Inhibition of cyclooxygenase-2: effects on renin secretion and expression in fetal lambs

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Inhibition of cyclooxygenase-2: effects on renin secretion and expression in fetal lambs. Am J Physiol Regul Integr Comp Physiol 284: R1012–R1018, 2003. First published November 27, 2002; 10.1152/ajpregu.00523.2002.—The importance of prostaglandins in the regulation of the renin-angiotensin system during development is not known. These experiments were conducted to examine the effects of prostaglandin synthesis inhibitors on basal and isoproterenol-induced plasma renin concentration and renin gene expression in the late-gestation fetal lamb. Eighteen lamb fetuses ranging in gestational age from 129 to 138 days underwent surgical insertion of femoral arterial and venous catheters under general endotracheal anesthesia. After a period of recovery, animals underwent an infusion of isoproterenol after administration of a saline bolus (control experiments); 24–48 h later a second study was performed after administration of NS-398, a cyclooxygenase (COX)-2 inhibitor, or saline for a second control study. Administration of COX-2 inhibitor significantly reduced baseline plasma renin levels and attenuated responses in fetal renin secretion to isoproterenol infusions. Renal cortical cells from animals receiving COX-2 inhibitor had significantly lower levels of renin mRNA compared with animals receiving only saline. Renal cortical cells in culture from animals receiving only saline exhibited increased levels of renin mRNA when treated with isoproterenol, forskolin, or IBMX. Only forskolin increased renin mRNA levels in renal cortical cells in culture from animals receiving COX-2 inhibitor. We conclude that prostaglandins play a stimulatory role in the regulation of the renin-angiotensin system and are necessary for β-adrenergic stimulation of renin secretion and gene expression in the late-gestation fetal lamb.

NS-398; isoproterenol; β-adrenergic stimulation; kidney; development

The renin-angiotensin system (RAS) is vitally important during fetal and neonatal life. Kidney morphogenesis and growth are dependent on the functional maturation of the RAS as is the maintenance of physiological homeostasis, including blood pressure regulation and body fluid balance (5, 10, 39).

Activity of the RAS is markedly increased in mammalian species during fetal and neonatal life compared with adults. This is particularly evident during late gestation and appears essential for survival (31, 32, 43). Mice with mutations of the angiotensinogen gene and those that are homozygous for knockout of the converting enzyme gene demonstrate significant abnormalities in kidney development and rarely survive beyond the infant stage (5, 22, 23, 29).

As in the adult, the major regulators of renin production and secretion in the fetus and newborn are considered to be renal perfusion pressure, delivery of sodium chloride to the macula densa, and β-adrenergic stimulation (6, 13, 25, 39). While these mediators of the RAS appear to exert their influence through a final common path involving CAMP, there are major gaps in our understanding of the local mechanisms involved in the regulation and modulation of the RAS during development (2, 13).

Evidence supporting a role for prostaglandins in the regulation of the RAS has been accumulating. Prostaglandins have been shown to stimulate renin secretion and expression in adult animals (7, 15). Furthermore, the inducible form of cyclooxygenase, cyclooxygenase-2 (COX-2), has been found in adult and fetal renal tissue, and immunohistochemistry has demonstrated strong COX-2 expression in epithelial cells of the cortical thick ascending limb in the region of the macula densa (15, 47). Animal studies have also demonstrated upregulation of COX-2 in the neonatal kidney at a time when renin is enhanced (8, 15, 47). Investigations in adult animals have found stimulators of renin release, such as decreased renal perfusion pressure and low sodium intake, to increase expression of COX-2 mRNA and protein in the macula densa (17, 18).

In addition, investigations utilizing prostaglandin synthase inhibitors during gestation have shown decreased basal plasma renin activity in mature fetuses as well as decreased renal blood flow, increased renal vascular resistance, and increased urinary excretion of sodium and chloride (14, 15, 25). In human pregnancy, maternal use of prostaglandin inhibitors has been as-
associated with fetal renal maldevelopment and oligohydramnios (15, 21).

We designed these experiments to further elucidate the potential role of prostaglandins in the regulation of the fetal RAS. In particular, we attempted to elucidate the effect of specific inhibition of COX-2 on basal and β-adrenergic-induced plasma renin concentrations and mRNA expression in renal cortical cells of the near-term fetal lamb.

