Regulation of renal cortical cyclooxygenase-2 in young rats

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Zhang, Ming-Zhi, Su-wan Wang, Huifang Cheng, Yahua Zhang, James A. McKanna, and Raymond C. Harris. Regulation of renal cortical cyclooxygenase-2 in young rats. Am J Physiol Renal Physiol 285: F881–F888, 2003.—Cyclooxygenase-2 (COX-2) is involved in kidney morphogenesis and is transiently elevated in the immature kidney. In adult rats, renal cortical COX-2 expression is tonically suppressed by mineralocorticoids (MC) and glucocorticoids (GC) and induced by chronic salt restriction. Young rats have low levels of GC and are in a state of relative volume depletion. The present study was designed to investigate the mechanisms underlying elevated cortical COX-2 expression in the immature kidney. Supplementation of GC or MC suppressed cortical COX-2 expression in suckling rats. GC suppression was completely prevented by a mineralocorticoid receptor antagonist. Salt supplementation suppressed cortical COX-2 expression in a dose- and time-dependent pattern in the suckling rats. Cortical COX-2 expression in the weaning rats was upregulated by a low-salt diet and downregulated by a high-salt diet. These results suggest that relative volume depletion and reduced GC levels are involved in elevated cortical COX-2 expression in the immature rodent kidney.

prostaglandin synthase G_{2}/H_{2}; mineralocorticoid; glucocorticoid; salt supplementation

PROSTAGLANDINS ARE INVOLVED in mammalian kidney development, and they also regulate renal vascular tone and salt and water homeostasis in adult kidneys (11, 12, 18, 22, 27, 37). Prostaglandin production depends on three key steps: 1) release of arachidonic acid from membrane phospholipids by phospholipase A_{2}; 2) conversion of the arachidonic acid to PGH_{2} by cyclooxygenase (COX); and 3) further metabolism by specific prostaglandin syntheses. Two separate gene products with COX activity have been described, COX-1 and COX-2 (5, 20, 29). The “constitutive” COX-1 gene encodes a 2.7- to 2.9-kb transcript of COX-1, whereas the COX-2 gene encodes a 4.5-kb transcript that is “inducible” and glucocorticoid sensitive. In the kidney, COX-1 has been localized to mesangial cells, arteriolar endothelial cells, parietal epithelial cells of Bowman’s capsule, collecting duct epithelial cells (both cortical and medullary), and medullary interstitial cells (33).

COX-2 has been localized to cells of the macula densa and surrounding cortical thick ascending limb of Henle (cTAL) and to regulated populations of interstitial cells in the inner medulla/papilla (13, 42).

In adult rat kidney, cortical COX-2 expression is tonically suppressed by mineralocorticoids (MC) as well as by glucocorticoids (GC) (43). Cortical COX-2 mRNA and protein expression are inversely related to dietary salt intake (13, 15–17, 39). Cortical COX-2 expression is also regulated by the renin-angiotensin-aldosterone system (3, 4). In previous studies of the ontogeny of renal cortical COX-2 expression, we reported that in rats and mice, COX-2 mRNA and protein were present in neonates, peaked in postnatal weeks 2 and 3, and declined to adult levels by postnatal month 3. However, the mechanisms regulating this elevated cortical COX-2 expression in immature kidney remain unclear (18, 44).

Given the regulation of renal cortical COX-2 expression in mature animals by alterations in intravascular volume, it is noteworthy that the suckling rat is in a state of relative volume depletion, and plasma levels of corticosterone (CS), the predominant circulating GC in rodent, are low (14, 34). In the present study, we found that supplementation of salt or adrenal steroids effectively suppressed renal cortical COX-2 expression in suckling rat kidneys, suggesting that relative volume depletion and low levels of CS contribute to elevation of cortical COX-2 expression in suckling rats.

