Active renin, prorenin, and renin gene expression after reduced renal perfusion pressure in term ovine fetuses

JON S. ROSNES,1 NANCY VALEGO,2 JINJUAN WANG,2 TIMOTHY ZEHNDER,2 AND JAMES C. ROSE1,2

1Department of Obstetrics/Gynecology and 2Department of Physiology/Pharmacology and Perinatal Research Laboratories, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27157

Rosnes, Jon S., Nancy Valego, Jinjuan Wang, Timothy Zehnder, and James C. Rose. Active renin, prorenin, and renin gene expression after reduced renal perfusion pressure in term ovine fetuses. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R141–R147, 1998.—We studied the pattern of plasma active renin concentration (ARC), prorenin concentration (PRC), renal renin concentration, and the renin mRNA levels in ovine fetuses subjected for 24 h to reduced renal perfusion pressure (RPP). The results obtained in five animals (133.8 ± 1.4 days of gestation) in which RPP was reduced by 10 mmHg were compared with those in seven control fetuses (130.3 ± 0.8 days of gestation) without pressure reduction. Plasma samples were obtained before and at intervals of 24 h after initiating reduced RPP. The plasma ARC increased within 60 min of reduced RPP, reaching a maximum (13.0 ± 4.7 vs. 115.7 ± 23.8, P < 0.01) at 3 h. The ARC then declined toward control values. In contrast, plasma PRC did not increase consistently until 4 h into reduced RPP, with maximal levels at 24 h (8.2 ± 2.4 vs. 87.7 ± 21.9, P = 0.016). Within the kidney PRC, but not ARC, increased significantly, by 2.5-fold. Reduced RPP also increased renal renin mRNA levels (P = 0.004). We conclude that a chronic reduction in RPP in the near-term ovine fetus increases renal PRC and is associated with increased plasma prorenin levels. The data suggest that the conversion of prorenin to active renin is an important regulation point of the renin ANG system during development.

renin angiotensin system; hypotension

THE FETUS DEPENDS on multiple maturational changes during development in utero for assurance of continued postnatal survival. One of the most important systems in this regard is the renin ANG system (RAS), which is integrally involved in the regulation of extracellular fluid composition, blood volume, and blood pressure.

In the near-term fetal sheep, numerous stimuli, including hypoxia (23), furosemide (10, 27), hemorrhage (6), and hypotension (10, 21), increase plasma renin activity (PRA).1 In acute experiments the elevation in PRA seen with suprarenal aortic occlusion is rapid, proportional to the magnitude of the reduction in renal perfusion pressure (RPP) (2), and strongly correlated to changes in renal renin content, suggesting that the synthesis and secretion of renin may be qualitatively correlated processes (19). Similar relationships between renal renin concentration (RRC) and renin mRNA expression have been experimentally observed as well (4). Taken together, acute stimulation of the renin gene results in increased RRC, which is similarly associated with elevations in PRA.

It is known that renin can be secreted in two forms, one of which is inactive and represents the inactive renin biosynthetic precursor, prorenin (9). Observations in human subjects exposed to prolonged dietary sodium restriction have shown that even though parallel changes occur in the secretion of both active renin and prorenin, the prominent secretory form is active renin (28). However, in vitro and in vivo secretion rates for renin appear to depend on renal renin content levels to a certain point, suggesting that despite increasing content, limited increases in active renin secretion may occur (19). In chronic studies in one-clip Goldblatt hypertensive rats with induced renal ischemia, elevations in renin gene expression have not consistently resulted in elevated active renin concentration (ARC) (14).2 These observations suggest that in response to certain stimuli, newly translated prorenin may be secreted immediately with lesser amounts secreted via the pathway that includes the processing necessary for the formation of active renin. Thus chronically elevated renin gene transcription may be associated with changes in the relationship between constitutive secretion of prorenin and the regulated secretion of active renin (18). Although the mature fetal kidney is able to respond in many ways similarly to the adult animal, the relationship between prorenin and active renin after chronic stimulation is less well understood.

