Effect of renal denervation on renin gene expression, concentration, and secretion in mature ovine fetus

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Received 26 August 1999; accepted in final form 17 February 2000.

Draper, Michael L., Jinjuan Wang, Nancy Valego, William A. Block, Jr., and James C. Rose. Effect of renal denervation on renin gene expression, concentration, and secretion in mature ovine fetus. Am J Physiol Regulatory Integrative Comp Physiol 279: R263–R270, 2000.—To determine the role of the renal nerves on renin secretion and expression in the mature ovine fetus, we performed bilateral renal denervation on eight fetuses of time-dated pregnant ewes (126.8 ± 0.6 days gestation) and compared renin in them to seven fetuses that underwent sham denervation (126.7 ± 0.6 days gestation). Fetal arterial and venous catheters were implanted, and after 5–7 days of recovery isoproterenol was infused. Plasma active renin was lower in denervated animals than in intact animals under basal conditions and at each dose of isoproterenol. Plasma prorenin levels were lower in denervated fetuses but unaffected by isoproterenol. Denervation did not change renal renin, prorenin, or renin mRNA, but it did block isoproterenol-induced increases in renin mRNA in renocortical cells in vitro. We conclude that the renal nerves are required for renin secretory mechanisms and responsiveness of renin mRNA to β-adrenergic stimulation but not for the expression of renin in the fetal kidney. We propose that one or more of the factors that maintain renin expression in the perinatal period may be absent or may be replaced by the renal nerves in the adult.

Kidney; development; β-adrenergic stimulation

OVER THE LAST DECADE, it has become increasingly clear that the renal nerves and the renin-angiotensin system play important roles in the morphogenesis and growth of kidneys throughout fetal development (10, 11, 36, 37). Whether these are direct effects of innervation itself or are secondary to renin release and/or its downstream products remains unclear.

The primary influences on renin production and secretion are considered to be renal perfusion pressure (mediated through renal baroreceptors), electrolyte changes (modulated through effects on the macula densa), and β-adrenergic stimulation through the renal sympathetic nerves (36). The importance of each of these three mechanisms of renin regulation is dependent on the specific demand placed on the system and may change during development (23).

In adult animals, the renal nerves promote the release of norepinephrine, which stimulates beta adrenoceptors and recruits second messengers such as cAMP which, in turn, stimulate renin gene expression and renin release. However, the effect of the renal nerves on renin in the developing fetus is less well understood. On the basis of studies in adult animals (13, 41), we postulated that renal denervation of the late gestation ovine fetus would cause a decrease in renin secretion, renin mRNA, and renal renin concentration. To further examine the importance of the renal nerves, we designed our study to answer the following specific question: does renal denervation in the late gestation ovine fetus affect the expression and secretion of renin, its immediate precursor, prorenin, and renin mRNA under basal conditions and in response to an acute β-adrenergic stimulus?

MATERIALS AND METHODS

This study was conducted on approval by and in accordance with guidelines established by the Animal Care and Use Committee at Wake Forest University School of Medicine.

Fifteen fetuses of time-dated pregnant ewes were randomly assigned to undergo either bilateral renal denervation (n = 8, 126.8 ± 0.6 days gestation) or sham denervation (n = 7, 126.7 ± 0.6 days gestation). Both singleton and twin pregnancies were used. In the case of twins, one of the fetuses underwent denervation, whereas the other underwent sham surgery without denervation (referred to hereafter as “intact”).

All animals were housed in an environmentally controlled facility with free access to food and water. Pregnant ewes were fasted for 24 h before surgery.