MATERIALS AND METHODS

Animals. Pregnant Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). Each litter was reduced to 8–10 pups just after birth. The pups were kept with their mothers until postnatal day 19 (P19). GC or MC supplementation was achieved with subcutaneous pellets (50% cholesterol) of DOCA or CS or daily injections to achieve a dose of 15 mg·kg^{-1}·day^{-1} (43). The glucocorticoid receptor (GR) antagonist RU486 and mineralocorticoid receptor (MR) antagonist spironolactone were given at doses of 7.5 and 20 mg·kg^{-1}·day^{-1}, respectively, by daily intramuscular injection. Salt supplementation was achieved via subcutaneous injection of sterile normal saline in the back of the suckling and weaning rats. The rats were weaned at P19 and then maintained on normal rat chow, rat chow deficient in sodium (LS; 0.02–0.03% Na^{+}), or rat chow with high salt (HS; 8% Na^{+}).

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NaCl for 1 wk (ICN Biochemicals, Costa Mesa, CA). LS diet-treated rats were given a single intraperitoneal injection of furosemide (1 mg/kg) before being placed on the LS diet. For dietary salt intake experiments, 24-h urine was collected before death. Urinary sodium excretion was determined using a flame photometer. The dams and pups were kept on a 12:12-h light-dark cycle. To determine plasma CS and aldosterone levels, all the animals were treated in the same way to collect blood samples. The animals were removed from the cage at 8 AM and immediately anesthetized with Nembutal at a dose of 70 mg/kg ip. Blood samples were collected from the heart, put into EDTA-coated tubes, and centrifuged. Plasma was collected and kept at -70°C until use. Plasma CS or aldosterone levels were determined using RIA kits (ICN Biochemicals).

**Immunohistochemistry.** In general, at the termination of an experiment, one kidney from each rat was removed for Western blot analysis and the other was perfused with fixative in situ for histology. Under deep anesthesia with Nembutal (70 mg/kg ip), the rats were first exsanguinated with 50 ml/100 g heparinized saline (0.9% NaCl, 2 U/ml heparin, 0.02% sodium nitrite) through a transcardial aortic cannula and fixed for COX-2 immunohistochemistry with 3.5% formaldehyde in an acidic solution (pH 4.5) containing phosphate, periodate, acetate, and sodium chloride as described (24). The acidified aldehyde fixatives were crucial for reliable preservation of immature kidney structure and COX-2 antigenicity. The fixed kidney was dehydrated through a graded series of ethanol, embedded in paraffin, sectioned (4 μm.), and mounted on glass slides. Immunohistochemical procedures were facilitated by creating compound blocks with multiple specimens that were sectioned and stained together.

COX-2 immunoreactivity (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 using Vectastain ABC-Elite (Vector, Burlingame, CA) with diaminobenzidine as chromogen, followed by a light counterstain with toluidine blue. The specificity of our COX-2 immunolocalization was confirmed by two fundamental tests (23). Staining was eliminated by preabsorption of the primary serum with COX-2 protein purified from the rat distal vas deferens epithelium (25), and COX-2-ir colocalized with COX-2 mRNA was detected by in situ hybridization (43).

**Immunoblotting.** Homogenates were prepared in 20 mM Tris-HCl, pH 8.0, with a proteinase inhibitor mixture (Boehringer Mannheim). After 10-min centrifugation at 10,000 g, the supernatant was centrifuged for 60 min at 100,000 g to sediment microsomes as described (13). The microsomes were resuspended in homogenizing buffer, mixed with an 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 being blocked overnight with 20 mM Tris-HCl, pH 7.4/500 mM NaCl/5% nonfat milk/0.5% Tween 20, the blots were incubated overnight at 4°C with rabbit polyclonal anti-murine COX-2 (Cayman Chemicals) at a dilution of 0.1 μg/ml. The primary antibodies were detected with peroxidase-labeled goat anti-rabbit IgG (Santa Cruz Biotechnology) and exposed on film by using enhanced chemiluminescence (Amerham International).

**Quantitative image analysis.** On the basis of the distinctive density and color of COX-2-ir in video images, the number, size, and position of stained cells were quantified using the BIOQUANT true-color windows system (R & M Biometrics, Nashville, TN) equipped with digital stage encoders that allow high-magnification images to be mapped to global coordinates throughout the whole section. The whole renal cortex from each section was quantified at ×160 magnification. Sections from at least three regions of each kidney were analyzed, and the average was used as data from one animal sample.

