Cyclooxygenase-2 contributes to elevated renin in the early postnatal period in rats

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Cyclooxygenase-2 contributes to elevated renin in the early postnatal period in rats. Am J Physiol Regul Integr Comp Physiol 284: R1179–R1189, 2003. First published January 30, 2003; 10.1152/ajpregu.00340.2002.—We asked whether cyclooxygenase (COX) activity controls the renin-angiotensin system in the postnatal period. During kidney development, renin peaked at postnatal days 0–1 at the mRNA, tissue protein [renal renin concentration (RRC)], and plasma renin concentration (PRC) levels and was widely expressed along proglomerular vessels. PRC and renin mRNA expression was elevated until weaning in the 4th postnatal week compared with adult rats. Renocortical COX-2 was restricted to Tamm-Horsfall protein-positive cells in the thick ascending limb of Henle’s loop, and cortical COX-2 mRNA and protein expression were elevated along with PRC in the 2nd and 3rd postnatal weeks. In contrast, cortical COX-1 expression was constant, but medullary COX-1 expression increased eightfold from the 1st to 4th postnatal week. A COX-2-selective blocker, parecoxib, and a nonselective blocker, indomethacin, given in a period with COX-2 induction from postnatal day 6 to day 12, markedly decreased PRC, but not renin mRNA or RRC. Inhibition of angiotensin AT1 receptors by candesartan from postnatal day 1 to day 5 increased COX-2 mRNA (2.5-fold), protein, and distribution, renin mRNA (7-fold) and PRC (20- to 70-fold), but had no influence on COX-1 mRNA. Thus, due to very low levels of expression, COX-2 is unlikely to be responsible for the birth peak of renin, but COX-2 activity supports renin secretion later in the suckling period. ANG II negatively feeds back on renocortical COX-2 expression in the 1st postnatal days with high activity of the renin system. We suggest that suckling in the rat is correlated to an enhanced, COX-2-mediated, secretory activity of renin-producing juxtaglomerular cells.

ANG II has important effects on structural development and functional differentiation of the kidney in the early postnatal life of many species. The key enzyme that initiates ANG I synthesis, renin, is strongly expressed in prenatal and neonatal rodent (12, 18, 28, 43), sheep (2), and human (33) kidneys, and accordingly there are elevated plasma levels of renin, ANG II, and aldosterone in late gestation and early postnatal life in several species (2, 7, 18, 32, 43). It is established in rodents that a high concentration of ANG II and aldosterone is crucial for sodium conservation in the first critical days of postnatal life (1, 5). Moreover, nephrogenesis depends critically on intact ANG II signaling (11, 13, 31, 39–41). Thus a phenotype with diminished kidney growth, vascular wall thickening, and atrophy of the inner renal medulla is virtually identical between ANG II receptor-deficient, angiotensinogen-deficient, and angiotensin-converting enzyme-deficient mice, and mice with blocked ANG II formation or ANG II type 1 (AT1) receptors. Little is known about the factor(s) responsible for the perinatal, ontogenetic surge of intrarenal renin expression and renin secretion. Data obtained mainly in sheep fetuses have shown that several mechanisms of renin control found in the adult kidney are also functional in late gestation. Thus tonic renal efferent sympathetic nerve discharge influences renin (8), renal perfusion pressure and ANG II correlate inversely to renin secretion (12, 35, 37), and cortisol enhances renin secretion (10). In the present study, we focused on a potential role for COX-mediated prostaglandin-dependent regulation of renin in the rat kidney in the early postnatal period. In adult rats, COX-2 activity is necessary for full responsiveness of renin after certain intrarenal stimuli such as reduction in renal blood flow (44), ANG II receptor blockade (4), a low luminal NaCl concentration in the loop of Henle (38), and furosemide treatment (20). COX-2 is strongly expressed in fetal and neonatal rat kidney (42, 48) and is also found in human fetal kidneys (21, 23). COX-2 is equally important for nephrogenesis as ANG II (24, 30). In the adult rat juxtaglomerular apparatus, COX-2 is constitutively expressed in few cells in the thick ascending limb of Henle’s loop (cTAL) (15) and is regulated concordantly with renin in various situations with chronic stimulation of the renin-angiotensin system (4, 15, 16, 19, 45). We therefore considered the possibility that renocortical COX-2 activity in the perinatal period drives stimulation of the renin-angiotensin-aldosterone system. To address this issue, we characterized the temporal and spatial correlation of COX

Candesartan; prostaglandin; parecoxib

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isoenzyme expression and renin expression in developing rat kidneys and, subsequently, performed intervention studies with COX inhibitors and ANG II receptor inhibitors.