Surgical Preparation

The ewes were sedated with ketamine intramuscularly (20 mg/kg) and then intubated and mechanically ventilated with 1–2% halothane in 100% oxygen. A midline laparotomy was then performed under sterile conditions to expose the gravid uterus. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
uterus, and a hysterotomy was made over the fetal hind-limbs, which were brought through the incision for placement of fetal femoral arterial and venous polyvinyl catheters (ID 1.0 mm, OD 1.7 mm) through groin incisions. These catheters were advanced into the fetal aorta and vena cava and secured in place, and the distal ends were brought out through the uterine incision, which was then closed with a chromic suture. A separate uterine incision was made over the fetal back, and a skin incision was made over the fetal spine. The flanks were dissected down to the kidney on each side where the renal pelvis, renal artery, and vein were visualized. In fetuses undergoing sham surgery, the dissec- tion was stopped at this point, but in those that underwent renal denervation, the nerves were ablated with both mechanical and chemical interruption (phenol irrigation). Gentamicin was instilled in this operative site, and the flank incisions were then closed with a chromic suture and an intrauterine pressure catheter was attached to the fetal back. Gentamicin was placed in the hysterotomy site, which was then closed with a chromic suture. The catheters were exteri- orized through a maternal flank incision where (after com- pletion of the surgical procedure) they were held at the ewe’s side in a sterile pouch within a mesh band around the ewe’s abdomen. Maternal femoral artery and venous catheters were also placed via a groin incision and tunneled subcuta- neously to exit with the fetal catheters.

Postoperative care. The ewes were awakened and allowed to recover for a mean of 6.4 days for denervated animals vs. 6.8 days for intact animals in pens with free access to food and water. During the first 3 postoperative days, the ewes re- ceived prophylactic intravenous ampicillin (1 g) and genta- micin (80 mg) daily. The ewes were inspected each day, and fetal well-being was assessed by daily arterial blood gas analysis. Catheters were flushed daily with saline and filled with 1,000 U/ml heparin.

Twenty-four hours before the in vivo portion of the exper- iment, the ewes were moved to metabolic carts in which they were given 10 ml iv ketamine (100 mg/ml), and the fetuses were then delivered by cesarean section and euthanized with an intravenous overdose of pentobarbital sodium.

In Vivo Stimulation

The experimental design involved infusing three sequen- tial doses of isoproterenol in random order (0.03, 0.06, and 0.12 µg · kg⁻¹ · min⁻¹; on the basis of estimated fetal weight) while simultaneously measuring fetal blood pressure, heart rate, and amniotic fluid pressure. These variables were re- corded on a polygraph interfaced with a personal computer for data storage.

A 5.5-ml baseline blood sample was drawn 5 min before each infusion. One-half of a milliliter was used for arterial blood gas and hematocrit determination, 1 ml was used for electrolyte studies, and the remaining 4 ml (in 0.12 ml so- dium EDTA; 11.2 g EDTA/100 ml) were saved for renin and prorenin determinations. Each infusion of isoproterenol lasted for 10 min; the total infusion volume was 1 ml. This rate avoided the possibility of volume-related effects on renin secretion. Before the infusions, the catheters were flushed with exactly the amount of isoproterenol necessary to fill the dead space in the catheter, the volume of which was mea- sured before the experiment. At the end of the 10-min infu- sion, a second 5.5-ml sample was drawn for the same mea- surements, and this sequence was repeated a second and third time with different doses of isoproterenol with 45-min recovery periods in between. 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. Samples for renin and prorenin were immediately centrifuged at 3,000 g for 10 min, and the plasma was retrieved and stored at −80°C. Samples for electrolytes were collected in a similar manner as renin and prorenin samples, but without EDTA, and they were allowed to clot before centrifugation. Arterial blood gases were collected in heparinized syringes and immedi- ately analyzed on a model BMS Mk2 blood gas analyzer calibrated with a 5% CO₂–20% O₂-balanced N₂ gas mixture at the ambient barometric pressure and at 39°C. Hematocrits were spun and read immediately after collection.

The morning after the in vivo experiment, the ewes were given 10 ml iv ketamine (100 mg/ml), and the fetuses were then delivered by cesarean section and euthanized with an intravenous overdose of pentobarbital sodium.

