

## Cortisol Infusion Decreases Renin, But Not PGHS-2, EP<sub>2</sub>, or EP<sub>4</sub> mRNA Expression in the Kidney of the Fetal Sheep at Days 109–116

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### ABSTRACT

Renal prostaglandins (PG), renin, and cortisol are necessary for normal kidney development and function during fetal life. We examined the effects of cortisol infusion before completion of nephrogenesis (d 109–116 gestation; 2.0–3.0 mg hydrocortisone succinate/24 h) on the renal mRNA expression of PGHS-2, the PGE<sub>2</sub> receptors, EP<sub>2</sub> and EP<sub>4</sub>, and renin in fetal sheep. Cortisol infusion raised plasma cortisol levels to  $42.8 \pm 6.0$  nmol/L compared with saline infusion levels of  $1.5 \pm 0.5$  nmol/L ( $p < 0.001$ ), but had no effect on fetal body weight, proportional kidney mass, or blood gases. Cortisol decreased significantly the relative expression of renin mRNA (saline:  $0.93 \pm 0.06$  units; cortisol:  $0.32 \pm 0.03$  units,  $p < 0.05$ ), however it had no effect upon the expression of PGHS-2, EP<sub>2</sub>, or EP<sub>4</sub> mRNA in fetal sheep kidney. Although there is substantial evidence that PGE<sub>2</sub> acting through either the EP<sub>2</sub> or EP<sub>4</sub> receptor stimulates renin

synthesis in the adult kidney, our results have demonstrated that before the completion of nephrogenesis, cortisol down-regulation of renin mRNA expression is independent of any change in the expression of PGHS-2, EP<sub>2</sub>, or EP<sub>4</sub> mRNA expression. During nephrogenesis, the insensitivity of PGHS-2, EP<sub>2</sub>, and EP<sub>4</sub> expression to down-regulation by cortisol may permit continued PG regulation of renal development and urine formation. (*Pediatr Res* 55: 637–644, 2004)

### Abbreviations

PG, prostaglandins  
RAS, renin-angiotensin system  
PGHS-1 –r -2, prostaglandin endoperoxide G/H synthase  
RT, reverse transcription

The first budding of the fetal sheep metanephric kidney begins between gestational d 27 and 30, and by d 41 it contains renin, angiotensinogen, and angiotensinogen converting enzyme and is producing urine (1). Nephrogenesis is completed by d 130, more than 2 wk before birth at 145–150 d (2). PG and the RAS are both endogenous factors responsible for renal development in fetal life, however, cortisol, which is present from early in fetal life and is necessary for many developmental aspects of the lung, gut, and other organs, has an uncertain role in the development or function of the fetal kidney (3).

Prostaglandin E<sub>2</sub>, synthesized by either isoform of PG endoperoxide G/H synthase (PGHS-1 or -2), is the primary PG

produced by the fetal sheep kidney during late gestation (4). Through interactions with the EP<sub>1–4</sub> receptor subtypes (5), PGE<sub>2</sub> regulates renal function and has been demonstrated to be essential for normal nephrogenesis (6). Renal PGE<sub>2</sub> also promotes the synthesis and secretion of renin (7). It is clear from several studies that the RAS also plays an important role in renal development and function in the fetus (8, 9). Although bilateral adrenalectomy has no apparent effect on fetal renal function until d 142 of gestation because of placental-maternal exchange mechanisms that maintain fetal electrolyte and fluid balance (3), infusion of cortisol increases allantoic fluid volume at 64 d (10) and may mature the fetal kidney (11), whereas acute administration of cortisol to the immature fetal sheep kidney (at 114 d) leads to natriuresis and diuresis (12). These data suggest that cortisol has an effect on fetal kidney function or maturation. The relationship of elevated (or rising) plasma cortisol concentrations with the fetal renal PG synthesis-receptor system and RAS may therefore be of interest.

Fetal infusion of cortisol (3.7–4.0 mg/d  $\times$  5 d) between days 130 and 135 decreased the mRNA expression of PGHS-2 (13).

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Furthermore, betamethasone administration to pregnant baboons reduced the expression of EP<sub>4</sub> mRNA in the fetal baboon kidney (14). Interestingly, cortisol infusion for 6 d to fetal sheep aged 94–100 d gestation did not alter the expression of renin mRNA within the kidney (15), but infusion of high (parturient) doses of cortisol for 48 h to fetal sheep on d 130, when formation of the kidney is complete, caused suppression of renin mRNA expression (16). Therefore, whereas acute cortisol infusion may lead to natriuresis and diuresis before the completion of nephrogenesis, it appears that in late gestation it also has the potential to suppress the expression of the PG synthesis-receptor system and renin. The age at which cortisol begins to regulate the PG synthesis-receptor system and renin mRNA expression and whether each system is affected similarly are unknown.

We therefore examined the effects of a preterm increase in circulating cortisol on the expression of mRNA for PGHS-2, EP<sub>2</sub>, EP<sub>4</sub>, and renin in the kidney of the fetal sheep. If, in this initial study, similar effects of cortisol were observed on the various mRNA species examined, it would lead to further examination of the effect of cortisol upon the relationship between the PG synthesis-receptor system and renin. Cortisol was infused from d 109–116 at concentrations that represent the increase observed during the last 5–7 d of gestation that are important for the maturation of several fetal organs, but not the high levels observed at parturition. We hypothesized that cortisol would decrease the mRNA expression of the components of the PG synthesis-receptor system and renin.