To further investigate the relationships among renin secretion, renal renin content, and renin gene expression during development, we evaluated the change in plasma levels of prorenin and active renin, the change in renal prorenin and active renin content, and the change in renin mRNA in near-term fetal sheep with chronically reduced RPP. The goals of this study were to determine whether 1) prolonged reduction in RPP alters the relationship between the plasma levels of prorenin and active renin, 2) any alteration in plasma prorenin or renin concentration is accompanied by changes in renal prorenin or ARC, and 3) chronically reduced RPP alters renin gene expression in utero.

MATERIAL AND METHODS

Animal Preparation

The study was conducted after protocol review and approval by the Animal Care and Use Committee at Bowman

1 Plasma renin activity (PRA) is a measure of the amount of ANG I (in ng) generated per milliliter of plasma when incubated with endogenous substrate only.

2 Active renin concentration (ARC) is a measure of the amount of ANG I (in ng) generated per milliliter of plasma per hour when incubated with excess exogenous substrate.
Gray School of Medicine. Ewes with known insemination dates and in which pregnancy was confirmed at 60 days by ultrasound were obtained from a local supplier. All animals were housed in an environmentally controlled facility with free access to food and water. Pregnant ewes were fasted for 24 h before being sedated with ketamine intramuscularly (20 mg/kg) and then intubated and mechanically ventilated with 1–2% halothane in 100% oxygen. These anesthetics also provided anesthesia to the fetus.

At surgery, a midline incision was made in the abdomen of the ewe, exposing the gravid uterus. A uterine incision was made over the fetal hindlimbs, allowing exposure of fetal lower extremities and adjacent lower abdomen. Polyvinyl catheters (1.0 mm ID, 1.7 mm OD) were introduced into the fetal femoral arteries bilaterally, advanced into the fetal aorta, and secured in place. The catheters were then passed through the uterine incision, and the hysterotomy was closed. Next, the uterus over the fetal lower thoracic and lumbar spine was opened, and a longitudinal skin incision was made overlaying the fetal spine. Further left flank dissection allowed the fetal left kidney and adjacent descending abdominal aorta to be exposed. Proximal to both renal arteries, a 6-mm inflatable Silastic occluder was placed around the aorta for the occluder group, while sham dissection or placement of a noninflatable occluder was performed in the control group. The inflation line was passed through a separate fetal skin incision, an amniotic catheter was secured to the fetal back, and all lines were passed through the hysterotomy incision. The uterus was closed. The fetal catheters were then brought through a separate maternal left flank incision to allow access in the recovery and study periods. The maternal abdomen was closed. Finally, a small incision in the left groin of the ewe allowed placement of femoral arterial and venous lines (1.3 mm ID, 2.3 mm OD), which were tunneled subcutaneously to exit the maternal flank incision with the fetal lines. All lines were kept in a sterile pouch within elastic netting that surrounded the abdomen. The ewes were examined daily throughout this protocol, and for the first 3 days amidipillin (1 g) and gentamicin (80 mg) were administered intravenously to the ewes. All lines were flushed with saline and filled with heparin, 1,000 U/ml solution, at least every 48 h. The recovery period was 4–5 days in each group before study.