MATERIALS AND METHODS

Animal preparation. All surgical and experimental protocols were reviewed and approved by the Animal Care and Use Committee at Wake Forest University School of Medicine and followed the newest guiding principles for research (1).

Pregnant ewes with known insemination dates were obtained from local suppliers. All animals were housed in an environmentally controlled facility with free access to food and water. Animals were allowed to acclimate to the facility for at least 3 days before undergoing surgical procedures. Before surgery, ewes were fasted for 24 h.

On the day of surgery, the ewes were sedated with ketamine intramuscularly (20 mg/kg) and then intubated and mechanically ventilated with 1–2% halothane in 100% oxygen. Under sterile conditions, a vertical midline incision was made over the ewe’s abdomen, and the gravid uterus was exposed. The fetal hindlimbs were then identified, and the uterus was incised over this region. The fetal hindlimbs were brought through the uterine incision, and polyvinyl catheters were placed within both fetal femoral arteries and one fetal femoral vein through groin incisions. These catheters were advanced into the fetal aorta and vena cava and secured in place. An amniotic fluid pressure catheter was also placed in the amniotic cavity and secured to a fetal hindlimb. The fetal hindlimbs were then returned to the uterine cavity, and 80 mg of gentamicin were injected into the amniotic fluid. The distal ends of all the catheters were brought out through the uterine incision, and the uterus was closed. The catheters were then led to the maternal flank, exteriorized, and held at the ewe’s side in a clean pouch within elastic netting around the ewe’s abdomen.

Maternal femoral arterial and venous catheters were also placed. This was done through a small groin incision. Once placed, these catheters were tunneled subcutaneously to exit the maternal flank with the fetal lines.

The animals were then awakened and allowed to recover for ~5 days in pens with free access to food and water before further study was done. During the first 3 postoperative days, the ewes received prophylactic intravenous antibiotics consisting of 1 g of ampicillin and 80 mg of gentamicin daily. Catheters were flushed every 24–48 h with saline and filled with heparin (1,000 U/ml). The ewes were inspected each day, and fetal well-being was assessed by daily arterial blood gas and pH analysis.

In vivo stimulation. Eighteen lamb fetuses ranging in gestational age from 129 to 138 days gestation were studied. All experiments were conducted while the ewes stood quietly in metabolic carts. Fetal heart rate, blood pressure, and amniotic pressure were monitored simultaneously and continuously during all experiments. These variables were recorded on a polygraph interfaced with a personal computer. Baseline values were obtained for 60 min preceding each experiment. All fetuses were studied on 2 separate days. The initial experiment was designed as a control study; 24–48 h later a second experiment was done during which COX-2 inhibitor was administered. Eight animals underwent a second control experiment and did not receive COX-2 inhibitor to provide renal tissue for control studies done in vitro.

On the first day of study, fetal blood samples were drawn at baseline, 90 min after a saline bolus, and at 15 and 45 min after a 15-min infusion of isoproterenol (0.06 µg/kg•min⁻¹). Each blood sample included a withdrawal of 0.7 ml of fetal blood for blood gas and pH determination and 3 ml for renin determination. After each blood sample was drawn from the fetus, an equivalent volume of blood was drawn from the ewe and transfused into the fetus to avoid hemorrhagic artifact over the study period. Arterial blood gasses and pH were immediately analyzed on a model ABL 5 blood gas analyzer calibrated with a 5% CO₂-20% O₂-balance N₂ gas mixture at the ambient barometric pressure and at 39°C. Samples for renin were placed in 100 µg of EDTA and centrifuged at 3,000 g for 10 min at 4°C. Plasma samples were then immediately stored at −20°C.

On the second day of study, COX-2 inhibitor (NS-398, Alexis) was given in place of saline (5 mg/kg). Fetal blood samples were repeated as described above.

Immediately after completing this study, 16 of the fetuses were delivered by cesarean section and killed with a lethal dose of pentobarbital sodium. Two of the fetuses receiving COX-2 inhibitor underwent cesarean delivery on the day after the last experiment. These tissues were not studied in the in vitro stimulation protocols. The fetal kidneys were removed immediately after delivery, decapsulated, and bisected longitudinally, and the cortex was dissected.