**Micrography.** Brightfield images from a Leitz Orthoplan microscope with a 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.

**Statistical analysis.** All values are presented as means ± SE. ANOVA and Bonferroni i-test were used for statistical analysis, and differences were considered significant when \( P < 0.05 \).

**RESULTS**

**Effects of salt supplementation on renal cortical COX-2 expression in suckling rats.** As previously reported, renal cortical COX-2 expression is tonically elevated in immature rat kidney compared with adult

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**A**

**Control**

| 3.2g/kg | 1.6g/kg | 0.8g/kg | 0.4g/kg |

**B**

**Control**

| 1d | 2d | 3d |

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**Fig. 1.** A: dose-dependent inhibition of cortical cyclooxygenase (COX-2) expression by salt supplementation. Suckling rats were treated with different doses of sterile normal saline for 7 days [postnatal days 7-14 (P7-P14)]. Maximal inhibition was achieved at an NaCl dose of 3.2 mg/g -1·day -1. B: time course of the effect of salt supplementation on cortical COX-2 expression at an NaCl dose of 3.2 mg/g -1·day -1. A and B, insets: representative experiments. CTRL, control; d, day; ir, immunoreactivity. \( **P < 0.01; n = 4. \)
kidney and is upregulated in mature rat kidney in response to chronic salt restriction (13, 44). To investigate whether elevated cortical COX-2 expression in the suckling rats was related to relative sodium deficiency, suckling rats were injected with sterile normal saline at different doses for 1 wk (from P7 to P14). As shown in Fig. 1A, cortical COX-2 expression was dose dependently suppressed by salt supplementation. Renal cortical COX-2 expression was not inhibited by salt supplementation with a dose of 0.8 mg NaCl·g body wt⁻¹·day⁻¹ but was inhibited almost twofold at a dose of 1.6 mg NaCl·g⁻¹·day⁻¹, and maximal inhibition was observed with a dose of 3.2 mg NaCl·g⁻¹·day⁻¹ (over 10-fold decrease vs. control).

To investigate the time course of the inhibitory effects of salt supplementation on cortical COX-2 expression, suckling rats were treated with normal saline at a dose of 3.2 mg NaCl·g⁻¹·day⁻¹, and all animals were killed at P14. As shown in Fig. 1B, suppression of COX-2 expression was already obvious 1 day after treatment and was maximal after treatment for 3 days. Treatment for 10 days (from P5 to P15) did not further suppress COX-2 expression but increased the kidney weight/body weight ratio (treatment: 0.0069 ± 0.0005

Fig. 2. Renal COX-2 immunostaining in P14 rats. In control rat kidneys, intensive COX-2-ir was found in many epithelial cells of macula densae and cortical thick ascending limbs (A, arrows). B: although markedly decreased, COX-2-ir was still found in the cortex with maximal inhibition by salt supplementation. Cortical COX-2 expression decreased significantly after treatment with corticosterone (CS) or DOCA from P7 to P14. Fewer COX-2-ir cells were found in CS-treated (C) than DOCA-treated animals (D). Both the glucocorticoid receptor (GR) antagonist RU486 (E) and the mineralocorticoid receptor (MR) antagonist spironolactone (G) blocked CS inhibition of cortical COX-2 expression. DOCA suppression of cortical COX-2 expression was slightly blocked by RU486 (F) but was completely blocked by spironolactone (H). A-H: 700-nm-wide magnification.
vs. control: 0.0059 ± 0.0003, *P* < 0.05). Immunohistochemistry data indicated that compared with untreated suckling rats (Fig. 2A), administration of 3.2 mg NaCl·g⁻¹·day⁻¹ for 3 days markedly reduced cortical COX-2 expression but reduced immunoreactive COX-2 was still detected in some macula densae and cortical thick ascending limb cells (Fig. 2B).