**Experimental Design**

All studies were conducted with the ewes standing quietly in a metabolic cart. Two groups of fetal sheep were studied: 5 fetuses comprised the occluder group (133.8 ± 1.4 days of gestation) and 7 fetuses the control group (130.3 ± 0.8 days of gestation). In each experiment, pressures transducers (Cobe), coupled to a polygraph (Hewlett-Packard) and interfaced with a personal computer system, continuously measured the fetal aortic systolic, diastolic, and mean arterial blood pressure (MAP) and the amniotic cavity pressure. On the days the occluder protocol was begun, completion of a baseline blood pressure recording of at least 30 min preceded occluder inflation. During this time the mean femoral blood pressure was averaged and a target blood pressure reduction of 10 mmHg from baseline mean femoral blood pressure was set for the 24-h experiment. Blood samples were obtained before occluder inflation and hourly for 6 h in the occluder group or hourly for 6 h in the control group. Lines were then closed and rechecked periodically to ensure continued reduction of mean femoral blood pressure. At 23 and 24 h after occluder inflation, or control group start time, two additional blood samples were obtained. The blood sampling procedure was standardized and included the withdrawal of 7 ml of fetal blood for plasma hormone levels, serum electrolytes, and arterial blood gas determinations. An equal volume of maternal arterial blood was infused into the fetus to avoid hemorrhagic artifact over the study period. The blood samples were divided into two aliquots, with 5 ml being placed into a chilled 15-ml centrifuge tube containing 0.15 ml sodium EDTA solution (12.2 g EDTA/100 ml) and 2 ml being placed into a clean chilled centrifuge tube for serum electrolyte determination. All tubes were kept on ice until centrifuged at 3,000 g for 8 min. Plasma samples, divided into four aliquots, and the serum electrolyte samples were placed in Eppendorf tubes and immediately stored at −80°C. Arterial blood gases were drawn into preheparinized 1-ml syringes at each sampling point and analyzed on a model BMS MK2 blood gas analyzer (Radiometer Instruments) calibrated with a 5% CO2-20% O2-balanced N2 gas mixture at our laboratory's barometric pressure and water vapor pressure at 39°C. Hematocrits were performed in duplicate.

After the study was completed, the ewes were given 10 ml intravenous ketamine (100 mg/ml), and the fetuses were delivered by cesarean section. The ewe was immediately killed on delivery of the fetus by rapid intravenous KCl. The fetuses were killed with an overdose of intravenous pentobarbital. The fetal kidneys were immediately harvested, decapsulated, weighed, and measured, and the cortex was dissected. Tissue was immediately placed in cryoprotective vials and frozen in liquid nitrogen for transport and subsequent storage at −80°C until extraction of mRNA and determination of tissue renin content.

**Blood Chemistries and Hormonal Determinations**

The plasma active renin (ARC) and prorenin concentrations (PRC) were determined by a modification of previously described methods (21). The plasma ARC was determined by the amount of immunoreactive (RIA) ANG I, in nanograms generated per milliliter of plasma per hour incubated. The plasma total renin concentration was obtained by measuring the active renin after treatment with bovine pancreatic trypsin (Sigma) in a dose chosen for maximal activation of renin by previous dose-response curve analysis in our laboratory (22). Each lot of trypsin used was tested by constructing a dose-response curve with pooled plasma and kidney homogenate. Once the optimal dose of trypsin was established for each, this dose was used for subsequent assays. The trypsin activation step took place at 4°C, pH 7.3, for 0.5 h. Trypsin inhibitor was added at room temperature for 15 min. Then excess renin substrate (adult nephrectomized sheep plasma) was added at 37°C for 2 h before the RIA procedure. The ANG I generated was measured with a commercially available RIA kit (ANG I, Iso-tec, Friendswood, TX). The plasma PRC was determined by subtraction of the ARC from the total renin concentration. Samples from both controls and occluder animals were analyzed simultaneously and in duplicate.

Plasma ANG 11 concentrations were determined at baseline, 4, and 24 h after ethanol extraction by RIA using a commercially available kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). These points were chosen based on plasma ANG 11 measurements. After thawing, the plasma samples were centrifuged in a refrigerated centrifuge at 2,000 g for 15 min. Plasma (1 ml) was extracted in 5 ml ethanol, and the tubes were mixed for 30 min and centrifuged again at 2°C. The ethanol was removed from each extraction tube, evaporated to dryness, and reconstituted in 1 ml assay buffer. Samples were corrected for radiolabel recovery, which averaged 65%. The interassay coefficient of variation was 19%, and the sensitivity of the assay, defined as two times the standard deviation of the value detected at 90% binding, was 3.5 pg/ml.
Measurements of renal tissue total and ARC were performed as previously described (22). Fifty milligrams of renal cortical tissue were submerged into a cold neutral buffer solution, containing 15 mM disodium EDTA, 5 mM dimercaprol, and 3.5 mM 8-hydroxyquinoline. This solution was then homogenized with a Bellco 5-ml tapered glass homogenizer. The homogenate was centrifuged at 100 g for 10 min, the supernatant collected, and a sample of the supernatant diluted with neutral buffer solution to a final concentration of 1 mg/ml. Tissue total and ARCs were determined by incubating aliquots of this mixture, at 37°C with nephrectomized sheep plasma, with and without previous activation with trypsin. Concentrations of ANG I were then measured with a commercially available RIA kit (ANG I, I-so-tex). The tissue ARC was expressed as the amount of ANG I generated per milliliter homogenate per hour of incubation per milligram wet tissue weight. Tissue PRC was determined by subtracting the tissue ARC from the total tissue concentration.

