixative containing 3.7% formaldehyde or 2.5% glutaraldehyde in an acidic solution (pH 4.5) with phosphate, 1,500 ml/kg heparin, and 0.02% sodium nitrite) was followed by 1,500 ml/kg of fixative containing 3.7% formaldehyde or 2.5% glutaraldehyde in an acidic solution (pH 4.5) with phosphate, periodate, acetate, and NaCl as described (16). The acidified aldehyde fixatives enabled reliable preservation of COX-2 antigenicity in combination with papillary structural integrity. The fixed kidney was dehydrated through a graded series of ethanol, embedded in paraffin, sectioned (4 μm), and mounted on glass slides.

COX-1-ir was immunolocalized with affinity-purified goat polyclonal anti-human COX-1 carboxy terminus antibody no. C-20 (Santa Cruz Biotechnology) at a 0.20–μg IgG/ml dilution. COX-2-ir was immunolocalized with affinity-purified rabbit polyclonal anti-murine COX-2 peptide (residues 570–598) antibody no. 160126 (Cayman Chemicals, Ann Arbor, MI) at a 0.25-μg IgG/ml dilution. The primary antibodies were localized by use of Vectastain ABC-Elite (Vector, Burlingame, CA) with dianaminobenzidine as chromogen, followed by a light counterstain with toluidine blue. The specificity of our COX-2 immunolocalization was confirmed by two fundamental tests (15). Staining was eliminated by preabsorption of the primary serum with COX-2 protein purified from the rat distal vas deferens epithelium (17); COX-2-ir colocalized with COX-2 mRNA detected by in situ hybridization (28).

Immunoblotting. Homogenates of papillae (4 ml/each papilla) were prepared in 20 mM Tris·HCl, pH 8.0, with protease inhibitor mixture (Boehringer Mannheim). After 10 min of centrifugation at 10,000 g, the supernatant was centrifuged for 60 min at 100,000 g to sediment microsomes as described (11). The microsomes were resuspended in homogenizing buffer, mixed with equal volume of 2× SDS sample buffer, and boiled for 5 min. The proteins were separated on 10% SDS gels under reducing conditions and transferred to Immobilon-P transfer membranes (Millipore, Bedford, MA). After a blocking overnight with 20 mM Tris·HCl (pH 7.4)-500 mM NaCl-5% nonfat milk-0.5% Tween 20, the blots were incubated for 3 h at room temperature with rabbit polyclonal anti-murine COX-2 (Cayman Chemicals) at a 0.25-μg/ml dilution or goat polyclonal anti-human COX-1 at a 1.25-μg/ml dilution. The primary antibodies were detected with peroxidase-labeled goat anti-rabbit IgG or rabbit anti-goat IgG (Santa Cruz Biotechnology) and exposed on film by use of enhanced chemiluminescence (Amersham International). The serial dilution method (see Fig. 2) was used for analysis of relative protein levels in critical experiments.

Cell culture. Renomedullary interstitial cells type 1 (renal papillary fibroblasts) were prepared according to the methods of Burger-Kentischer et al. (2). Female C57BL/6 mice (20 g) were killed under deep anesthesia with Nembutal (70 mg/kg ip), and the kidneys were rapidly removed, washed with Ringer solution, decapsulated, and cut tangentially. The renal papilla was excised, minced, and placed in 10 ml of Ringer solution containing 1 mg/ml collagenase (Boehringer Mannheim, Mannheim, Germany). The suspension was agitated at 37°C for 2 h, with short interruptions every 15–20 min, during which tissue pieces were passed repeatedly through the tip of a 10-ml plastic pipette. The suspension was centrifuged at 200 g for 3 min, and the pellets were resuspended in Dulbecco’s modified Eagle’s-Ham’s F-12 medium (DMEM/F-12; Gibco, Eggstein, Germany) at 1:1 supplemented with 10% fetal calf serum (FCS; Boehringer Mannheim), 50 μl/ml penicillin, and 50 μg/ml streptomycin (Sigma). Cells were plated in 75-ml flasks and grown in DMEM/F-12 (1:1) containing 10% FCS. After four passages, >90% of the cells displayed oil-red-O-positive lipid droplets, characteristic of interstitial cells.