## METHODS

**Animals and surgery.** All procedures were approved by the Adelaide University Animal Ethics Committee. In 14 pregnant ewes at 103 d gestational age, vascular catheters filled with 50 IU/mL heparinized saline (heparin sodium, David Bull Laboratories, Mulgrave, Australia; saline, 0.9% NaCl solution, Baxter Healthcare, Old Toongabbie, Australia) were inserted into both the fetal and maternal carotid artery and jugular vein. All surgical procedures were performed under general anesthesia induced with an i.v. injection of sodium thiopentone (1.25 g/mL, Boehringer Ingelheim, NSW, Australia) and maintained with 3–4% inhalational halothane in oxygen. Fetal catheters were exteriorized through an incision in the ewe's flank. During surgery, antibiotics (procaine penicillin, 250 mg/mL; procaine hydrochloride, 20 mg/mL, Lyppards, Adelaide, Australia) were administered to both ewe and fetus. Fetal catheters were exteriorized through an incision in the ewe's flank. Ewes were housed in individual pens within animal holding rooms

with a 12-h light-dark cycle. Water was available *ad libitum*, and ewes were fed once daily.

From 109–116 d gestation, 2.0–3.0 mg cortisol/4.4 mL/24 h (hydrocortisone succinate, SoluCortef, Upjohn, Rydalmere, Australia) ( $n = 6$ ) or 4.4 mL/24 h saline ( $n = 8$ ) was infused into the fetal jugular vein. Fetal blood samples were collected daily from all animals between 107 and 116 d gestational age in heparinized tubes. Fetal arterial blood samples (0.5 mL) were also collected daily, for the measurement of blood pH, Po<sub>2</sub>, Pco<sub>2</sub>, oxygen saturation, and Hb content (ABL 520 analyzer, Radiometer, Copenhagen, Denmark).

**Postmortem and tissue collection.** Ewes and fetuses were killed with an overdose of sodium pentobarbitone (Lyppards, Castle Hill, NSW, Australia) at 116 d gestation. Fetal sheep were delivered by hysterectomy, weighed, and decapitated. Fetal kidneys were weighed and a sample containing both renal cortical and medullary tissue was collected from the mid-glandular section of one kidney from each fetus. These samples were snap frozen in liquid nitrogen and stored at –80°C.

**Cortisol RIA.** Cortisol was extracted from fetal plasma using dichloromethane (2 mL, AnalaR, Merck Pty. Ltd., Kilsyth, Victoria, Australia) (17) and measured using the Orion Diagnostica cortisol kit (Orion Diagnostica, Espoo, Finland), as described previously (18).

**Real-time RT-PCR.** To ensure that the relative contribution of renal cortical and medullary RNA to the whole kidney RNA sample examined was equal, total RNA was extracted from 50–100 mg of each tissue using Sigma Chemical Tri-reagent, as described previously (19, 20) (Sigma Chemical, St. Louis, MO, U.S.A.). Extracted RNA was resuspended in Tris-EDTA buffer (TE: 10 mM Tris, 1 mM EDTA, pH 7.4), and the RNA concentrations determined by spectrophotometry. Renal cortical and medullary RNA from each kidney were then combined in equal proportions, treated twice with DNase I, (Ambion DNA-free, Ambion, Austin, TX, U.S.A.) and RNA concentration determined by spectrophotometry. In this initial study, our aim was to assess the effect of cortisol infusion upon total target mRNA in combined tissues. Primers for each gene (Table 1) were designed with the assistance of Primer Premier 5.0 Software (Premier Biosoft International, Palo Alto, CA, U.S.A.) and were optimized for annealing temperature (64–70°C) and reverse transcription RNA concentration. Correct product size and sequence were confirmed by agarose gel electrophoresis and sequencing.

RNA (500 ng) was reverse transcribed using the TaqMan reverse transcription (RT) reagents kit (Applied Biosystems, Foster City, CA, U.S.A.), containing 1× RT buffer (TaqMan