Serum electrolytes were measured using an automated chemical analyzer (Astra-8, Beckman, Fullerton, CA).

**Determination of Renal Renin mRNA**

Extraction of renal tissue total RNA. Samples of kidney cortical tissue (100 mg) from both occluder and control fetuses were homogenized in 1.5 ml TRIzol reagent (GIBCO, BRL) using a high-speed polytron for 30–60 s. Chloroform (0.3 ml) were homogenized in 1.5 ml TRIzol reagent (GIBCO, BRL) and then centrifuged at 12,000 g for 10 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 resuspended in RNase-free water. RNA concentration was determined by A_{260} readings. The integrity of all RNA samples was determined by electrophoresis in 1.5% agarose gels containing 6.6% formaldehyde.

Construction of RNA probe. Plasmid pGEM-RW1, previously constructed by our laboratory, containing 870 bp of a fragment of renin cDNA (nucleotide position 116–986), was linearized with EcoRI and in vitro transcribed using SP6 RNA polymerase, producing an antisense renin RNA probe.

Synthesis of sense RNA. Sense RNA was synthesized in 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 rRNA asin ribonuclease inhibitor, 20 µl of ATP, CTP, GTP, and UTP, 2 µl CiaI linearized renin template DNA, 2 µl T7 RNA polymerase, and 41 µl nuclelease-free H2O. After incubation, 5 units 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 and quantified by A_{260} 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/T7 RNA polymerase using the procedure described by Melton et al. (11), with minor modifications. ANG II reagents were purchased from Promega unless otherwise stated. Antisense renin probe (specific activity 6–9 × 10^6 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 the following components at room temperature in sequence: 4 µl transcription 5× buffer, 2 µl of 100 mM dithiothreitol, 1 µl rRNA asin ribonuclease inhibitor, 4 µl ATP, 2.5 mM each 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. After the in vitro transcription reaction was performed, 0.1–0.2 unit of RQ1 RNase-free DNase was added into the reaction mixture and incubated at 37°C for 15 min. The probe was purified by using a G-50 Sephadex Quick Spin Column (Boehringer Mannheim). Purified probe (1 µl) was used to determine the counts per minute.

RNase protection assay. Total RNA (20 µg) was analyzed for each sample by using an RNase protection assay kit (Ambion). Samples and standards (1, 5, 10, 20, and 40 pg) were mixed with 32P-labeled antisense renin probe (10^5 counts·min⁻¹·reaction⁻¹) 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 units RNase A and 200 units 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 using a PDI 325oe scanning densitometer (Protein and DNA Imageware System, Huntington Station, NY) and quantitated using ImageMaster software (Pharmacia Biotech). A standard curve was generated by plotting known amounts of renin sense mRNA standards against obtained integrated optical density (OD × area) in the protected bands.

**Data Analysis**

Two-way ANOVA was used for statistical analysis of the data to provide both group and time evaluation. When significant effects were found, Newman-Keuls testing was used to identify specific differences. A significance level of 0.05 was used to reject the null hypothesis in all cases. Values are reported as means ± SE.

**RESULTS**

**Mean Femoral Arterial Blood Pressure Change Over Time**

Baseline MAP in the occluder and control groups was not different (53.9 ± 1.6 vs. 50.4 ± 1.8 mmHg; Fig. 1). Femoral MAP reduction in the occluder group was 10.01 ± 0.55 mmHg. MAP did not change in the control group.