Micrography. Brightfield images from the Leitz Orthoplan microscope with DVC digital RGB video camera were digitized by the Bioquant image-analysis system and saved as computer files. Contrast and color level adjustment (Adobe Photoshop) were performed for the entire image; i.e., no region- or object-specific editing or enhancements were performed.

RESULTS

Renal papillary COX in adult rats under control conditions. Strong COX-1-ir was apparent in collecting duct epithelial cells in the inner medulla and papilla and in the epithelium covering the tip of the papilla (Fig. 1A and B); it was absent from the urothelium lining the renal pelvis. Nearly all renal medullary interstitial cells (RMIC) in the papilla displayed strong COX-1-ir (Fig. 1B), but RMIC in the proximal one-half of the inner medulla showed less COX-1 staining, and no COX-1-ir was detected in RMIC of the outer medulla. Because no changes in levels or localization of COX-1 were noted in the experiments below, no further results pertaining to COX-1 are presented.

COX-2-ir was not detected in papillary epithelial cells: it was detected neither on the papillary surface nor in collecting ducts nor vasa recta nor Henle’s loops.
Strong COX-2-ir was present in nearly all RMIC at the tip of the papilla (Fig. 1C), but COX-2 staining intensity in RMIC was significantly diminished in the outer one-half of the inner medulla and absent in the outer medulla. At higher magnification, immunopositive processes from the interstitial cells were observed to encircle the thin limbs and/or vasa recta, creating rows of puncta apposed to the bases of the epithelial cells (Fig. 1D, arrowheads).

Western (immunoblot) analysis was used to provide semiquantitative estimates of COX-2 levels. On the basis of prior evidence that virtually all COX-2-ir in cellular homogenates sedimented in the microsome fraction, baseline control levels of COX-2 were compared with the use of microsomes from equivalent wet weight tissue samples of adult rat renal papilla and cortex. In typical results from two rats (Fig. 2), the bands produced by loading 50-μl samples of cortex microsomes were barely detectable (lanes 1 and 2), whereas three smaller samples of papilla microsomes (0.5, 1.5, and 4.5 μl) loaded in adjacent lanes produced substantial bands. Because the bands generated by 0.5 μl of papilla microsomes (lanes 5 and 8) were darker than the bands from 50 μl of cortex (lanes 1 and 2), we conclude that COX-2 protein in the papilla was significantly more than 100-fold greater than in the cortex. In experiments to test regulation of COX-2 in the papilla as presented below, effects in the cortex were monitored concurrently to provide contrast and to control for the efficacy of the execution.

Fig. 1. Immunolocalization of cyclooxygenase (COX)-1 (A and B) and COX-2 (C–F) in renal papillae of adult rats. COX-1 immunoreactivity (COX-1-ir) is present not only in the papilla but extends up into the outer medulla (A); COX-2-ir is limited to the distal one-half of the papilla in control rats (C) but is both darker and present in more renal medullary interstitial cells (RMIC) of the inner medulla after 2 wk of deoxycorticosterone acetate (DOCA) supplementation (E). Higher magnifications reveal that collecting duct (D) epithelium stains for COX-1 (B) but not COX-2 (D and F); epithelia of the thin limbs (T) and vasa recta (V) do not stain for either COX. Slender processes of RMIC wrapping around the thin limbs also exhibit COX-2-ir (D and F, arrowheads). Field widths: left = 2,800 μm; right = 212 μm.

Fig. 2. Western blot of COX-2 expression in microsomes prepared from adult rat kidney. Cortex lanes (C1 and C2) loaded with 50 μl of microsome suspension compared with papilla lanes (P1 and P2) loaded with much smaller samples ranging down to 0.5 μl demonstrate that COX-2 in the papilla is >100× cortex based on wet wt.
Adrenal steroid effects. Under control conditions, COX-2 expression in the rat renal cortex was reported to be tonically downregulated by adrenal steroids (28). Consequently, the low levels of COX-2 demonstrated in cortex (Fig. 3A, top, lanes 1 and 2) were dramatically upregulated 14 days after ADX (lanes 3 and 4) and were downregulated to control levels by CS replacement in ADX littermates (lanes 5 and 6).