MATERIALS AND METHODS

In vivo protocols. All procedures conformed with the Danish national guidelines for the care and handling of animals and with the published guidelines from the National Institutes of Health. Female Sprague-Dawley rats had free access to standard pathogen-free rat chow (Altromin-1310, Lage, Germany, Na+ 2 g/kg, Cl− 5 g/kg) and tap water. Dams and pups were housed in a 12:12-h light-dark cycle. Pups were nursed by their dams and were weaned at 3 wk of age. Rat dams and pups were killed by decapitation, mixed trunk blood was sampled in EDTA-coated vials, and organs were rapidly removed and frozen in liquid nitrogen and stored at −80°C. At gestational day 17, blood was pooled from several embryos after rapid decapitation. All other samples were obtained separately from single rats. At selected postnatal stages, pup kidneys were dissected in cortex and medulla before freezing. Candesartan (a kind gift from Astrazeneca, Gothenburg, Sweden) was dissolved (10 mg/ml stock solution) in Na2CO3 solution and injected subcutaneously in the neck fold of rat pups from postnatal day 1 to day 5 (1 mg·kg−1·day−1). Indomethacin (Sigma, Redovre, Denmark) was dissolved in sesame oil and injected subcutaneously in the neck fold of pups from postnatal day 6 to day 12 (3 mg·kg−1·day−1). Dynastat (Parecoxib) (Pharmacia) was dissolved in isotonic glucose (20 mg/ml) and given at 3 mg·kg−1·day−1 from postnatal day 6 to day 12. Control pups were injected with vehicle (Na2CO3, sesame oil, and isotonic glucose).

Solution hybridization and RNase protection assays. Tissue samples (150–200 mg) were homogenized (Polytron PT300, Kinematica) and total RNA was isolated with the Qiagen RNeasy midikit according to the instructions (Qiagen, Albertslund, Denmark). RNA was eluted in pure water, and the yield was quantified by measuring optical density at 260 nM (GeneQuant II, Amersham Pharmacia). mRNA levels were estimated by solution hybridization followed by A/T1 RNase protection assay using plasmids and protocols as described (19). mRNA-cRNA hybrids were separated by denaturing PAGE. Autoradiography (Biomax film, Kodak) was performed at −80°C for 6 h to 3 days. Subsequently, protected probes were excised, and radioactivity was quantitated in a beta counter.

RIA for plasma and tissue renin concentrations. Plasma renin concentration (PRC) was measured by ultramicroassay of generated ANG I using renin standards as described (25). Five serial dilutions from the same plasma sample were assayed in duplicate for all samples. Only when at least three of the dilutions were linear was the measurement accepted. Renin concentration is expressed in Goldblatt units (GU) compared with renin standards from the National Institute for Biological Standards and Control (Hertfordshire, UK). Tissue renin concentration was measured by homogenizing kidney tissue in 1–3 ml of buffer (0.1 mol/l Tris·HCl, pH 7.2; 10 mmol/l EDTA, 1 mmol/l DTT, 2 mg/ml human serum albumin, 0.1 mmol/l PMSF, 0.1% Triton X-100). The homogenate was centrifuged at 20,000 g for 10 min at 4°C. Aliquots of the supernatant were diluted and used for determination of protein concentration and renin concentration.