**Table 1.** Primers used for real-time RT-PCR

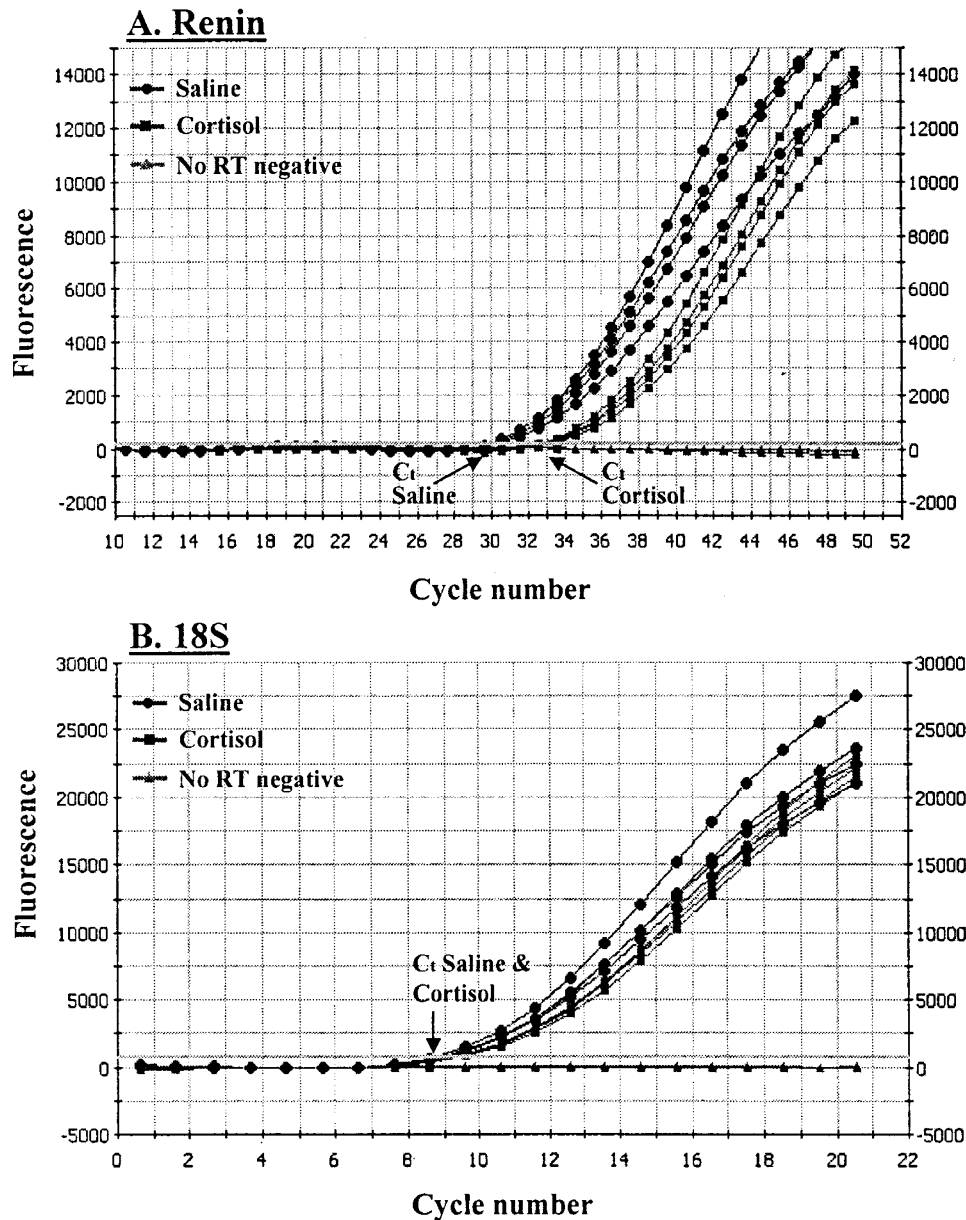
Accession no.	Gene	Forward primer	Reverse primer	Size
M11188*	18S	5'-(36) CTACACATCCAAGGAAGGC (56)-3'	5'-(150) GACTCATTCCAATTACAGGGC (129)-3'	115nt
U68486	PGHS-2	5'-(1234) GGCCAGGAGTACAACATCAGC (1255)-3'	5'-(1356) ATTCCTACGAGCGACC (1338)-3'	123nt
AF035416	EP <sub>2</sub>	5'-(11) GCAACTTCAGCGTCATCGTCAA (32)-3'	5'-(129) CCTCCGCATGGATGCCCT (111)-3'	119nt
AF035418	EP <sub>4</sub>	5'-(7) CGCAAGGAGCAGAAGGAGACGA (28)-3'	5'-(179) CCGAAGAAGAGCAGGATGAAGGTG (156)-3'	173nt
L43524	Renin	5'-(824) CTGGCAGATCAGAATGAAAGGG (845)-3'	5'-(954) CCATGAGCAGTCTCAGGGAGC (934)-3'	131nt

\* No ovine sequence was available for the 18S gene. The rat (M11188), human (M10098, U034321), mouse (X00686), and rabbit (X06778) sequences were aligned and primers were designed from the regions of the highest homology.

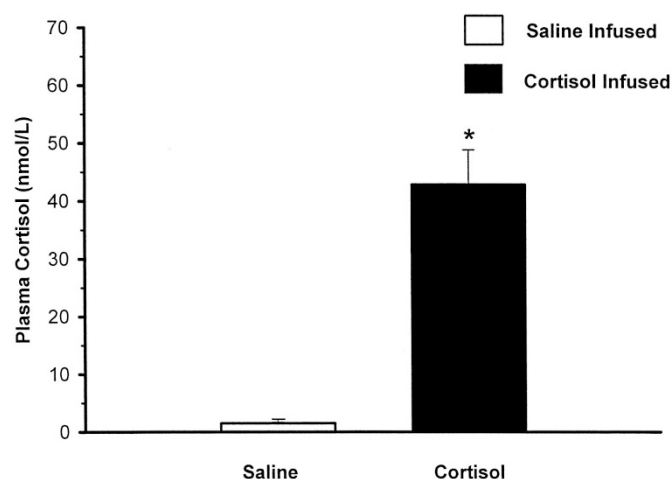
RT buffer), 5.5 mM MgCl<sub>2</sub>, 500 μM deoxyNTP, 2.5 μM random primers, 0.4 U/μL RNase inhibitor, and 1.25 U/μL reverse transcriptase (MultiScribe) in a total volume of 100 μL. Control mixtures were prepared for each RNA sample containing no reverse transcriptase and a no-template control also included. A six-point standard curve was constructed by serial dilution of an RNA sample from one control animal and was reverse transcribed in parallel with the experimental samples. The RT thermal cycle was conducted using the Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, CA, U.S.A.) as follows: 25°C (10 min), 48°C (30 min), and 95°C (5 min) before freezing at -20°C.