In papillae from these same kidneys, COX-2 responses to steroids were quite different from cortex (Fig. 3A, bottom). Control papillae displayed substantial COX-2 expression (lanes 1 and 2) that decreased 50–60% after ADX (lanes 3 and 4); CS replacement reversed the decrease but did not reach control levels (lanes 5 and 6). Variations of steroid replacement with CS alone (±times and/or ±dosage) were unable to restore papillary COX-2 to control levels. Histologically, the numbers and location of papillary COX-2 cells in ADX rats were similar to controls, but the intensity of staining was decreased (not illustrated).

Because MC also are eliminated by ADX, the aldosterone analog DOCA was administered via subcutaneous pellets to ADX and adrenal-intact rats (Fig. 3B). It should be noted that the magnitude of the responses required adjustment of sensitivity in this Western blot and others shown below; it is necessary to evaluate each experimental response relative to the density of control bands in the same figure. Specifically, the control and CS samples in Fig. 3B (lanes 1–3) were equivalent to the dark bands in Fig. 3A (lanes 1, 2, 5, and 6), but these samples appear as very light bands with the shorter exposure used to avoid overexposure of lanes 4–7 in Fig. 3B. Thus DOCA supplementation for 14 days increased papillary COX-2 5- to 10-fold in control rats (Fig. 3B, lanes 4 and 5); similar upregulation was apparent in DOCA-supplemented ADX rats (lanes 6 and 7). In histological sections from these specimens (Fig. 1, E and F), COX-2-ir in RMIC of the DOCA-supplemented papilla was only slightly stronger than controls; however, many more RMIC of the inner medulla also exhibited strong COX-2 expression. Collecting ducts and other epithelia remained completely negative, as did interstitial cells of the outer medulla.

The roles of MC receptors (MR) and glucocorticoid receptors (GR) in regulation of renal papillary COX-2 expression were confirmed and compared by daily administration of specific competitive inhibitors for 2 wk with mature rats with intact adrenals (Fig. 3C). Compared with control (lanes 1 and 2), the MR inhibitor spironolactone decreased papillary COX-2 expression by 50–70% (lanes 3 and 4), whereas the GR inhibitor RU486 caused no appreciable changes (lanes 5 and 6).

Salt deprivation from 2 wk on a low-sodium diet did not have dramatic effects alone. Cortical COX-2 expression was moderately stimulated (Fig. 4A, top, lanes 3 and 4), and papillary COX-2 expression was moderately depressed (Fig. 4A, bottom). However, the COX-2 responses of salt-deprived rats to DOCA supplementation were quite different from controls. In contrast to the 5- to 10-fold stimulation of papillary COX-2 observed in salt-replete rats (Fig. 3B), papillary COX-2 barely reached control levels in DOCA-supplemented,

![Fig. 3](image-url) Steroid effects on COX-2 expression. A: rat renal cortex and papilla after 2 wk of adrenalectomy (ADX) without and with corticosterone (CS) replacement. B: renal papillary COX-2 expression after supplementation with CS or DOCA in intact rats, and DOCA replacement in ADX rats. C: COX-2 in renal cortex after 2 wk of treatment with steroid receptor antagonists spironolactone [mineralocorticoid receptor (MR) inhibitor] or RU486 [glucocorticoid receptor (GR) inhibitor].

![Fig. 4](image-url) Effects of volume contraction on COX-2 in renal papilla and cortex. A: 2 wk of low-salt diet (LS) with DOCA. B: water deprivation (WD; 42 h) and ADX (5 days + 42 h).
salt-deprived rats (compare lanes 5 and 6 with lanes 1 and 2); i.e., the response was severely blunted.

Water deprivation. On the basis of reports that water deprivation (WD) caused upregulation of papillary COX-2 (26), we sought to determine whether this response depended on endogenous MC. In rats with intact adrenals, control levels of papillary COX-2 (Fig. 4B, bottom, lane 1) increased two- to threefold after 42 h of WD (lane 2). As expected, basal levels of papillary COX-2 were reduced in ADX rats (lane 4); WD stimulated slight upregulation of COX-2 in ADX rats (lane 3). Histologically, the increased COX-2-ir in the papilla of WD rats was entirely contained in interstitial cells (not illustrated); no evidence of COX-2-ir was observed in collecting duct or other epithelial cells.