**Dynamics of plasma CS and aldosterone and cortical COX-2 levels during postnatal development.** To investigate the effects of adrenal steroids on renal cortical COX-2 expression in suckling rats (*P7* to *P14*), weanling rats (*P21* to *P28*), and adult rats (2 mo old), we first determined plasma levels of CS and aldosterone at *P7*, *P14*, *P21*, and *P28* and in the adult rats. As shown in Fig. 3A, plasma CS concentration levels were very low in *P7* and *P14* rats but rapidly increased to adult levels by *P21*. Plasma CS levels were comparable in adult rats treated with a normal diet, HS diet, COX-1 inhibitor, COX-2 inhibitor, and various combinations (data not shown). In contrast, plasma aldosterone levels were relatively stable at the same periods of time (Fig. 3B). Plasma aldosterone level varies inversely with dietary salt intake in mature kidney (data not shown). To investigate whether an inverse correlation exists between serum GC and COX-2 expression, renocortical COX-2 expression was tonically suppressed by CS inhibiting cortical COX-2 expression during postnatal development; *n* = 8. Effects of adrenal steroid supplementation on renal cortical COX-2 expression during postnatal development of the rats. We previously reported that renal cortical COX-2 expression was tonically suppressed by MC as well as GC in adult rats (43). Plasma CS levels are very low in suckling rats (Fig. 3A; Ref. 12). To investigate whether relative deficiencies in MC and/or GC were involved in the elevated renal cortical COX-2 expression of the suckling rats, pups at *P7* were treated with different doses of CS and DOCA pellets for 1 wk. In preliminary studies, it was determined that the doses that produced maximal COX-2 suppression were 1,500 and 50 mg/kg for CS and DOCA, respectively. After treatment with this dose of CS for a week, both body and kidney weight in CS-treated animals were significantly lower, whereas in DOCA-treated animals, both body and kidney weight were significantly higher compared with control [body wt (g): control: 21.06 ± 1.74; CS: 17.44 ± 1.31, *P* < 0.01 compared with control; DOCA: 27.97 ± 1.51, *P* < 0.01 compared with control; kidney wt (mg): control: 144.33 ± 2.49; CS: 121.5 ± 0.85, *P* < 0.01 compared with control; DOCA: 176.67 ± 6.24, *P* < 0.01 compared with control; *n* = 6 in each group]. Similar to adult animals, both CS and DOCA suppressed cortical COX-2 expression in the suckling rats (Figs. 2, C and D, and 4A). As indicated by both Western blot analysis and immunohistochemistry, CS was relatively more effective than DOCA in suppressing cortical COX-2 expression in the suckling rats.

To investigate which receptor pathway(s) were involved in the suppressive effects of MC or GC on renal cortical COX-2 expression, suckling rats at *P6* were treated for 8 days with either the GR antagonist RU486 or the MR antagonist spironolactone with CS or DOCA added at *P7*. As shown in Figs. 2 and 4B, CS suppression of cortical COX-2 expression was partially blocked by either the GR antagonist RU486 or the MR antagonist spironolactone (Renocortical COX-2 expression in control rats is designated as 100. CS: 2.9 ± 1.7; CS + RU486: 36.9 ± 8.5, *P* < 0.01 compared with CS; CS + spironolactone: 30.4 ± 5.4, *P* < 0.01 compared with CS; *n* = 4), whereas DOCA suppression of cortical COX-2 expression was only minimally affected by RU486 but prevented by spironolactone (DOCA: 11.5 ± 2.9, DOCA + RU486: 18.4 ± 5.8; NS compared with DOCA; DOCA + spironolactone: 154.9 ± 4.5, *P* < 0.01 compared with DOCA; *n* = 4). These data suggest that CS inhibits cortical COX-2 expression by activating both MR and GR, whereas DOCA's effects are mainly mediated by activation of MR in the suckling rats.

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![Fig. 3. Dynamics of plasma total CS (A) and aldosterone (aldo; B) levels during postnatal development; *n* = 8. C: renocortical COX-2 expression during postnatal development. Each time point has 2 representative lanes standing for 2 individual animals. As indicated, renocortical COX-2 levels are much higher in *P7* and *P14* rats than those in *P21*, *P28*, and adult (A) animals.](http://ajprenal.physiology.org/)

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Spironolactone not only blocked DOCA suppression, but it further significantly by RU486.