**Plasma Active Renin Over Time**

Changes in plasma ARC during 24 h of reduced RPP are shown in Fig. 2. Baseline ARC was similar in the occluder and control groups. Although ARC did not change in control fetuses, occluder fetuses exhibited elevated plasma ARC beginning within 1 h of occluder inflation (19.5 ± 4.1 vs. 53.0 ± 18.4 ng ANG I·ml⁻¹·h⁻¹). Peak ARC was observed 3 h after occluder inflation (13.0 ± 4.7 vs. 115.7 ± 23.8 ng ANG I·ml⁻¹·h⁻¹), P < 0.001. Thereafter, plasma ARC gradually fell. Compared with control fetuses, significantly elevated ARC persisted at 24 h (14.5 ± 2.4 vs. 43.2 ± 12.8 ng ANG I·ml⁻¹·h⁻¹).
Plasma Prorenin Over Time

The time course for changes in plasma PRC is shown in Fig. 3. Baseline prorenin levels were not different in the occluder and control groups, averaging 13.0 ± 2.3 ng ANG I·ml⁻¹·h⁻¹. No significant change in plasma PRC was noted in the control group. In contrast, in the occluder group, plasma PRC significantly increased 5 h after occluder inflation and remained elevated throughout the study period. Maximum plasma PRC occurred at 24 h (82.2 ± 2.4 vs. 87.7 ± 21.9 ng ANG I·ml⁻¹·h⁻¹).

RRC Change

Renal prorenin was significantly elevated in the occluder fetuses (23.5 ± 4.6 vs. 59.2 ± 10.9 ng ANG I·mg⁻¹·h⁻¹, P < 0.05) after 24 h of experiment. There was no significant difference between groups for concentrations of active renin.

Renal Renin mRNA Levels

Renal renin mRNA levels were approximately fourfold higher in the occluder group after 24 h of reduced RPP (7.32 ± 1.57 vs. 28.38 ± 4.71 pg/20 µg total RNA) compared with values in the control group (Fig. 4).

Plasma ANG II, Serum Electrolytes, and Animal Data

Baseline levels of ANG II were not different between the groups (30.7 ± 3.2 vs. 29.0 ± 4.7 pg/ml). Plasma ANG II levels increased over sixfold at 4 h (Table 1) and were highly correlated with ARC observed during the occluder manipulation (Fig. 5). In contrast, no significant correlation between plasma prorenin and ANG II level was noted, nor was there a change in the ANG II concentration in the control group during the experiment. In Table 1 are data from the occluder and control groups for gestational age, fetal weight at necropsy, right and left kidney weight of the fetuses at necropsy, fetal arterial blood gases (pH, PO₂, PCO₂), and serum Na⁺ and K⁺ levels. There were no significant group or time effects for these variables.

DISCUSSION

The present study demonstrates that prolonged reduction of renal artery perfusion pressure in the near-term ovine fetus significantly alters the relationship between plasma ARC and PRC, distinctly favoring an
increase in plasma PRC. Immediately after the reduction in RPP, there is a marked increase in plasma ARC. Subsequently, a reversal of prominent plasma form of renin developed, characterized by slightly elevated plasma ARC and markedly elevated PRC. In the chronic phase, renin mRNA levels increase and are associated with similar directional changes in both active and prorenin content. These observations suggest that extended periods of reduced RPP in the near-term fetus result in increased prorenin content, which is accompanied by a change in the pattern of secretion of active renin and prorenin.

Although the clearance of plasma active renin and prorenin was not measured directly, it is theoretically possible that the plasma concentration changes observed in this study are secondary to alterations in clearance. However, we know of no manipulation that results in opposite changes in the plasma clearance of active renin and prorenin. Opposing changes in clearance would be necessary to produce the plasma changes observed in the absence of any change in secretion. More likely, the rate of active renin and prorenin synthesis and the degree of conversion of prorenin to active renin change together with an increasing demand for active renin.

Reduction of RPP initially produced significant elevations of plasma ARC that were not accompanied by changes in plasma PRC. The pattern of ARC change is not unexpected given that acute renal ischemia in fetal and adult animal models has produced significant...