PCR reaction mixtures (50 μL) were prepared containing 1× SYBR Green buffer, 3 mM MgCl<sub>2</sub>, 1 mM dNTP mix with

dUTP substituted for dTTP, 0.025 U/μL Taq polymerase (AmpliTaQ Ddd), 0.01 U/μL Uracil N Glycosylase (Amp Erase UNG), 0.2 μM sense primer, 0.2 μM antisense primer, and 2 μL cDNA using the SYBR Green PCR core reagents kit (Applied Biosystems). Aliquots (50 μL) in triplicate of each cDNA sample were transferred to a 96-well optical plate (iCycler iQ PCR plates, Bio-Rad Laboratories). Wells were sealed using optical tape (iCycler iQ optical grade tape, Bio-Rad Laboratories), and the plate centrifuged briefly. Fluorescence measurements for each experimental run were standardized either using the experimental plate or an external well factor plate. The thermal cycle protocol used for amplification was 50°C (2 min), 95°C (10 min), and 40× 95°C (15 s) and 70°C (60 s), or appropriate annealing temperature (30 s) fol-



**Figure 1.** Representative amplification vs cycle plot for renin mRNA (A, upper figure) or 18S r RNA (B, lower figure) from kidney from saline-treated (circles) and cortisol-treated (squares) fetal sheep. C<sub>T</sub> is the threshold cycle. The no RT (reverse transcriptase) negative control (triangles) hovers around the zero fluorescence line for all cycles indicating no random amplification. Each line represents a different fetus. Cortisol treatment caused a significant increase in the threshold cycle required for renin amplification (meaning lower abundance for its mRNA), whereas there was no effect of cortisol relative to saline treatment on the reference gene 18S r RNA amplification.



**Figure 2.** Plasma cortisol levels in cortisol-infused (closed bars,  $n = 6$ ) and saline-infused (light bars,  $n = 8$ ) fetal sheep. Data are shown as mean  $\pm$  SEM. \*Significant difference ( $p \leq 0.05$ ) between the saline- and cortisol-infused groups.

lowed by 72°C (30 s). Sample fluorescence was measured each cycle during either the 70°C or 72°C step. Melt curve data were then collected immediately, using the thermal protocol 95°C (1 min) and 55°C (1 min), and then fluorescence was measured as sample temperature was increased by 1°C increment/12 s.

**Immunocytochemistry.** Kidney sections (5  $\mu$ m) from cortisol and saline-infused control animals were prepared and mounted on pretreated slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA, U.S.A.) to localize PGHS-2 using immunocytochemical techniques as described previously (21). In brief, we used the Zymed Histostain Plus immunostaining kit (Zymed Laboratories, South San Francisco, CA, U.S.A.), which utilizes an avidin-biotinylated secondary antibody, a streptavidin-horseradish peroxidase conjugate, and an Immunopure metal enhanced diaminobenzidine (DAB) substrate (Pierce Chemical, Rockford, IL, U.S.A.) as the chromagen. The rabbit-anti mouse PGHS-2 primary antibody (Cayman Chemical, Ann Arbor, MI, U.S.A.) was used at a concentration of 1:600. According to the manufacturer, the PGHS-2 antibody is specific for ovine, human, murine, monkey, guinea-pig, and rat PGHS-2 and does not cross-react with PGHS-1 from all species. The PGHS-2 antibody has been used previously to localize PGHS-2 in sheep tissues (22). Photomicrographic images were captured from an Olympus BHS microscope with a 20 $\times$  DPlanApo objective using a Panasonic KR222 camera connected to VideoPro imaging software (Leading Edge, Hove, SA, Australia).

**Data analysis.** After the PCR run was completed, melt curve analysis was used to confirm that there was one amplified product. Baseline fluorescence was determined, and a threshold value assigned at 10 SD above the mean baseline fluorescence.

This was used to assign a threshold cycle ( $C_T$ ) for each well (Fig. 1). The mean  $C_T$  was then calculated for each sample from the triplicate replications. A maximum variation between the  $C_T$  measurements of the three aliquots was set at 0.6  $C_T$ , and data points outside this value were excluded from further analysis. The difference in the mean abundance of renal 18S rRNA between the cortisol and control groups was 0.2  $C_T$  and 18S rRNA was therefore used as the reference gene. The ratio of target gene mRNA abundance to 18S rRNA abundance was determined using a modification of the data analysis method recently developed and validated for real-time PCR measurement of relative gene expression (23). One sample from the saline-treated group was designated a control and the remaining samples (saline and treated) were then compared with this control sample using the equation below. The PCR reaction efficiency ( $E$ ) for each primer set was determined from the slope of the standard curve [ $E = 10^{(-1/\text{slope})}$ ]. The relative gene mRNA expression for each sample was then determined:

$$\text{Ratio} = \frac{(E_{\text{target}})^{\Delta C_T(\text{Control} - \text{Experimental})}}{(E_{\text{ref}})^{\Delta C_T(\text{Control} - \text{Experimental})}}$$

Data are expressed as mean  $\pm$  SEM, and results from the cortisol and control groups were compared using  $t$  test for normally distributed data. A probability level of 5% ( $p < 0.05$ ) was considered significant.