either GR antagonist RU486 or MR antagonist spironolactone (SP), effect of CS on cortical COX-2 expression was partially reversed by

creased gradually during postnatal development (44). Renocortical COX-2 expression was expressed as COX-2-ir area/cortex area. As shown in Fig. 5, renocortical COX-2 expression in normal rats decreased gradually during postnatal development (P14: 0.00493 ± 0.00055; P28: 0.00245 ± 0.00028, P < 0.01 compared with P14; adult: 0.00035 ± 0.00006, P < 0.01 compared with P14 and P28; filled bars, n = 8). CS suppressed renocortical COX-2 expression in all developmental stages; the suppressive effects being more potent in the suckling rat than in the weanling and adult animals [P14: 0.00006 ± 0.00001 vs. 0.00493 ± 0.00055 (control), over 90% suppression; P28: 0.00172 ± 0.00021 vs. 0.00245 ± 0.00028 (control), ~30% suppression; adult: 0.00027 ± 0.00008 vs. 0.00035 ± 0.00006 (control), over 20% suppression; gray bar, n = 8]. Cortical COX-2 expression was 0.00048 ± 0.00005 vs. 0.00493 ± 0.00055 of control (~90% suppression) at P14 after DOCA treatment for 1 wk. At P28 and adult animals, renocortical COX-2 was undetectable after DOCA treatment. Therefore, DOCA suppression of cortical COX-2 expression increases with development (Fig. 5, open bar).

Effect of dietary salt intake on renal cortical COX-2 expression in weanling rat. In other experiments, rats were weaned at P19 and then fed a normal-salt diet, LS diet, or HS diet for 1 wk. The effectiveness of the LS diet and HS diet was confirmed by 24-h urinary sodium excretion and body weight gain during the treatment period. After treatment for 6 days, 24-h urinary sodium excretion was undetectable in LS-treated animals but increased nearly 20 times in HS-treated animals compared with those on a normal-salt diet (Table 1). Body weight was significantly lower in the LS-treated animals than normal- or high-salt diet-treated animals (Table 1).

Western blot analysis showed that COX-2 expression increased significantly in LS-treated animals (Fig. 6A, lanes 3 and 4). In contrast, COX-2 expression was barely detectable in HS-treated animals (Fig. 6A, lanes 5 and 6). To investigate whether salt supplementation by subcutaneous injection of sterile normal saline is a

The immature kidney is more sensitive to CS than aldosterone, whereas the mature kidney is more sensitive to aldosterone than CS (34). To investigate whether adrenal steroid suppression of renal cortical COX-2 expression is related to kidney sensitivity to adrenal steroids during postnatal development, suckling rats (P7-P14), weanling rats (P21-P28), and adult rats were treated with CS or DOCA for 1 wk, the kidneys were perfused, and COX-2 immunohistochemistry was performed. Cortical COX-2 expression was quantified using the BIOQUANT true-color windows system (44). Renocortical COX-2 expression was expressed as COX-2-ir area/cortex area. As shown in Fig. 5, renocortical COX-2 expression in normal rats decreased gradually during postnatal development (P14: 0.00493 ± 0.00055; P28: 0.00245 ± 0.00028, P < 0.01 compared with P14; adult: 0.00035 ± 0.00006, P < 0.01 compared with P14 and P28; filled bars, n = 8). CS suppressed renocortical COX-2 expression in all developmental stages; the suppressive effects being more potent in the suckling rat than in the weanling and adult animals [P14: 0.00006 ± 0.00001 vs. 0.00493 ± 0.00055 (control), over 90% suppression; P28: 0.00172 ± 0.00021 vs. 0.00245 ± 0.00028 (control), ~30% suppression; adult: 0.00027 ± 0.00008 vs. 0.00035 ± 0.00006 (control), over 20% suppression; gray bar, n = 8]. Cortical COX-2 expression was 0.00048 ± 0.00005 vs. 0.00493 ± 0.00055 of control (~90% suppression) at P14 after DOCA treatment for 1 wk. At P28 and adult animals, renocortical COX-2 was undetectable after DOCA treatment. Therefore, DOCA suppression of cortical COX-2 expression increases with development (Fig. 5, open bar).