Tissue culture. Renin-containing fetal renal cortical cells were prepared in a similar manner as previously described from a fraction of the remaining fetal renal cortex (4, 39, 42). Fetal renal cortical tissue was minced and then incubated in dissociation buffer (in mM: 130 NaCl, 5 KCl, 2 CaCl₂, 10 glucose, 20 sucrose, and 10 Tris, pH 7.4) with 0.1% collagenase. When these incubations were completed, the dispersed cells from each incubation were pooled and filtered. Single cells were collected, washed, and resuspended in dissociation buffer and then further separated using Percoll. The cells were resuspended in culture medium (RPMI 1640) containing 0.66 U/ml insulin, 100 U/ml penicillin, 100 U/ml streptomycin, and 2% fetal calf serum. NS-398 was not added to this medium. Then cells were plated onto standard plastic tissue culture plates at an average density of 1 × 10⁶ cells/cm² and cultured at 37°C in a humidified atmosphere containing 5% CO₂.

In vitro stimulation. After culture for 20–24 h, the cells were incubated with serum-free medium containing vehicle, isoproterenol (1 × 10⁻⁴ M), forskolin (5 × 10⁻⁶ M), or IBMX (5 × 10⁻⁵ M) for 4 h. The cells were then harvested and RNA was extracted using Trizol reagent.

RNA extraction. Fetal renal cortical cells were homogenized in 1.5 ml Trizol reagent (LIFE Technologies, Gaithersburg, MD) with the use of a high-speed polytron for 30–60 s. Chloroform (0.3 ml) was added to the homogenate, and the homogenate was mixed, incubated for 5 min, and then centrifuged at 12,000 g for 15 min. The aqueous phase was transferred to a fresh tube, and the RNA was precipitated by adding 0.9 ml isopropanol and centrifuging at 12,000 g for 10 min. The pellet was washed with ethanol, dried, and dissolved in RNase-free water. RNA concentration was determined by absorption of ultraviolet light at 260 nm (A₂₆₀) readings. The integrity of all RNA samples was determined
by electrophoresis in 1.5% agarose gel containing 6.6% formaldehyde.

**Synthesis of sense RNA.** Sense RNA was synthesized in a 100-μl reaction mixture at 37°C for 1 h. The reaction mixture contained 20 μl transcription (5× buffer); 10 μl of 100 mM dithiothreitol; 5 μl RNasin RNase inhibitor; 20 μl of ATP, CTP, GTP, and UTP; 2 μl Clal-linearized renin template DNA; 2 μl T7 RNA polymerase; and 41 μl nuclelease-free H2O. At the end of the incubation, 5 μl of RNAse-free DNase were added to the reaction to digest the template DNA. The transcribed product was purified with G-50 Sephadex Quick Spin Column (Boehringer Mannheim) and quantified by an A260 reading. Aliquots of sense RNA were stored at −70°C. Sense renin mRNA was used as a standard for the RNase protection assay (RPA) for quantification of renin mRNA.

**Labeling of antisense RNA probe.** The in vitro transcription reaction was performed with linearized template and SP6 RNA polymerase as described by Melton et al. (28) with minor modifications. Antisense renin probe (specific activity 6–9 × 10⁶ counts·min⁻¹·μg⁻¹) was synthesized in a 20-μl reaction mixture at 37–40°C for 1 h. The reaction mixture was prepared by adding in sequence the following components at room temperature: 4 μl transcription (5× buffer); 2 μl of 100 mM dithiothreitol; 1 μl RNasin RNase inhibitor; 4 μl each of 2.5 mM ATP, GTP, and UTP; 2.4 μl of 100 mM CTP; 1 μl EcoRI-linearized renin template DNA; 5 μl (50 μCi) of [α-³²P]CTP; and 1 μl SP6 RNA polymerase. At the in vitro transcription, 0.1–0.2 U of RQ1 RNAse-free DNase was added into the reaction mixture and incubated at 37°C for 15 min. The probe was purified with the use of a G-50 Sephadex Quick Spin Column (Boehringer Mannheim). Purified probe (1 μl) was used to determine the counts per minute.