## RESULTS

Plasma cortisol was significantly increased by cortisol infusion (saline: 1.5  $\pm$  0.5 nmol/L, cortisol: 42.8  $\pm$  6.0 nmol/L,  $p < 0.001$ ) (Fig. 2), however the infusion of cortisol did not significantly alter mean fetal arterial  $P_{O_2}$ ,  $P_{CO_2}$ , pH, Hb, or oxygen saturation (Table 2). Fetal body weight (saline: 2.15  $\pm$  0.08 kg, cortisol: 2.14  $\pm$  0.09 kg,  $p > 0.05$ ), total renal mass (saline: 17.3  $\pm$  1.1 g, cortisol: 16.08  $\pm$  0.8 g,  $p > 0.05$ ), and renal proportion of body weight (saline: 0.8  $\pm$  0.02%, cortisol: 0.8  $\pm$  0.03%,  $p > 0.05$ ) were also unaltered by cortisol infusion.

Cortisol infusion did not alter the relative expression of mRNA for PGHS-2 (saline: 0.70  $\pm$  0.09 units, cortisol: 1.14  $\pm$  0.29 units) (Fig. 3A) or for the EP<sub>2</sub> (saline: 1.19  $\pm$  0.31 units, cortisol: 1.61  $\pm$  0.35 units) (Fig. 3B) or EP<sub>4</sub> (saline: 1.15  $\pm$  0.08 units, 1.08  $\pm$  0.10 units) (Fig. 3C) receptors. The relative expression of renin mRNA was significantly reduced by fetal cortisol infusion (saline: 0.93  $\pm$  0.06 units, cortisol: 0.32  $\pm$  0.03 units) (Fig. 4).

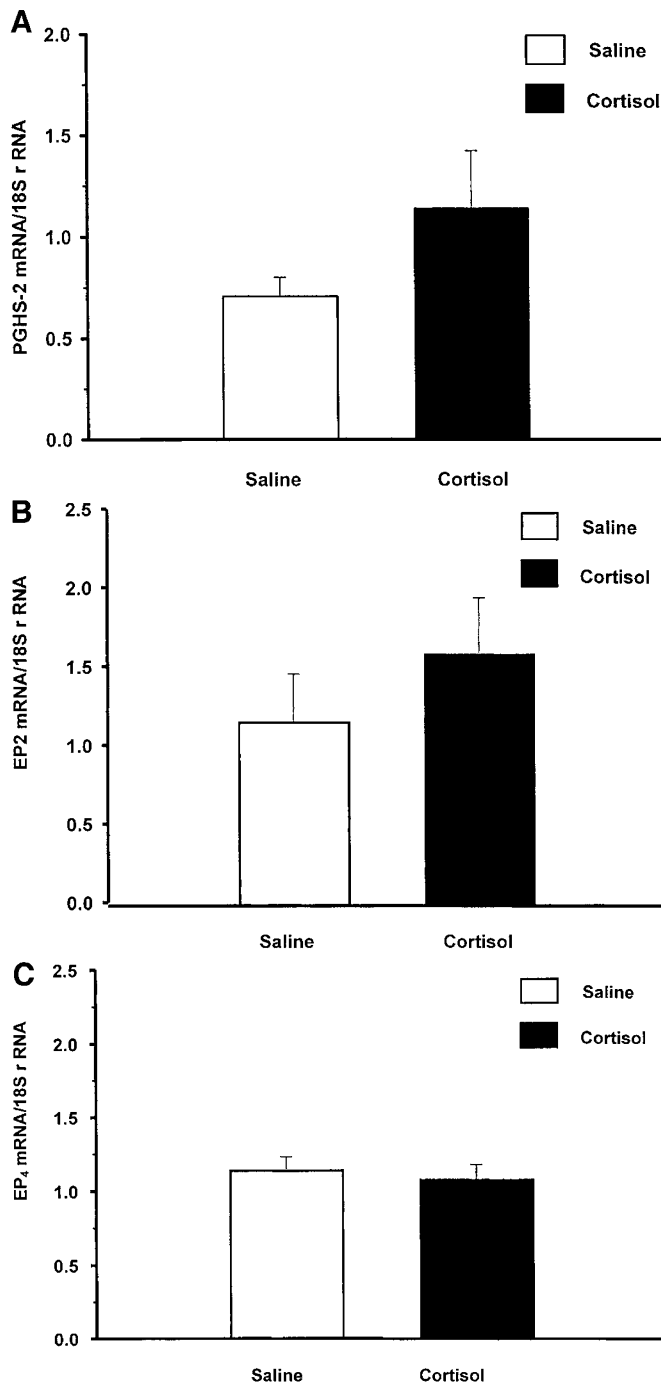
A similar distribution of staining for PGHS-2 was observed in the cortical and medullary regions of kidneys from saline and cortisol fetuses (Fig. 5). PGHS-2 immunoreactivity (PGHS-2-ir) was localized to the macula densa and in some of the cells within the glomeruli and distal and proximal tubules