Immunohistochemical and immunofluorescence analysis of kidney sections. For immunolabeling, rat pup kidneys were perfused through the left cardiac ventricle with freshly prepared 3% paraformaldehyde solution in PBS (pH 7.35) for 5 min. Processing of tissue for immunohistochemical analysis was essentially as described in detail previously (36). Primary antibodies used were polyclonal rabbit anti-mouse COX-2 antibody (Cayman Chemicals, AH Diagnostics), polyclonal goat anti-rat COX-2 (Santa Cruz, AH Diagnostics), polyclonal rabbit anti-mouse COX-1 antibody (Cayman Chemicals, AH Diagnostics), polyclonal rabbit anti-rat Tamm Horsfall glycoprotein (THP) antibody (a gift from Dr. J. Hoyer, Philadelphia, PA), and a polyclonal rabbit anti-mouse renin antibody (28). For immunoperoxidase labeling the sections were incubated for 1 h with horseradish peroxidase (HRP)-conjugated rabbit secondary antibody directed against the relevant species (Dako). Signals were visualized by incubation for 2–30 min with 0.01% diaminobenzidine and 0.02% H2O2. Double-immunofluorescence labeling for THP and COX-2 was performed on cryostat sections (5 μm) by using first COX-2 antibody (Santa Cruz, 1:500) and rabbit anti-goat Cy3-conjugated antibody for 1 h (Dianova, 1:250). After several washes, the sections were incubated with rabbit anti-rat THP antibody, which was then visualized with goat anti-rabbit Cy2-conjugated antibody (Dianova, 1:100). Sections were inspected in a Leica DMRB microscope equipped with interference contrast optics and an HBO fluorescent lamp. Photos were captured with a digital camera (Spot 32, Diagnostic Instruments, Munich, Germany) and processed with Meta View 3.6a software (Universal Imaging, West Chester, PA).

Western immunoblotting. Tissue samples (~100 mg) were homogenized in buffer as used for tissue renin determination, centrifuged at 14,000 g at 4°C for 10 min, and the supernatant was split in 100-μl aliquots kept at −80°C. Protein concentration was determined spectrophotometrically (Bio-Rad protein assay reagent) using serial dilution of BSA as a standard. The samples were mixed with TRIS-SDS-loading buffer (1/4 vol) and 1/10 vol 0.6 mol/l DTT, boiled for 2 min, and subsequently separated by SDS-PAGE (7–10% gel) at 150–200 V for 30–40 min. The gel was equilibrated with transfer buffer (Tris-glycine-SDS with 20% ethanol) and proteins were electroblotted (Bio-Rad) onto polyvinylidene difluoride Immobilon membranes (Millipore) at 0.8 mA/cm² for 1 h. Subsequently, the membrane was air dried, blocked in Tween-Tris-buffered saline (TTBS) with 5% dry milk (16 h at 4°C), washed in TTBS, and subsequently incubated with primary antibody (goat anti-rat COX-2, Santa Cruz) in TTBS with 2% dry milk, 1 h. Bound secondary antibody was detected by chemiluminescence (Renaissance kit plus, Dupont) and exposed to X-ray film (Biomax, Kodak) for 10 s to 1 min. Preabsorption negative controls were made by incubating primary antibody in dilutions as used for the assay with peptide used to raise the antibody (10 μg/ml for 1.5 h at room temperature).

Statistics. All values are given as means ± SE. Unpaired Student’s t-test was used to determine statistical difference when two groups of data were compared. P < 0.05 was considered statistically significant.