**RPA.** Total RNA (20 μg) was analyzed for each sample with the use of an RPA kit (RPA II, Ambion). Samples and standards (1, 5, 10, 20, and 40 pg) were mixed with 32P-labeled antisense renin probe (10⁵ cpm/reaction) and 20 μl hybridization buffer (80% deionized formamide, 100 mM sodium citrate (pH 6.4), 300 mM sodium acetate (pH 6.4), and 1 mM EDTA). The mixtures were heated at 90°C for 5 min and incubated at 45°C for 16 h. After hybridization, 200 μl RNase digestion buffer containing 5 U RNase A and 200 U RNase T1 were added into each reaction and incubated at room temperature for 1 h. The reactions were stopped and precipitated by adding RNase inactivation and precipitation mixture. The products of the RPA were pelleted by centrifugation at 12,000 g for 15 min and fractionated by 5% polyacrylamide–8 M urea minigel. Wet gels were exposed to Fuji medical X-ray film in an intensifying screen cassette overnight at −70°C. The autoradiogram was scanned with the use of a scanning densitometer, and the resulting signal was quantified with the use of ImageMaster software (Pharmacia Biotech). A standard curve was generated by plotting known amounts of renin sense mRNA standards against integrated optical density (OD × area) in the protected bands.

**Measurement of plasma active renin concentration.** Plasma active renin concentration (ARC) was measured as a function of the amount of ANG I generated from angiotensigen. All materials were included in the kit purchased from NEN Life Science Products (¹²⁵I-ANG I Radioimmunoassay Kit; NEN 104). To measure renin concentration independent of endogenous angiotensigen, the method was slightly modified from that described for renin activity. Excess renin substrate (0.5 ml of adult nephrectomized sheep plasma) was added to each aliquot (0.1 ml) of previously frozen plasma, as well as the enzyme inhibitors, dimercaprol and 8-hydroxyquinoline, and maleate buffer (pH 6.0, to ensure a constant pH at the optimum for renin activity). One milliliter of the mixture was then incubated in a shaking water bath at 37°C while the rest was kept at 4°C for 1 h. The ANG I generated was measured by RIA using standards, ¹²⁵I-ANG I, rabbit anti-ANG I, and anti-rabbit gamma globulin solution included with the kit. All samples from an animal were analyzed simultaneously and in duplicate. Results are expressed as net (37°C ng/ml minus 4°C ng/ml) nanograms of ANG I generated per milliliter plasma per hour of incubation.

**Measurement of tissue ARC.** Approximately 100 mg of renal cortical tissue were minced and submerged in 4 ml of cold saline. The minces were homogenized (Tri-R-Stir; model K43) on ice for ~1 min using a Teflon pestle. The homogenate was centrifuged at 2,100 g for 10 min, and the supernatant was collected. An aliquot was taken for protein determination, and the supernatant was diluted to 0.25 mg/ml with saline containing 5.2 mM 2,3-dimercapto-1-propanol (BAL), 0.59 mM 8-hydroxyquinoline, and 10 mM disodium EDTA. This dilution was frozen and stored at −80°C until assayed. ARC was determined as for plasma and is expressed as nanograms of ANG I generated per milligram of protein per hour of incubation.

**Measurement of prostaglandins.** Prostaglandin E₂ (PGE₂) is readily converted in vivo to an unstable 13,14-dihydro-15-keto metabolite. For this reason, we measured the metabolites as an estimate of the actual PGE₂ content. In this assay, all of the PGE₂ metabolites are converted to a single stable derivative, bicyclo-PGE₂, which can be quantified by enzyme immunoassay (Enzyme Immunoassay Kit; catalog no. 514531; Cayman Chemical, Ann Arbor, MI). Tissue was prepared according to instruction with the kit. Briefly, frozen kidney cortex was homogenized with the Ultrasonic Homogenizer. Protein was precipitated with ethanol, and the pH of the supernatant was adjusted to 4 with 1 M acetate buffer. This supernatant was then passed through an activated C₁₈ reverse-phase cartridge (no. 400020; Cayman Chemical), rinsed, and eluted with ethyl acetate-1% methanol. The samples were dried in a Savant Speedvac and frozen until assayed. After reconstitution, they and the standard curve were derivatized by overnight incubation with a carbonate buffer, then diluted appropriately, and assayed using an acetylhdrolyase-linked bicyclo-PGE₂ tracer and anti-serum to bicyclo-PGE₂. The end product of the reaction of acetylhdrolyase with Ellman’s reagent is strongly absorbent at 412 nm. The intensity of this was measured by a Molecular Devices Kinetic Microlate Reader at 405 nm.

**Data analysis.** Data are expressed as means ± SE and were analyzed with two-way ANOVA (multiple comparisons). Paired Student’s t-tests were used to compare appropriate data. Differences were considered significant at the level of P < 0.05.