**Table 2.** Fetal arterial blood gas status in saline- and cortisol-infused fetal sheep throughout cortisol infusion

Group	$P_{O_2}$ (mm Hg)	% $O_2$ Saturation	$P_{CO_2}$ (mm Hg)	pH	Hb (g/L)
Saline ( $n = 8$ )	22.5 $\pm$ 0.9	68.0 $\pm$ 2.2	46.2 $\pm$ 1.3	7.35 $\pm$ 0.01	8.8 $\pm$ 0.3
Cortisol ( $n = 6$ )	23.6 $\pm$ 0.7	73.4 $\pm$ 1.2	46.1 $\pm$ 0.7	7.37 $\pm$ 0.01	8.7 $\pm$ 0.3

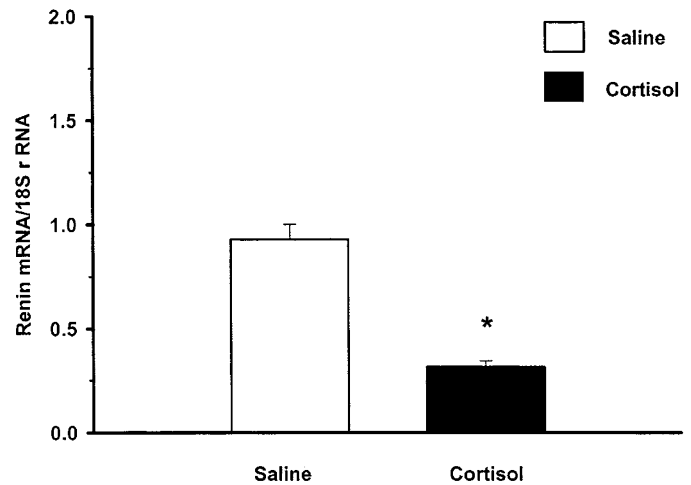
Data are shown as mean  $\pm$  SEM.





**Figure 3.** Plasma cortisol and fetal sheep renal mRNA of the PG synthesis-receptor system. (A) The renal expression of PGHS-2 mRNA/18S r RNA in cortisol-infused fetal sheep (closed bars,  $n = 6$ ) was not different from the saline-infused fetal sheep (open bars,  $n = 8$ ). Data are shown as mean  $\pm$  SEM. (B) Renal EP<sub>2</sub> mRNA/18S r RNA expression was not different between the saline-infused fetal sheep (open bars,  $n = 8$ ) and cortisol-infused fetal sheep (closed bars,  $n = 6$ ). Data are shown as mean  $\pm$  SEM. (C) Renal expression of EP<sub>4</sub> mRNA/18S r RNA was not different between the saline-infused fetal sheep (open bars,  $n = 8$ ) and cortisol-infused fetal sheep (closed bars,  $n = 6$ ). Data are shown as mean  $\pm$  SEM.

within the cortical region of the kidney (Fig. 5, A and C). PGHS-2-ir was localized extensively in the collecting ducts and the ascending and descending limbs of the loop of Henle in the medullary region of the kidney from saline and cortisol



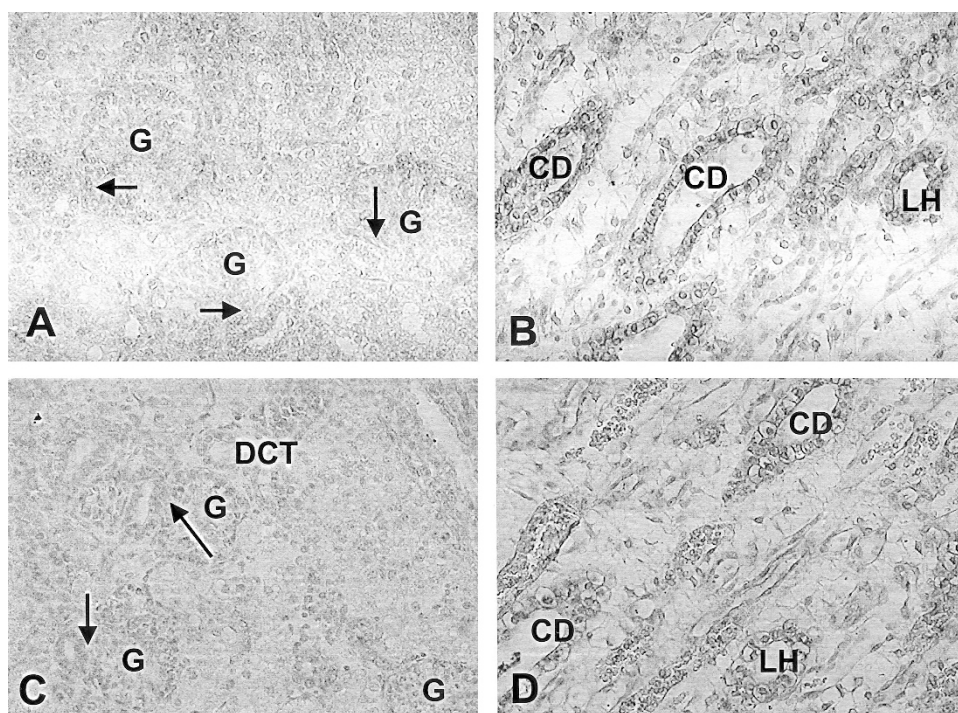
**Figure 4.** The renal expression of renin mRNA/18S r RNA in saline-infused fetal sheep (open bars,  $n = 8$ ) compared with cortisol-infused fetal sheep (closed bars,  $n = 6$ ). Data are shown as mean  $\pm$  SEM. \*Significant difference ( $p \leq 0.05$ ) between the saline- and cortisol-infused groups.

fetuses (Fig. 5, B and D). There was also positive staining for PGHS-2 in the endothelial cells lining blood vessels within the cortex and medulla. PGHS-2-ir was not present in the interstitial cells of the medulla.