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Table 1. Effects of dietary salt intake on body weight and urinary sodium excretion

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<th>Normal Diet</th>
<th>Low-Salt Diet</th>
<th>High-Salt Diet</th>
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<tr>
<td>Body weight gain, g</td>
<td>15.23 ± 3.11</td>
<td>8.42 ± 0.63*</td>
<td>14.25 ± 1.20</td>
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<tr>
<td>24-H urinary sodium excretion, mM</td>
<td>0.51 ± 0.33</td>
<td>Undetectable</td>
<td>10.17 ± 0.72*</td>
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Values are means ± SE. The rats were weaned at postnatal day 19 and then treated with different diets for 1 wk. Twenty-four-hour urine was collected before death. Each group has 6 samples. *P < 0.001 compared with normal-diet group.

reliable method to supplement salt in weanling rats as well as in suckling rats to suppress cortical COX-2 expression, normal saline at a dose of 3.2 mg NaCl·g⁻¹·day⁻¹ was given to weanling rats for 1 wk (P19-P26). As shown in Fig. 6B, salt supplementation by subcutaneous injection of normal saline suppressed cortical COX-2 expression as effectively as by HS diet. These data suggest that normal saline administration by subcutaneous injection is an effective way to supplement salt in both suckling and weanling rats and that cortical COX-2 is more sensitive to salt supplementation in weanling rats than in the suckling rats.

DISCUSSION

The major findings in the current investigation are the following. 1) CS supplementation effectively suppresses cortical COX-2 expression in the sucking rats when plasma CS levels are very low. 2) The suppressive effect of GC supplementation on renal cortical COX-2 expression decreases with maturation. 3) The suppressive effect of MC supplementation on renal cortical COX-2 expression increases with maturation. 4) Salt supplementation suppresses cortical COX-2 expression in both sucking and weanling rats.

Compared with normal rat chow, which contains 0.3–0.5% NaCl, maternal milk is a relatively LS diet (0.15% NaCl). Interestingly, sodium concentration in the milk does not vary with maternal salt intake. The dams maintain normal levels of sodium in the milk by mobilization of sodium from bones and muscles (7, 35). In view of this limited sodium intake and physiological requirement for sodium conservation for optimal growth, the young rats are in a state of relative volume depletion, which is reflected by an activated renin-angiotensin system (RAS) (31, 38, 40, 41). In adult rats, renal cortical COX-2 expression is induced by volume depletion (13). Renal cortical COX-2 expression is elevated in the immature kidney (44). Suppression of renal cortical COX-2 by salt supplementation in the sucking and weanling rats suggests that relative volume depletion is a factor in the elevated renal cortical COX-2 expression seen in the immature kidney. Elevated renal cortical COX-2 expression in the immature kidney is important for normal postnatal development of the kidney. Administration of a COX-2 inhibitor started during pregnancy until weaning significantly impaired development of the renal cortex and reduced glomerular diameter in both mice and rats (18). An identical phenotype was reported in COX-2 null mice (6, 18, 27, 28). Increased activity of the RAS is important for kidney morphogenesis and growth (8, 9, 30). In late gestation of lambs, renin secretion and gene expression at baseline, as well as in response to β-adrenergic stimulation, appear to be dependent on COX-2-derived prostaglandins, supporting a tonic stimulatory role for COX-2-derived prostaglandins in the local regulation of the fetal RAS (26).

In adult rats, renal cortical COX-2 expression increases after adrenalectomy (36, 43). Replacement with either MC or GC suppressed the increased COX-2 expression induced by adrenalectomy. Treatment of normal adult rats with the MR antagonist spironolactone or the GR antagonist RU486 induced cortical COX-2 expression, although spironolactone was more effective than RU486 (43). Therefore, both MR and GR are involved in the regulation of cortical COX-2 expression in the adult animals. In the suckling rats, CS levels are much lower than those in weanling and adult rats (Fig. 3A; Ref. 14). In the present study, we found that both cortical COX-2 and renin (data not shown) expression and kidney growth were suppressed after CS supplementation in the suckling rats. These results suggest that low CS levels are involved in elevated cortical COX-2 expression in the immature kidney.