**RESULTS**

Fetal arterial pH and blood gasses were similar between animals receiving COX-2 inhibitor (pH 7.31 ± 0.03, PO₂ 45.1 ± 3.4 mmHg, PO₂ 17.7 ± 1.1 mmHg) and those undergoing control protocols (pH 7.30 ± 0.05, PO₂ 45.5 ± 6.5 mmHg, PO₂ 19.3 ± 7.0 mmHg). These remained stable during all experiments. Baseline fetal mean arterial blood pressures (MAP) were also similar during all experiments (51.4 ± 3.6 vs. 49.3 ± 2.1 mmHg in control and COX-2-inhibited animals, respectively). Administration of isoproterenol (Isuprel), COX-2 inhibitor, or both did not result in any...
statistically significant changes in MAP (55.2 ± 3.8, 48.5 ± 3.5, and 50.4 ± 2.7 mmHg, respectively).

Baseline fetal heart rates were similar during both control and COX-2-inhibited protocols. Both groups exhibited a significant rise in fetal heart rate after the administration of Isuprel (Fig. 1).

Administration of COX-2 inhibitor resulted in significantly lower basal fetal plasma renin concentrations (Fig. 2). During control studies, fetal plasma renin concentrations were significantly increased in response to isoproterenol stimulation. In animals receiving COX-2 inhibitor, no significant change in fetal plasma renin concentration was observed after isoproterenol stimulation (Fig. 3).

There were no significant differences in mean levels of active renin from renal cortical tissue from fetuses receiving COX-2 inhibitor (4,195.813 ± 999.853 ng ANG I·mg protein⁻¹·h⁻¹) compared with animals undergoing control protocols (4,766.463 ± 1,284.225 ng ANG I·mg protein⁻¹·h⁻¹). As expected, mean PGE₂ levels in the fetal kidney cortex in controls were significantly higher than in animals exposed to COX-2 inhibitor (Fig. 4).

Renin mRNA levels in the renal cortical cells of animals infused with COX-2 inhibitor were lower than in those of saline-infused animals (Fig. 5). Renal cortical cells in culture from fetuses undergoing control protocols were found to have significantly increased levels of renin mRNA when treated with isoproterenol, forskolin, or IBMX compared with treatment with vehicle alone. Only forskolin increased renin mRNA levels in the cells from COX-2 inhibited fetuses (Fig. 6).

**DISCUSSION**

The results of these studies support a tonic stimulatory role for COX-2-derived prostaglandins in the local regulation of the fetal RAS late in gestation. 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 during this phase of development. Thus the data suggest that the
upregulation of the RAS in the perinatal period may be related to the increase in COX-2 expression observed at this time (32, 43).

These findings are consistent with other in vivo and in vitro investigations of the effect of prostaglandins on renin secretion and gene expression. Prostaglandins given in the renal artery have been shown to have a stimulatory effect on renin secretion, and isolated perfused kidneys, isolated glomeruli, and juxtaglomerular cells in culture have all been found to increase renin secretion and renin gene expression when exposed to prostaglandins (7, 19, 24, 39).

The stimulatory effects of prostaglandins are likely mediated through stimulation of cAMP formation. Prostaglandins, specifically PGE2, have been shown to increase intracellular cAMP in juxtaglomerular cells (3, 14, 40). Recent evidence has suggested that this may occur through EP4 receptors present in the glomerulus (2, 19, 37).

Previous investigations of the role of prostaglandins in the renin response to physiological stimuli have predominantly focused on NaCl delivery to the macula densa. Animals given a diet low in salt have increased expression of renocortical COX-2 mRNA and protein (16, 18). Furthermore, in vivo studies have demonstrated an inhibition of the renin secretory response to decreased salt intake as well to furosemide, a blocker of salt transport to the macula densa, after administration of prostaglandin inhibitors (14, 36). Preparations of isolated perfused juxtaglomerular apparatus treated with COX inhibitors show marked inhibition of the stimulatory effect of decreased macula densa NaCl concentration on renin release (12, 39). In addition, COX-2 knockout mice fail to exhibit stimulation of the RAS with low salt intake (46). Kammerl et al. (20) recently reported a blunted response in renin secretion but not gene expression in rats treated with the selective COX-2 inhibitor rofecoxib and a low-salt diet.