Because ADX profoundly modulated the effects of WD on COX-2 expression in both cortex and papilla (compare lane 2 with 3 in Fig. 4B), we postulated that DOCA replacement would reverse these effects and that the speed of such a reversal would indicate the immediacy of the mechanism. For example, a rapid reversal would suggest direct effects of DOCA on COX-2 expression. To test these hypotheses, ADX rats received DOCA replacement either at the time of or before 2 days of WD (Fig. 5).

In the cortex, COX-2 was elevated after ADX (Fig. 5, top, lane 1) and was further stimulated by 2 days of WD before death (lane 2). DOCA replacement for 2 days (initiated at the onset of WD) did not have significant influence on cortical COX-2 (lane 3), but DOCA administered for 3 or 4 days (initiated either 1 or 2 days before WD) effectively suppressed cortical COX-2 to control levels (lanes 4 and 5).

In the papilla, COX-2 was present at reduced levels after ADX (Fig. 5, bottom, lane 1) and was stimulated only slightly by WD in the absence of steroids (lane 2). Whereas cortical COX-2 showed dramatic responses to DOCA as described above (Fig. 5, top), the papillary response was graded (Fig. 5, bottom). Two days of DOCA produced a slight increase (Fig. 5, bottom, lane 3) that increased gradually over the next 2 days (lanes 4 and 5). Thus the response of papillary COX-2 to DOCA replacement was gradual over several days rather than rapid, and, therefore, the hypothesis of direct steroid action on papillary COX-2 expression was not supported.

In Fig. 5, Effects of DOCA replacement on renal cortex and papilla COX-2 expression in ADX (7 days) rats that were WD for the last 2 days. DOCA was administered 2, 3, and 4 days (2d, 3d, and 4d, respectively) before death.

RMIC in vitro. As reported previously, COX-2 is expressed by cultured RMIC under control conditions, and hypertonic medium induces significant COX-2 up-regulation (9). To test for direct influences of steroids on COX-2 synthesis, RMIC isolated from mice were treated with CS or DOCA at incremental physiological doses under isotonic and hypertonic conditions (Fig. 6, A and B). Basal COX-2 levels without added steroid were at least fivefold increased in the medium made hypertonic (500 mosmol/kgH2O) by addition of NaCl. Addition of DOCA from 0.01 to 1.0 mg/ml had no effect on COX-2 expression in either isotonic or hypertonic media (Fig. 6B). In contrast, addition of CS over a similar range of concentrations produced dose-dependent reductions in both isotonic and hypertonic media (Fig. 6A).

Further experiments with cultured interstitial cells investigated the nature of osmotic factors that influence COX-2 expression (Fig. 6C). Hypertoncity produced by addition of 200 and 250 mosmol/kgH2O NaCl upregulated COX-2 (lanes 2 and 3, respectively), but equivalent hypertoncity generated by added urea had no effect (lanes 4 and 5). Similarly, 50 mosmol/kgH2O of added urea did not diminish the upregulation caused by 200 mosmol/kgH2O of NaCl (lane 6), and 200 mosmol/kgH2O of urea was not significantly augmented by 50 mosmol/kgH2O of NaCl (lane 7).

DOCA response in Brattleboro rats. Because the in vitro experiments seemed to indicate that COX-2 expression in RMIC responded to electrolyte toxicity
rather than to direct MC stimulation, an additional test was performed giving DOCA to Brattleboro rats with diabetes insipidus due to hereditary vasopressin deficiency (Fig. 7).  

Brattleboro rats administered cholesterol pellets (sham control) for 7 days displayed normal levels of cortical COX-2 (Fig. 7, top, lane 2); cortical COX-2 was severely downregulated in rats receiving DOCA pellets (lanes 1 and 3). In contrast, papillary COX-2 (Fig. 7, bottom) was present at very low levels in sham control Brattleboro rats (lane 2) and was not influenced at all by DOCA supplementation (lanes 1 and 3).  