## DISCUSSION

The results of this study indicate that although exposure to elevated glucocorticoids during nephrogenesis has no effect on the expression of either PGHS-2 mRNA or protein localization or mRNA for the EP<sub>2</sub> or EP<sub>4</sub> receptors in the kidney of the fetal sheep, it does decrease renin mRNA expression. There are two particularly interesting implications of these results. Firstly, the mechanism by which cortisol down-regulates renin mRNA is independent of any change in the expression of the enzyme or receptors believed to mediate prostaglandin regulation of renin expression in the adult. Secondly, the insensitivity of PGHS-2, EP<sub>2</sub>, and EP<sub>4</sub> mRNA expression to down-regulation by cortisol before the completion of nephrogenesis may be protective, thereby facilitating continued PG regulation of fetal kidney development and function.

Glucocorticoids have a range of effects on the mesonephric and metanephric kidney. In the sheep, glucocorticoid administration very early in gestation results in a reduction in sodium and chloride excretion from the mesonephric kidney (24). However, by d 64, when the mesonephric kidney is no longer functional and the metanephric kidney is actively synthesizing RAS proteins and producing urine, dexamethasone administration increased fetal urine flow rate (10). Later, at 114 d gestation, acute (4 h) intrafetal cortisol infusion increased GFR, and decreased proximal tubule sodium reabsorption, resulting in diuresis and natriuresis (12). Older fetal sheep (133 d gestation) in this study also displayed decreased proximal tubule sodium reabsorption, but were able to compensate as distal tubule sodium reabsorption matured, such that no diuresis or natriuresis occurred (12). Betamethasone administration to fetal sheep at a similar gestational age increased renal Na, K-ATPase activity in two studies, and this increase may ex-



**Figure 5.** Photomicrographs of sections of kidneys from saline- (*A* and *B*) and cortisol-infused (*C* and *D*) fetal sheep at 116 d of gestation, stained for PGHS-2, where the brown cytoplasmic staining is a positive signal for PGHS-2-immunoreactivity and cell nuclei are counterstained blue with Mayer's hematoxylin. *A* and *C*: Photomicrographs of the kidney cortical region immunostained for PGHS-2 from saline-infused (*A*) and cortisol-infused fetuses (*C*). There is a similar distribution of PGHS-2 immunoreactivity in the cortical region of the kidneys from the saline- and cortisol-infused fetuses, where PGHS-2 is localized to the macula densa (identified by the arrows) and some cells within the glomerulus (*G*) and distal convoluted tubules (*DCT*). *B* and *D*: Photomicrographs of the kidney medullary region immunostained for PGHS-2, from saline-infused (*B*) and cortisol-infused fetuses (*D*). There is a similar distribution of PGHS-2 immunoreactivity in the medullary region of the kidneys from the saline- and cortisol-infused fetuses, where PGHS-2 is localized to the loop of Henle (*LH*) and the collecting ducts (*CD*).

plain the increased capacity for distal tubule sodium reabsorption (25, 26). More recently, studies have shown that early (d 27) fetal exposure to glucocorticoids decreases the number of nephrons in adult sheep (27).

The influence of adrenal steroids on both the renal expression of mRNA or protein for the PG synthesis-receptor system and renin may depend upon the gestational age of the fetus or adulthood. In adult rats, adrenalectomy decreased PGHS-2 protein in the papilla by half, an effect that was partially ameliorated by glucocorticoid replacement, whereas mineralocorticoid treatment indirectly (perhaps *via* interstitial electrolyte hypertonicity) increased PGHS-2 protein by five times over control (28). Our data in the fetus are in contrast to these as cortisol treatment had no effect upon immunoreactive PGHS-2 in medullary or cortical regions of the fetal sheep kidney. In other studies, cortisol infusion (3.7–4.0 mg/d) between d 94 and 100 had no effect upon renin mRNA expression in the fetal kidney (15). We have demonstrated a significant decrease in renin mRNA expression when cortisol is infused from d 109 to 116, and, in late gestation (d 130), 48-h infusions of high levels of cortisol (72 mg/d) resulted in a decrease in the renal expression of renin (16). Furthermore, both plasma renin activity and plasma angiotensin II were decreased after either maternal or intrafetal betamethasone administration in late gestation (26).

In the adult kidney, it has been conclusively established that PGE<sub>2</sub>, acting *via* the EP<sub>2</sub> or EP<sub>4</sub> receptor, increases renin

synthesis and secretion from the juxtaglomerular apparatus (5). A recent study has demonstrated an association between the ontogenic increase in PGHS-2 mRNA abundance and that of renin mRNA in the fetal sheep renal cortex between gestational d 105 and 135 (29). In two separate studies, nonspecific inhibition of prostaglandin synthesis by indomethacin decreased plasma renin activity in fetal sheep (29, 30), and more recently specific acute inhibition of PGHS-2 by nimesulide also decreased basal and stimulated plasma renin concentrations (31). Interestingly, infusion of 3.7–4.0 mg/d cortisol to fetal sheep between d 130 and 135 decreased the relative abundance of PGHS-2 mRNA in fetal sheep cortex tissue (13). The absence of any effect of cortisol on PGHS-2 in our study, however, demonstrates that the capacity of cortisol to down-regulate renal PGHS-2 mRNA expression is also dependent on gestational age. Our results may further suggest that the effect of PG on renin synthesis in the fetal kidney also change with increasing gestational age, although it will require other experiments to determine this as testing this possibility was not an aim of this study.