RESULTS

Temporal and spatial correlation of renin with COX expression during rat kidney development. Renin mRNA transcripts were detected in fetal rat kidney at the earliest point of measurement, embryonal day 17. Renin mRNA levels rapidly rose from embryonal day 17 to day 19 and peaked at the day of birth. Renin...
mRNA levels then progressively declined through subsequent developmental stages and reached a stable level after weaning at postnatal day 21 (Fig. 1A). No unspecific hybridization with yeast RNA was observed with renin antisense probe or with other antisense probes in any of the assays. All probes were completely digested in the absence of template RNA (Fig. 1A). Rat β-actin mRNA levels were determined as a control of RNA quality. However, there was a significant decrease of β-actin mRNA levels through early kidney development with very high levels in the embryonic kidney. Thus renin mRNA values were not normalized with respect to β-actin, and it can be concluded that the relatively low level of renin mRNA expression in late embryonic kidneys are not due to poor RNA quality. PRC changed with developmental stage in a pattern basically similar to renin mRNA, with the exception that the peak level was observed at postnatal day 1 when PRC was 30-fold higher (111 ± 13 × 10⁻⁵ GU/ml, n = 6) compared with embryonal day 17 (3.7 ± 0.5 × 10⁻⁵ GU/ml, n = 2 litters) (Fig. 1B). After the birth peak, PRC then stabilized at an elevated level until weaning, when normal adult rat levels were measured (at postnatal days 28 and 56). Renal tissue renin concentrations (RRC) increased 100 times at postnatal day 1 (25.6 ± 2.3 mGU/mg protein) compared with embryonal day 19 (0.23 ± 0.18 mGU/mg protein) and remained relatively stable at this level at later stages (16.4 ± 1.5 mGU/mg protein at postnatal day 8 and 21.9 ± 2.8 mGU/mg protein at postnatal day 56) (not shown). A time course study of COX isoform expression, with the same RNA samples as used for renin measurements, revealed that COX-2 was stably expressed in late embryonal and early postnatal rat kidneys. COX-2 then increased strongly between postnatal days 3 and 7 (Fig. 1C) and stayed at this level until

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Fig. 1. A: developmental change of renin mRNA in pre- and postnatal rat kidney. Autoradiograph shows results of a RNase protection assay of whole kidney total RNA for renin mRNA. The last 2 lanes show a negative control and the renin antisense probe in the absence of template RNA. Hybrids were cut out of the gels, and radioactivity was assayed in a beta counter. The figure shows quantitative evaluation of the gels. Day of birth is usually at embryonic days 20–21 (E20–E21). Day of birth is designated postnatal day 0 (P0). Each column is the mean value of 2 separate determinations. B: plasma renin concentration (PRC) during rat pre- and postnatal development. Plasma was analyzed by incubation of serially diluted samples with surplus of purified rat renin substrate followed by RIA of generated ANG I. Each column is the mean value ± SEM of 3–6 determinations from separate pup plasma samples. GU, Goldblatt units. C: cyclooxygenase-2 (COX-2) mRNA expression in rat kidney during late pre- and postnatal development. Autoradiograph depicting the changes of COX-2 mRNA expression in rat kidneys (inset) and quantitative evaluation of the gels (bottom). Hybrid bands were cut out of the gel and counted in a beta counter. Day of birth is usually at E20–E21. Day of birth is designated P0. Each column is the mean of 2 separate determinations. D: developmental changes of COX-2 protein expression in postnatal rat kidneys analyzed by Western immunoblotting. Immunoblot analysis of kidney lysates (75 μg protein/lane) at different developmental stages showing induction of COX-2 protein in the suckling period (until P21). E: immunoperoxidase analyses of renin and COX-2 localization in adjacent sections of rat kidneys from P2. COX-2-positive cells are observed in juxtaglomerular area in terminal part of cTAL at junction to distal convoluted tubule (left). The next section of the kidney is immunolabeled for renin (right). Renin immunoreactive protein is observed both in the distal afferent arterioles in cells that are close in contact with COX-2-positive loop of Henle cells and in more proximal arteriolar segments that are not in contact with COX-2-positive cells. Magnification, ×300.
weaning after 3 wk of age. COX-2 levels declined at postnatal day 28 and further decreased at postnatal day 56 basically as PRC and renin mRNA. In contrast, COX-1 was expressed at stable levels when whole kidneys were assayed (not shown). COX-2 induction was confirmed at the protein level by Western immunoblotting (Fig. 1D). A single protein band was detected in extracts from whole kidney (using 100 μg of protein) at an antibody dilution of 1:2,000. The immunolabeled protein had a molecular mass compatible with the expected size of COX-2 (72 kDa), and labeling was absent when the primary antibody was omitted or when it was preabsorbed with the peptide used for immunization (not shown). COX-2 protein was developmentally regulated in the kidney, with the highest levels in the end of the 1st postnatal week through to the 3rd postnatal week (Fig. 1D).