DISCUSSION  

The relative importance of prostanoids derived from COX-1 vs. COX-2 for modulation of renal salt and water excretion is still a subject of active investigation. Although COX-1 is expressed in vasculature, mesangial cells, and collecting duct, studies in animals and humans with COX-2-selective inhibitors have suggested an important, if not predominant, role for COX-2-derived prostanoids. PGs are known to regulate renal sodium resorption by their ability to inhibit active transport of sodium in both the thick ascending limb and collecting duct and to increase renal water excretion by blunting the actions of vasopressin (24). All of these effects are inhibited by COX-2 inhibitors (10). The cellular source of COX-2-derived prostanoids that promote natriuresis remains uncertain, but it is possible that they may in large part be derived from the medullary interstitial cells. These cells are closely associated with medullary blood vessels and renal tubules and may provide PGs that both inhibit tubule sodium resorption and also dilate the vasa recta, thereby increasing medullary flow and leading to decreased medullary interstitial toxicity.  

The present studies were designed to investigate adrenal steroid regulation of cyclooxygenase expression in rat renal papilla; responses in the cortex were evaluated concurrently for contrast and control. COX-1 levels were stable, whereas COX-2 expression in the papilla generally exhibited moderate changes that were opposite from those in the cortex. Administration of the MC DOCA induced significant upregulation of papillary COX-2 in normal rats, but the response to DOCA was blunted by salt deprivation, and there was no response to DOCA in Brattleboro rats.  

Fig. 7. Effects of DOCA treatment on renal COX-2 expression in Brattleboro rats with diabetes insipidus due to hereditary vasopressin deficiency (BB Di/Di rats). BB Di/Di rats were treated with DOCA via subcutaneous embedding for 1 wk. Renal cortical and papillary microsomes were prepared, and Western blot analysis was performed. Lanes 1 and 3, DOCA treatment; lane 2, control. DOCA suppressed renal cortical COX-2 expression but did not stimulate papillary COX-2 expression.
suppress COX-2 expression at sites of inflammation (5, 19); both glucocorticoids and MC suppress COX-2 in cultured rat mesangial and Madin-Darby canine kidney (MDCK) cells (22) as well as in renal cortex when administered to intact rats (28). Total depletion of steroids by ADX produced dramatic amplification of cortical COX-2, suggesting that steroids tonically suppress COX-2 under normal conditions. These data were consistent with the hypothesis that COX-2 transcription is directly regulated by steroids.

However, from our experiments, it soon became apparent that the response of papillary COX-2 to steroids was more complicated. RMIC in culture responded in a canonical dose-dependent fashion to exogenous CS, i.e., higher CS led to lower COX-2 expression, but DOCA had no effect on the COX-2 in RMIC. In situ, rather than stimulating papillary COX-2, ADX caused partial downregulation of papillary COX-2, and glucocorticoid replacement with CS after ADX only partially restored papillary COX-2 levels. Thus the response in cultured RMIC was opposite that in the papilla in situ, and the direction of response in situ was opposite the canonical glucocorticoid suppression of COX expression.

Furthermore, DOCA supplementation in normal rats or MC replacement after ADX stimulated COX-2 expression in papillary interstitial cells more than fivefold over control. Although experiments with spironolactone and RU486, specific inhibitors of the steroid receptors, clearly demonstrated that COX-2 upregulation in the papilla depended on activation of the MR, additional experiments suggested that this effect depended on increased electrolyte concentration in the medullary interstitium. The manyfold upregulation of papillary COX-2 required normal or high-salt diets. The decreased baseline of papillary COX-2 produced by a low-salt diet approximately doubled in response to DOCA but remained less than control levels with a normal diet.

Direct measurements showed that the fraction of interstitial osmolality due to electrolytes was significantly reduced with a low-salt diet; urea concomitantly increased to compensate (6). Thus we postulate that the COX-2 response to DOCA normally is obtained through the MC stimulation of sodium resorption into the medullary interstitium, and the response is blunted in low-salt-diet rats because of the paucity of interstitial salt.