Table 1. Comparison data for occluder and control groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Occluder</th>
<th>Control</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, days</td>
<td>133.8 ± 1.5</td>
<td>130.3 ± 0.8</td>
<td>P = 0.05</td>
</tr>
<tr>
<td>Fetal wt, kg</td>
<td>3.32 ± 0.45</td>
<td>3.26 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney wt, g</td>
<td>Right</td>
<td>11.6 ± 1.4</td>
<td>13.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>12.1 ± 1.0</td>
<td>13.1 ± 1.0</td>
</tr>
<tr>
<td>Baseline pH</td>
<td>7.32 ± 0.27</td>
<td>7.37 ± 0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Ending pH</td>
<td>7.31 ± 0.02</td>
<td>7.34 ± 0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline ( \text{PO}_2 ) Torr</td>
<td>21.0 ± 3.2</td>
<td>19.4 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Ending ( \text{PO}_2 ) Torr</td>
<td>16.9 ± 1.8</td>
<td>19.8 ± 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline ( \text{PCO}_2 ) Torr</td>
<td>50.6 ± 3.2</td>
<td>50.0 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Ending ( \text{PCO}_2 ) Torr</td>
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<td>49.7 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
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<td>30.7 ± 3.2</td>
<td>NS</td>
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<td>ANG II, 4 h, pg/ml</td>
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<td>33.9 ± 6.0</td>
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<td>ANG II, ending, pg/ml</td>
<td>67.2 ± 16.8</td>
<td>31.0 ± 3.4</td>
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<tr>
<td>Serum Na(^+), meq/l</td>
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<td>138.4 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Serum K(^+), meq/l</td>
<td>4.08 ± 0.16</td>
<td>3.76 ± 0.14</td>
<td>NS</td>
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</table>

NS, nonsignificant.

Fig. 4. A: standard curve for renin sense RNA in RNase protection assay. Inset: renin sense RNase protection assay blot with standard ranges from 40 to 2.5 pg. B: RNase protection assay blot of renin mRNA in control (left 2 lanes) and occluder (right 2 lanes) fetuses.

Fig. 5. Relationship between plasma renin concentration and plasma ANG II levels in occluder group (n = 5). Points used represent baseline, 4, and 24 h of occluder inflation for each fetus. Pearson correlation coefficient 0.81, P < 0.001.
almost immediate, elevations of renin activity after suprarenal aortic occlusion (1, 2, 5, 6, 18, 28). In addition, the absence of significant and immediate change in plasma PRC is consistent with data from Toffelmire et al. (28), in which five adult human subjects exhibited changes in plasma ARC, without changes in plasma prorenin levels in response to acute stimulation. We speculate that the initial elevations in plasma ARC, present in the acute phase of reduced RPP, reflect release of stored renin from mature secretory granules within the juxtaglomerular (JG) cells. In agreement with this concept are histological observations indicating that stimulation of renin secretion increases the number of JG cells within the kidney or along the afferent arteriole (3, 16). Such stimulation may also increase secretion from existing granules (3, 13) or increase renin gene transcription in previously functioning JG cells (15). The lack of significant change in circulating concentrations of prorenin during the initial hour is intriguing in light of previous reports suggesting that newly synthesized renin is immediately released as prorenin through a constitutive pathway (7–9, 26). In vitro pulse-labeling experiments incorporating [35S]methionine into protein in incubated rat kidney cortical slices (8, 20) have found that newly synthesized renin is detected in the medium as prorenin within 1 h, whereas >2 h is required before detection of the isotope in mature renin. If this is the case, the lack of plasma prorenin elevation observed initially may be secondary to a required increased transcription of mRNA and translation to new protein in sufficient quantity before significant changes in plasma PRC can be detected. Similar changes (28) showing a delay in plasma concentration have been described by others using prolonged dietary sodium restriction or amiloride treatment.