At the time of maximal renin expression and distribution (postnatal day 2), it was investigated whether renin and COX-2 were localized in adjacent cells in serial sections of rat kidneys (Fig. 1E). COX-2 immunoreactivity was detected in cells in the loop of Henle close to glomeruli in the differentiated zone (Fig. 1E, left). Most of these cells were spatially associated with renin-positive cells in the afferent arteriole, but renin immunoreactivity in the afferent arterioles typically extended far beyond the contact point with COX-2-positive cells in the juxtaplomerular area (Fig. 1E, right). Thus renin immunoreactivity often included the entire afferent arteriole, portions of the interlobular artery, and scattered foci also in the arcuate artery (Fig. 1E, right). Of note, proximal convoluted tubules also displayed a distinct, but weaker, staining of the cytoplasm for renin as shown previously with this and other antisera (3, 28). Immunoreactivity for renin at this site in the neonatal period is likely to reflect renin filtered at the glomerulus and then taken up by endocytosis rather than by locally transcribed and translated renin, because renin transcripts are restricted to the renal vessels at this time (12).

Thus renin mRNA, tissue renin stores, renin secretion, and renin immunoreactivity are all strongly induced in rat kidney preglomerular vessels shortly before, during, and in the 1st days after birth. PRC and renin mRNA are elevated compared with adult rats, until the time of weaning at postnatal day 21. The birth peak of renin expression and distribution does not correlate with COX-2 induction, but the prolonged phase of elevated PRC and renin expression during suckling is paralleled by renocortical COX-2 induction. Moreover, in contrast to early postnatal stages, COX-2-positive cortical cells are closely associated with most renin-positive vascular cells in the juxtaplomerular apparatus at this time.

Cellular localization of COX isoenzyme immunoreactivity in early postnatal rat kidneys. Next, we did a more comprehensive analysis of COX isoform distribution during the period of postnatal induction. At postnatal day 2, immunostaining for COX-2 yielded a strong labeling of straight tubules in the deep, differentiated kidney cortex (Fig. 2A). Immunolabeling was restricted to groups of cells in the tubular wall including peri-macula densa region of juxtamedullary nephrons, whereas the macula densa itself was immunonegative. As the kidney matured with age by cortical expansion, there was a marked increase in the number of labeled cells in cortical tubules (Fig. 2A). In the late 2nd postnatal week, the immunopositive tubules were situated in the medullary rays and in scattered foci in all layers of the cortical labyrinth. Most glomeruli were in contact with COX-2-positive tubules at postnatal day 14. In the medulla, a positive immunostaining for COX-2 was discernible at postnatal day 2 and maintained at day 7 through to day 14 (Fig. 2D). Labeling was associated with intertubular cells, most likely medullary interstitial cells, as observed in rats by previous investigators (15, 17, 48). At the time of COX-2 peak expression and distribution at postnatal day 14, actin-normalized COX-2 mRNA level was 10 times higher in cortex compared with medulla, as determined by RNase protection assay (210 ± 58 vs. 22 ± 6 arbitrary units) (Fig. 2E, top) \((n = 3)\).

Immunostaining for COX-1 yielded an immunopositive signal from inner medullary collecting duct cells and from interstitial cells (Fig. 2B). There was a more intense staining reaction in the medulla in late renal development compared with early stages (Fig. 2B). In cortex, immunostaining for COX-1 was associated with connecting tubules, cortical collecting ducts, and glomeruli (Fig. 2C). In contrast to COX-2, actin-normalized COX-1 mRNA level was 14 times higher in medulla compared with cortex at postnatal day 28, when medullary immunoreactivity for COX-1 was strong (421 ± 57 vs. 30 ± 17 arbitrary units, Fig. 2E) \((n = 3)\).