Additional evidence for the importance of papillary electrolyte toxicity in the regulation of COX-2 expression was generated by experiments using Brattleboro rats with inherited diabetes insipidus. These vasopres*sin-deficient rats produce copious dilute urine and, although their papillary interstitium is very hypotonic, have approximately normal levels of papillary interstitial sodium (4). In our studies, cortical COX-2 in the Brattleboro rats appeared normal and was strongly suppressed by DOCA supplementation. Papillary COX-2 was severely downregulated in control Brattleboro rats and showed virtually no response to DOCA. Polyuria continued unabated in the DOCA-treated Brattleboro rats, presumably indicating no change in papillary toxicity. Thorough discussion of these experiments is beyond the scope of this communication; however, this lack of response to DOCA strongly suggests that the DOCA effect on COX-2 in normal rats is not mediated by a direct action of MR on the COX gene.

The differences in cortical and medullary COX-2 expression in response to steroids are probably twofold. First, there may be a direct effect of glucocorticoids and mineralocorticoids on macula densa and cTaLH cells, because these cells express GR and MR, whereas medullary interstitial cells do not (at least they do not express MR). Second, systemic effects of mineralocorticoids would favor opposite effects on expression (macula densa/cTaLH COX-2 increases with volume depletion and/or decreased NaCl resorption, which will occur in the absence of MC, whereas medullary interstitial COX-2 increases with increased toxicity, which will occur in response to aldosterone).

WD. Upregulation of papillary COX-2 has been reported in response to the induction of renal medullary hypertonicity by a high-salt diet and/or WD (26, 27). Our results with cultured RMIC demonstrated that COX-2 was upregulated when the medium was made hypertonic with salt or impermeant solutes but not affected by hypertonicity due to urea, a solute to which these cells are permeable. We postulated that hypertonicity could be a factor in the response to DOCA in vivo.

In control rats, WD for 42 h caused a robust threefold increase in papillary COX-2. The WD effect was significantly blunted by ADX. However, further experiments demonstrated that the recovery from ADX was gradual. DOCA must be present for at least 1 day before WD to enable any significant upregulation of papillary COX-2, and even 2 days before WD were insufficient to allow the full response. These data defined a time frame of several days for DOCA to become effective and indicated that a direct influence of steroids on transcriptional regulation of papillary COX-2 is unlikely.

We postulate that COX-2 expression increases preferentially in the inner medulla with water restriction because medullary toxicity increases, which is a stimulus for COX-2 expression in medullary interstitial cells in vitro. With more severe water restriction and associated volume depletion, cortical COX-2 expression will also increase, but in our experience this occurs later than what is seen in the medulla.

**MC effects.** MC act by binding to cytoplasmic receptors (MR) that enter the nucleus and bind to response elements to stimulate gene transcription; presumably, similar interaction with other elements can inhibit other genes. In the kidney, MR are present in collecting duct epithelium in the cortex and medulla; MC stimulate upregulation in these tubules of two proteins involved with sodium transport, the epithelial cell sodium channel (ENaC) that situates in the apical membrane and the Na⁺-K⁺-ATPase that localizes to the basolateral membrane. These proteins play active roles in the exchange of serum potassium for urinary sodium. After implantation of DOCA pellets similar to ours, it has been shown that upregulation of ENaC and
Na\(^+\)-K\(^+\)-ATPase can be detected in 4–6 h and continues until a plateau is reached by 24 h. Upregulation of these transport proteins correlates initially with increased sodium retention and potassium excretion in the urine, but the phenomenon known as “MC escape” leads to the return of sodium balance in a few days (13).

Upregulation of papillary COX-2 expression coincides with MC escape; i.e., it is detectable by 2 days, rises through day 7, and remains elevated as long as the MC/MR stimulus is present. Although many questions remain regarding the mechanisms responsible for MC escape, experiments have demonstrated that MC effects in the renal papilla include increased oncotic and hydrostatic pressures (8). We postulate that COX-2 also would be upregulated in response to hyper-oncoticity in the papilla, and because PGE\(_2\) has been shown to reduce both sodium and water uptake from inner medullary collecting ducts (1), production of PGs by papillary interstitial cells could contribute to the mechanism of MC escape. Future investigations may illuminate the equilibrating factors that integrate upregulated papillary COX-2 with MC escape.

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