With more prolonged stimulation the pattern of plasma renin concentration is changed and prorenin becomes the primary form detected. However, this shift to proenzyme prevalence that increased plasma levels 10-fold in our study is still accompanied by a significant elevation in plasma ARC. Increases in plasma PRC have been described in adult animals after chronic stimulation (29); however, there is disagreement as to whether plasma prorenin elevations are paralleled by changes in plasma ARC (28, 29). In support of our findings of elevated plasma ARC in the chronic phase, Pratt et al. (20) have shown that renal ischemia leads to an overall increase in the rate of prorenin processing to mature renin, whereas Cantin et al. (3) propose that ischemic JG cells are more abundant, smaller in size, and have increased secretory activity. In the present study there is a markedly disproportionate increase in plasma PRC compared with ARC, suggesting that posttranslational factors may regulate or limit the proportion of prorenin that is converted to active renin. In adult subjects, chronically stimulated renin secretion increases the proportion of plasma active renin (28). In contrast, in the ovine fetus, we find that the proportion of renin as plasma ARC decreased. One hypothesis that could explain this observation is that the ability of the fetus to cleave prorenin to active renin may be rate limited, perhaps because of immaturity or limited induction of the enzymatic systems necessary for the conversion of tissue prorenin to active renin.

In response to reduced RPP, renin mRNA increases. It is unknown, however, whether the increase in renin mRNA results from an increase in renin gene transcription, an increase in the number of cells transcribing the renin gene, or from factors that serve to prolong renin mRNA lifespan. At the same time, there was a marked increase in renal PRC accompanied by a nonsignificant increase in tissue ARC of 1.2-fold. Under basal conditions a correlation among renin mRNA, PRC, and renin secretion (4, 21) has been described. However, under stimulated conditions (19) a loss of correlation between renin secretory rate has been shown as renal renin activity increases. Our data are consistent with others (3, 12, 14, 24) that recorded elevations in renal renin mRNA with only small elevations of plasma ARC. The nonsignificant increases in active renin content and the significant increase in plasma ARC may in part be explained by limited enzymatic conversion of prorenin to active renin in the response to an imposed stimulus. More importantly, the relatively greater increase in prorenin content could significantly contribute to the increase in plasma PRC observed. Thus one may propose a model in which prolonged reduction of RPP in the fetus stimulates renin gene transcription and subsequent translation of protein, the ultimate effect of which is to increase the plasma levels of prorenin because posttranslational conversion of prorenin to active renin may be limited within the kidney. In support of this proposal, Norling et al. (17) have shown that chronic cyclosporine treatment can disturb the intracellular processing of renin. We are, however, unaware of any data other than the present suggesting that limited processing may be operational in the fetus.

Although reduction in RPP is thought to be the primary stimulus for the RAS changes observed in this study, we cannot exclude that some of the effects may have been secondary to macula densa activation produced by reductions in renal blood flow. Whereas the macula densa may participate in the stimulation of renin gene expression to imposed hypoperfusion (25), chronic studies in near-term ovine fetuses (1) in which distal aortic blood flow was reduced 70%, with only a transient reduction in perfusion pressure, have shown no persistent significant increase in PRA. In addition, from previous experiments (unpublished data) in our laboratory and others (1), using a comparable set of ovine fetuses with indwelling carotid and femoral artery catheters used to monitor both proximal occluder and distal occluder arterial blood pressure, respectively, we have observed that inflation of the occluder was associated with elevation of the carotid MAP over baseline values even though plasma ARC and ANG II levels declined from their peak levels to levels consistently above baseline values. This effect was noted to last throughout the 24-h experimental period. Therefore, in the current study, despite probable activation of the carotid baroreceptors, renal and plasma renin
concentrations increased. Data from Opsahl et al. (18) suggest that PRA is increased primarily through reductions in RPP.

We found no significant change in pH, Pco₂, pO₂, or electrolytes in the experiment. This suggests that these variables were not responsible for the alterations in plasma renin level or form. The increase in plasma ANG II levels was noted to closely follow the changes in plasma ARC levels in the occluder group at the three time points measured. Thus ANG II production is unrelated to PRC in the fetal lamb.

In summary, this study suggests that chronic reduction in RPP in the near-term fetal sheep stimulates renin gene transcription, which results in time-dependent changes in renal and plasma PRC and ARC. The data suggest that posttranscriptional processing of renin may be an important point of regulation in the RAS during development.

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Address for reprint requests: J. S. Rosnes, Dept. of Obstetrics and Gynecology, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

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