In the next series of experiments, loops of Henle were identified by immunochemical labeling for the cTAL marker THP (Cy2, green fluorescence) in combination with labeling for COX-2 (Cy3, red fluorescence). The vast majority of COX-2-positive cells were colocalized (yellow fluorescence) with THP, and the COX-2-positive cells were only a fraction of cTAL cells even in the late 2nd postnatal week, when there was massive expression of COX-2 (Fig. 3A). The immunofluorescence labeling confirmed that neither THP nor COX-2 is detected in either stage I nephrons (renal vesicle) or stage II (S-shaped bodies) in the nephrogenic zone. COX-2 labeling was first encountered at the estimated level of stage III nephrons, where capillary loops are being formed in the glomerulus, but where the loop of Henle has no lumen (Fig. 3, B1 and B2). In late stage III and stage IV the loop of Henle attains a lumen, and strong COX-2 labeling is associated with these stages (Fig. 3, B3 and B4). COX-2 (and THP) was first detected in a juxtaplomerular position of stage III glomeruli cells near the future macula densa. At this stage, labeling for COX-2 was intense in the cytoplasm and not restricted to the nuclear membrane. In stage IV and V nephrons, COX-2 immunoreactivity was detected in all peri-macula cells in cTAL and, notably, always together with THP. The subset of cTAL cells adjacent to glomeruli that did not express either
Fig. 2. Immunohistochemical analyses of the regional and cellular localization of cyclooxygenase-1 (COX-1) and COX-2 isoenzymes in early postnatal rat kidneys by using immunoperoxidase labeling of perfusion-fixed rat kidney sections.

A: in kidney cortex at P2, scattered foci of COX-2-positive cells were observed in loops of Henle in the deep, juxtamedullary cortex, whereas the nephrogenic zone at the outer border of the cortex was largely negative for COX-2. The number of COX-2-positive foci markedly increased at P7 and at P14, where loops of Henle were positive in all parts of the cortex. Magnification, ×75.

B: at P7, COX-1 immunoreactivity was predominantly observed in the renal medulla, and immunostaining was restricted to collecting ducts and interstitial cells. COX-1 labeling intensity increased with age as seen in medulla from P14 and P29. Magnification, ×300.

C: in kidney cortex, COX-1 was primarily detected in cortical collecting ducts, glomeruli, and connecting tubules. Left: kidney cortex from P22; right: kidney cortex from P29. Magnification, ×300.

D: in the kidney medulla, COX-2 immunoreactivity was detected exclusively in the medullary interstitial cells. Micrograph shows inner medulla from P14. Magnification, ×300.

E: developmental change of COX-1 and COX-2 mRNAs in kidney cortex and medulla in the suckling period (until P21) and after weaning (P28). Autoradiographs show the results of RNase protection assays for COX isoforms using total RNA from kidney cortex (right) and medulla (left) for hybridization. Both isoforms were determined in 1 RNA sample, and hybrids were cut out of the gel for counting in a beta counter. See text for quantitative evaluation.
Fig. 3. Double-immunofluorescence analyses of COX-2 (red fluorescence) and Tamm-Horsfall glycoprotein (THP) (green fluorescence) localization in the rat kidney cortex during early postnatal kidney development. A: at P3, COX-2-positive areas are scattered in foci in the kidney cortex. The foci are restricted to THP-positive-cells (double-staining, yellow fluorescence). Only a fraction of THP-positive tubules (green) are also COX-2 positive (yellow). At P14, both COX-2 and THP-specific fluorescence signals have a wider distribution compared with P3, and a larger proportion of THP-positive tubules are COX-2 positive. Magnification, ×75. B: no COX-2 labeling was associated with stage I (renal vesicle) and II nephrons (S-shaped body) but was first encountered in stage III nephrons where capillary loops were being formed in the glomerulus and the loop of Henle has no lumen (1, 2). In late stage III and stage IV nephrons, the loop of Henle attained a lumen, and strong COX-2 labeling was associated with these stages (3, 4). At all stages, COX-2 was expressed together with THP. Magnification, ×300.
COX-2 or THP are assumed to be macula densa cells (Fig. 3B4).