Glucocorticoids have predominantly been reported as having a negative regulatory effect on PGHS-2 mRNA expression, although it has also been reported that in cultured amnion cells, cortisol specifically up-regulates PGHS-2 expression (32). The mechanisms by which glucocorticoids suppress PGHS-2 mRNA expression are not considered to be direct—no glucocorticoid response elements have been detected in the pro-

moter region of PGHS-2 in any species studied thus far (33), and there is evidence that the effects of glucocorticoids on PGHS-2 mRNA are mediated *via* interference with a glucocorticoid sensitive transcription factor (34) and also by decreasing mRNA stability (35). It is possible, therefore, that between 109 and 116 d gestation, PGHS-2 mRNA expression is not down-regulated by cortisol because at this age the factors responsible for PGHS-2 glucocorticoid sensitivity are not expressed in the kidney.

Activity of the prostaglandin synthetic enzyme, PGHS-2, is essential to normal renal development. In the human fetus, PGHS-2 mRNA is present in the kidney during the first trimester and increases its abundance with each trimester and into the early neonatal period, whereas PGHS-1 mRNA expression does not change during gestation (36). Fetal mice in which the PGHS-2 gene has been deleted develop severe renal tubule dysgenesis, an event not observed with PGHS-1 null fetuses (6, 37). Administration of specific PGHS-2, but not PGHS-1, inhibitors to fetal and newborn mice (much of nephrogenesis occurs postnatally in mice) caused an identical phenotype in terms of arrest of renal development. In these mice, PGHS-2 was localized by immunohistochemistry to the S-shaped bodies and macula densa in the cortex (38). Thus, it is clear that PG derived from PGHS-2 are important for nephrogenesis.

Prostaglandin E<sub>2</sub> is the most abundant of the renal PGs and is synthesized all along the nephron (39), where it regulates vasoactivity and water and salt handling properties in the adult (40, 41). The receptors for PGE<sub>2</sub> (EP<sub>1-4</sub>) are found throughout the adult kidney in both vascular and tubular sites in adult animals (5). The role of EP<sub>2</sub> in adult renal function is controversial. EP<sub>2</sub> is localized in the nephron of the adult rat kidney to the descending thin limb of the loop of Henle, and outer medulla vasa recta (42) and to the renal cortical arteries and arterioles in the human kidney (43). At present, the specific role of the EP<sub>2</sub> receptor in the adult kidney is unclear, although two gene deletion studies have suggested a role for this receptor in the regulation of renal salt reabsorption (44, 45). We are the first to report the presence of EP<sub>2</sub> mRNA in fetal sheep kidney, which opens the possibility of a unique role for it in kidney function and development during the fetal period (20).

EP<sub>4</sub> receptor expression is abundant within the glomerulus of the adult kidney (46, 47). A recent study (14) demonstrated expression of EP<sub>4</sub> mRNA in the fetal sheep kidney, the relative abundance of which increased 6-fold between gestational d 100 and 138. Through increasing intracellular cAMP production, the EP<sub>4</sub> receptor regulates glomerular hemodynamics, and promotes the synthesis and secretion of renin within the juxtaglomerular apparatus. Further along the nephron, EP<sub>4</sub> promotes tubular cAMP-stimulated salt and water reabsorption (5). We recognize that other levels of the PG synthesis-receptor system may be affected by cortisol treatment that were not studied or detected here, such as the phospholipases or isomerases, the levels of these proteins, or the actual production of PG. Frequently, though, a reliable scan of the PG system can be made by following the changes in mRNA abundance, which was the intention of this initial study.

Activity of the RAS increases substantially from mid to late gestation in the fetal sheep, and is higher during fetal and neonatal life than in the adult (48). Appropriate activity of the RAS has been demonstrated to be essential for normal renal development and function. Blockade of the AT<sub>1</sub> receptor with the receptor antagonist losartan in neonatal rats resulted in structural abnormalities of the arteriolar vascular tree, and reduced both the size and number of nephrons developed (8). Functionally, administration of captopril to block AII production in late gestation fetal sheep increased fetal renal blood flow, but concurrently decreased renal vascular resistance thereby reducing GFR, sodium excretion, and urine output (9).

Future studies exploring in greater detail the roles of PGE<sub>2</sub> and its receptors at specific points along the nephron or vascular tree in the developing kidney and their interactions with the RAS and cortisol should prove interesting. A greater understanding of the developmental and functional role of these regulators will help predict fetal renal responses to stressors such as undernutrition or hypoxemia (20), potential long-term effects of stressors upon adult blood pressure regulation, periods of development during which PGHS-2 inhibitors may be administered to mothers without causing alterations in fetal kidney development or function (38), or the impact of glucocorticoid administration for fetal lung maturation upon the developing kidney.

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