The role of prostaglandins in the RAS response to renal hypoperfusion and treatment with ANG I-converting enzyme inhibitors has also been examined. However, the evidence in support of a significant role for prostaglandin formation in the regulation of the RAS under these stimuli has been conflicting. Placement of a clip on the renal artery of adult mice has been shown to result in an increase in expression of COX-2 mRNA and protein as has treatment with an ANG II antagonist (17, 45). Conclusive evidence linking this rise in COX-2 gene expression with the parallel rise in renin is lacking. Mann et al. (26) recently found admin-
istation of a selective COX-2 inhibitor to animals with a renal artery clip to have no effect on renin gene expression. Furthermore, Kammerl et al. (20) were unable to demonstrate an effect of COX-2 inhibition on renin secretion or gene expression in response to treatment with the ANG I-converting enzyme inhibitor ramipril or both ramipril and a low-salt diet. These findings underscore the current lack of understanding regarding the mechanisms and physiological circumstances under which prostaglandins interact with the RAS.

The role of prostaglandins in renin secretion and gene expression in response to β-adrenergic stimulation in the fetus has not been previously examined. Our findings support a necessary role for COX-2-derived prostaglandins in this response. Isoproterenol has been found to act directly on β-receptors on the juxtaglomerular cells to stimulate renin secretion by a cAMP-mediated mechanism (13, 43). As described above, there is compelling evidence to support a mechanism of action for prostaglandins that also is mediated through cAMP. We found a significant reduction in the renin response to β-adrenergic stimulation despite the direct action of isuprel with the renin-containing cells of the juxtaglomerular apparatus. On the basis of these data, COX-2-derived prostaglandins must be an integral cofactor for this response.

In addition to providing evidence to support an essential role for COX-2-derived prostaglandins in the β-adrenergic stimulation of the juxtaglomerular cells, these data suggest a broader role for COX-2-derived prostaglandins in the local regulation of the RAS. Perhaps there is a COX-2-derived prostaglandin-dependent tonic level of cAMP within these cells. One may further speculate that COX-2-derived prostaglandins may be a significant component of any stimulus of the RAS that is mediated by a mechanism involving cAMP formation.

Alternatively, COX-2-derived prostaglandins may have other actions that are distal to the effects of cAMP. It is interesting to note that fetal renal cortical cells in culture exposed to COX-2 inhibitor did not demonstrate an increase in renin mRNA when treated with IBMX, a known inhibitor of cAMP degradation. However, when treated with forskolin, an activator of adenylyl cyclase, these same cells demonstrated a significant increase in renin mRNA. This supports a role for COX-2-derived prostaglandins that is proximal to activation of adenylyl cyclase and is integral to the intracellular increase in cAMP seen with β-adrenergic stimulation. This is further supported by the failure of these same cells to exhibit a change in renin mRNA when treated with isoproterenol and suggests a role for COX-2-derived prostaglandins in β-adrenergic receptor expression and activation. These concepts require further investigation.

It is also interesting to note that tissue active renin levels were not significantly different between animals exposed to COX-2 inhibitor and those subjected to control protocols. The juxtaglomerular cells of the fetal kidney are the major source of renin synthesis and storage during ontogeny, and the lack of disparity in tissue levels of active renin between these animals most likely represents a large pool of stored renin in both groups (11).

While these studies contribute to the understanding of the local control of the RAS during development, many aspects of this system remain unclear. These studies have focused on late gestation. Animal studies have found immunoreactive COX-2 to appear first in midgestation (15, 41). Furthermore, gestational age-dependent responses in renin secretion have been observed in the presence of stimuli such as hemorrhage, hypoxia, furosemide infusion, hypotension, and isoproterenol infusion (9, 30, 32–35). However, chronic stimulation of the RAS can induce renin secretion in immature animals to a pattern resembling that of more mature fetuses (35). Elucidation of the functional maturation of COX-2 as well as the interactions of prostaglandins with other modulators of the RAS, such as nitric oxide, during all stages of development is needed.

Continued work in this area will allow better understanding of the regulation of renin in the developing as well as adult individual. The activity and intrarenal distribution of the RAS varies dramatically between fetal and adult life (11, 38). However, fetal and neonatal patterns of renin secretion and expression have been found in adult animals under a variety of physiological and pathological stimuli (8, 11). This plasticity emphasizes the importance of defining the mechanisms involved in the developmental regulation of the RAS. Finally, our observations may provide some insight into the implications of the use of prostaglandin inhibitors during human pregnancy as well as the relationship between fetal disturbances of the RAS and adult diseases such as hypertension.

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