Effect of COX inhibition on renal renin parameters in the postnatal period. In the next experiments, we tested the hypothesis that COX-2 activity dictates early postnatal renin expression and secretion. Rat pups were given COX inhibitors or drug solvents in the period with maximal COX-2 induction and elevated renin expression and secretion. Rat kidneys tested the hypothesis that COX-2 activity dictates renin mRNA level in response to candesartan (Fig. 5B). COX-1 mRNA was not affected by candesartan (not shown). The effect of candesartan on COX-2 mRNA was confirmed at the protein level by Western immunoblots (Fig. 5B). In response to candesartan there was a marked increase in the spatial distribution of COX-2 immunoblotting. Thus COX-2-immunoreactive cells were located in the differentiated region of cortex at postnatal day 5 both in control rats (Fig. 6A) and in the candesartan-treated animals (Fig. 6B), thus sparing the nephrogenic zone. Double-immunofluorescence labeling for COX-2 and THP showed colocalization of both proteins in cTAL cells also at the high levels of COX-2 expression seen in response to candesartan (Fig. 6C, top). Often the whole perimeter of the loop was COX-2 positive (Fig. 6C, top). In some instances only part of the THP-positive cells were also COX-2 positive (Fig. 6C, bottom), showing that not all cTAL cells are recruited to express COX-2 by ANG II receptor inhibition. We did not observe COX-2-positive cells that were THP negative after candesartan treatment. Thus it can be concluded that AT1-receptor inhibition recruits cTAL cells to express COX-2 in the postnatal period in the rat.

DISCUSSION

Late stages of nephrogenesis require intact function of the renin-angiotensin-aldosterone system, which has been documented in multiple experimental set-
nings (6, 11, 13, 31, 39, 40, 41). Renin, the rate-limiting enzyme, is induced in mammalian kidney in a species-specific temporal pattern, but in a largely similar spatial pattern, during fetal and, in some species, postnatal development. Little is known about the physiological intrarenal signals that direct the recruitment, redistribution, and secretory activity of granular renin-producing cells in the perinatal period. In rats, nephrogenesis begins at embryonic days 10–11, continues through birth at embryonic days 20–21, and normally ceases at postnatal days 6–8 (36). In the present study, we found a concordant peak of renin mRNA, intrarenal renin content, and PRC shortly before, during, and after birth, that corresponded to a widespread distribution of renin immunoreactivity in the preglomerular vessels, as previously reported (3, 9, 12, 28). We confirmed a marked shift in localization of renin in the first 10 postnatal days from interlobar, arcuate, and cortical radial arteries to progressively smaller afferent segments (3, 12, 22, 28, 33, 34). Our data indicate that parallel with this shift in renin localization, there was a maintained elevation of renin expression and secretion in the period until weaning at postnatal day 21. Whereas the birth peak of renin did not correlate with cortical COX-2 expression, the prolonged phase of increased renin expression and plasma renin occurred simultaneously with a markedly enhanced expression of COX-2, limited to the kidney cortex. Moreover, in contrast to the first postnatal days of development, when renin is widely distributed and COX-2 expression is low, there was a close spatial congruence between juxtaglomerular renin-positive cells and COX-2-positive cells at later stages, as previously suggested (42, 48). After the redistribution of renin to the juxtaglomerular cells, a relatively larger influence of COX-2 for renin control could be expected. In accordance with this notion, COX-2 inhibitors significantly decreased PRC in the period with maximal COX-2 induction, suggesting an important contribution of COX-2 activity to maintain renin secretion in the suckling period. Thus two phases of renin regulation may exist in the postnatal period: an early birth peak of renin, which is independent of COX-2 activity, and a later phase, where COX-2 is upregulated and exerts control of renin secretory activity. In sheep, data suggest that a birth peak of cortisol is responsible for the concordant activation of renin (10). The reason that renin mRNA or renin stores did not change after COX inhibition could simply be the slower turnover time of these parameters, when considering the short time of treatment. Thus, in COX-2 knockout mice with permanently abol-ished COX-2 activity, a lower level of active renin is present in the adult isolated juxtaglomerular apparatus, but renin is localized at the expected site in the distal afferent arterioles of adult mice. This supports the notion that COX-2 activity is not required to govern the early postnatal redistribution of renin, but that COX-2 contributes to maintain the differentiated adult phenotype of renin expression (46). The effect of COX inhibition on renin secretion in the postnatal period in rats is in contrast to findings in piglets (32), but in accordance with findings in sheep fetuses, where administration of nonselective indomethacin (26) and COX-2-selective NS-328 (27) to the fetal circulation led to a decrease in fetal plasma renin activity. A decrease of renin mRNA was shown in primary cultures of sheep