The immature kidney is more sensitive to GC compared with the mature kidney (34). The affinity of the isolated or cloned MR is similar for aldosterone, CS, cortisol, or DOCA regardless of the tissue source (1, 10). Cortisol and CS circulate at concentrations of 100–1,000 times that of aldosterone, yet they normally produce few, if any, MC effects. This specificity of MR action is achieved by the presence of type II 11β-hydroxysteroid dehydrogenase-2 (11β-HSD2), which converts biologically active CS to inactive 11-keto forms in the rodents (19). In adult rats, 11β-HSD2 is colocalized with MR to renal distal nephron epithelial

Fig. 6. A: effects of dietary salt intake on cortical COX-2 expression in weanling rats. Rats weaned at P19 were treated with different dietary salt diets for 1 wk. Low-salt (LS) diet (lanes 3 and 4) induced and high-salt (HS) diet (lanes 5 and 6) inhibited cortical COX-2 expression. B: effects of saline subcutaneous injection on renal cortical COX-2 expression in weanling rats. Weanling rats were injected subcutaneously with sterile normal saline at a dose of 3.2 mg·g⁻¹·day⁻¹ for a week (P19-P26). Salt supplementation by saline subcutaneous injection effectively suppressed cortical COX-2 expression as well as HS diet in the weanling rats.
cells including cTAL, distal convoluted tubule, connecting tubules, cortical collecting tubules, and outer medullary collecting tubules (2, 21). When kidney 11β-HSD2 activity is low, CS binds to both MR and GR, resulting in increases in MC function. 11β-HSD2 expression in renal cortex is much higher in the mature kidney than in the immature kidney (32). Therefore, 11β-HSD2 might play an important role in determining kidney sensitivity to GC during postnatal development. As both MR and GR are involved in tonic suppression of cortical COX-2 expression by adrenal steroids in adult animals, we hypothesized that CS supplementation would effectively suppress cortical COX-2 expression by activation of both MR and GR because of low 11β-HSD2 levels in the immature kidney but would less effectively suppress cortical COX-2 expression in the mature kidney because of high 11β-HSD2 levels and activity. As shown in Figs. 2, E and G, and 4B, CS suppression of renal cortical COX-2 expression was partially blocked by both MR and GR antagonists in the suckling rats. Therefore, GC supplementation suppresses cortical COX-2 expression by activation of both MR and GR in the immature kidney. In contrast, DOCA suppression of renal cortical COX-2 expression in the immature kidney was minimally blocked by the GR antagonist but was significantly blocked by the MR antagonist (Figs. 2, F and H, and 4B). Figure 5 showed that CS suppression of renal cortical COX-2 expression diminishes with maturation, whereas DOCA suppression of renal cortical COX-2 increases with maturation.

In mature rats, plasma aldosterone levels increase many times after chronic LS treatment. In the present study, we found that plasma aldosterone concentration in the mature rat increases over 100 times after chronic LS-diet treatment (data not shown). Although suckling rats are in a relative volume depletion status, plasma aldosterone levels are comparable to the levels in adulthood, suggesting that the aldosterone response to volume depletion is attenuated in immature rats. The immature kidney is less sensitive to aldosterone compared with the mature kidney (34). The present data that renal cortical COX-2 is less sensitive to MC suppression in suckling rats than in weaning and adult rats are in accordance with kidney sensitivity to MC. Therefore, relative MC deficiency (compared with aldosterone levels in adult rats with volume depletion) and attenuated cortical COX-2 response to MC are involved in elevated cortical COX-2 expression in suckling rats. These findings suggest that elevated renal cortical COX-2 expression due to GC and/or MC deficiency and attenuated cortical COX-2 response to MC facilitates postnatal kidney development.

In summary, the present study indicates that relative volume depletion, relative GC and/or MC deficiency, and attenuated cortical COX-2 response to MC are responsible for elevated cortical COX-2 expression in immature kidneys. Elevated cortical COX-2 expression seen in the macula densa and cortical thick ascending limb of immature rodent kidneys is decreased in response to either salt supplementation or administration of GC or MC, suggesting that COX-2 expression is also under physiological control in the immature kidney. The association between the increased COX-2 expression seen in the immature kidney and the developmental defects noted in COX-2−/− mice remains to be determined.

**DISCLOSURES**

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