**Table 1. Plasma renin concentration**

<table>
<thead>
<tr>
<th>Litter</th>
<th>Control Vehicle-Na₂HCO₃</th>
<th>Candesartan, 1 mg·kg⁻¹·day⁻¹</th>
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<tr>
<td>1 (n = 8)</td>
<td>32.2 ± 3.3(n = 4)</td>
<td>2,481.9 ± 217*(n = 4)</td>
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<tr>
<td>2 (n = 8)</td>
<td>162.7 ± 9(n = 3)</td>
<td>2,624 ± 425*(n = 5)</td>
</tr>
<tr>
<td>3 (n = 7)</td>
<td>27.3 ± 4.0(n = 3)</td>
<td>2,023 ± 297.2*(n = 4)</td>
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Results are means ± SE. Values are renin concentrations in 10⁻⁶ Goldblatt units/ml plasma. *P < 0.05.
which makes a direct comparison of the data difficult with RNA isolated from quick-frozen kidney tissue. However, our data on renin mRNA were obtained in fetal renocortical cells after previous systemic NS-398 (27). It is known from mice that aldosterone is necessary for life in the fetal period, and that COX-2 expression in these cells is supported by the morphological data, showing that renal COX-2 induction depends on external factors. The notion that renal COX-2 induction occurs during suckling is consistent with the critical need for conservation of NaCl balance in the postnatal period in the rat. It is important to note that aldosterone is necessary for life in the first postnatal days (1), which underlines the critical need for conservation of NaCl during suckling. Thus COX-2-mediated stimulation of renin secretion, induced by a low NaCl intake during suckling, might play an important role to sustain NaCl balance in the postnatal period in the rat. The notion that renal COX-2 induction depends on external factors is supported by the morphological data, showing that COX-2 expression first becomes significant as the loops of Henle have a lumen and probably are functional. The peri-macula densa region is strongly COX-2 positive, first seen in nephrogenic stage III glomeruli, whereas COX-2 was not observed in the majority of THP-negative macula densa cells as previously reported (42). Also in the adult kidney, only a minority of macula densa cells, identified as brain-type neuronal nitric oxide synthase-positive cells, were reported to be COX-2 positive (17).

Our data suggest that ANG II suppresses renocortical COX-2 expression in the first postnatal days, which is an interplay also observed in adult rats (4, 45). In parallel, renin mRNA and renin secretion were strongly enhanced by AT₁-receptor inhibition as previously observed (31, 40), suggesting the operation of a strong negative-feedback mechanism between ANG II and renin already at birth in rats.

COX-1 and COX-2 were both expressed in the kidney medulla. COX-2 was selectively localized to the medullary interstitial cells, as previously observed by other investigators (14, 15, 48), and was detected as early as postnatal day 2. COX-2 expression in the renal medulla is stimulated by water restriction in vivo (47, 48) and by hyperosmolality in cultured renal medullary cells in vitro (14, 47). COX-2 activity has been shown to sustain cell survival during hyperosmotic conditions (14, 47), and, of note, COX-2 expression in the inner medulla coincides with the increase in medullary toxicity that is known to occur during the first 3 wk after birth. In the first 4 postnatal weeks, COX-1 mRNA and immunoreactivity increased markedly in the kidney medulla and was expressed in the collecting duct epithelium and interstitial cells. The significance of COX-2 as well as COX-1 could therefore be closely linked to the development of ability to concentrate urine. Less is known about the factors that control COX-1 expression and the separate physiological roles of COX-1 compared with COX-2 in the renal medulla.

In summary, we have shown that, after a marked birth peak, renin secretion and renin expression are maintained at an elevated level during the period of suckling in the rat. Renocortical induction of COX-2, seen in the 1st postnatal week, correlates with moderately elevated plasma renin and renin expression during suckling. Inhibition of COX-2 in the 2nd postnatal week significantly decreases PRC, whereas blockade of ANG II AT₁ receptors leads to a strong stimulation of COX-2 and renin. We suggest that the birth peak of renin is COX-2 independent and that a low NaCl in-
take during suckling induces COX-2 in the cortical loop of Henle, which supports an increased secretory activity of the juxtaglomerular granular cells.

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