In Vitro Stimulation of Renin-Containing Cells

Cortical cell dissociation and dispersion. Immediately af- ter the kidneys were removed from the fetuses, they were put into cold dissociation buffer (130 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 10 mM glucose, 20 mM sucrose, 10 mM Tris·HCl, pH 7.4) on ice, and they were decapsulated, bisected longitudi- nally, and the medulla was removed. Portions of cortex were saved in cryoprotective vials and frozen in liquid nitrogen for storage at −80°C for later extraction of renin mRNA and determination of renal renin content and for immunohisto- chemistry. The remainder of the cortex was finely minced and washed in dissociation buffer. The minced, washed cortex was then placed in dissociation buffer containing 0.1% collagenase (Type 2, Worthington, Biochemical, Freehold, NJ), with 100 U DNase I (Sigma, St. Louis, MO), 0.25 mg/ml soybean trypsin inhibitor (Sigma), and 0.1% bovine serum albumin (A7638; Sigma). The tissue fragments were incubated at 37°C for three consecutive 30-min periods. After each period, the spent solution containing dispersed cells was collected and replaced with fresh enzyme solution. The en- zyme activity in the spent solution was inhibited by adding fetal bovine serum to a 20% final concentration. The medium from each consecutive 30-min incubation was filtered through a 20-µm nylon mesh. The dispersed cells were re- covered by centrifugation at 400 g for 10 min and resus- pended in dissociation buffer.

Enrichment of renin-containing cells. The dispersed renal cortical cells were separated on a continuous Percoll gradient as previously described (15, 18, 39). Standard isosmotic Per- coll (12 ml, Pharmacia) and cell suspension (2–4 × 10⁷ cells in 18 ml dissociation buffer) were gently mixed in a 50-ml ultracentrifuge tube to form a 40% Percoll solution (vol/vol). The tube was centrifuged at 27,000 g for 20 min in a fixed angle rotor (SS34). A tube containing standard density beads (reconstituted according to directions from Pharmacia Fine Chemicals AN, Uppsala, Sweden) was also centrifuged and used to determine the density of the cell layers. Three bands of cells were seen after centrifugation. The second band of cells (density 1.067) was collected, washed free of Percoll, and resuspended in RPMI 1640 medium containing 100 U/ml penicillin, 100 µg/ml of streptomycin, and 0.66 U/ml of insul- lin. The cells were stained with trypan blue to confirm vi- ability, counted, diluted in RPMI 1640 medium to a final concentration of 0.5–1.0 × 10⁶ cells/ml, and plated on culture plates at 1 ml of medium/10 cm² surface area.

Incubation and isoproterenol stimulation of renin-contain- ing cells. The plated cells were incubated at 37°C in a hu- midified atmosphere containing 5% CO₂ for 18–20 h. Then the medium was removed and replaced with fresh medium containing 10⁻⁴ M isoproterenol or vehicle. The cells were incubated for an additional 2 h and then harvested for later
extraction of total RNA as described in Determination of Renal Renin mRNA. Prior studies in our laboratory have shown that 2 h was the optimal amount of time to detect renin mRNA responses to β-adrenergic stimulation in isolated fetal renal cortical cells (40).

Measurement of Active Renin and Prorenin

Plasma active renin and prorenin concentrations (ARC and PRC, respectively) were determined by a modification of previously described methods (24). Plasma ARC was measured as a function of the amount of ANG I generated in the presence of excess angiotensinogen (nephrectomized sheep plasma). Plasma total renin concentration (TRC) was obtained by measuring the active renin after treatment with bovine pancreatic trypsin (Sigma #T-9128) in a dose chosen for maximal activation of renin (23). Each lot of trypsin was tested by constructing a dose-response curve with pooled plasma or kidney homogenate. Once the optimal dose of trypsin was established for each, this dose was used for subsequent assays. The trypsin activation was at 4°C and pH 7.3 for 0.5 h. Trypsin inhibitor (Sigma #T-9128) was added at room temperature for 15 min. An aliquot of this mixture was incubated in a shaking water bath at 37°C for 2 h with excess renin substrate (adult nephrectomized sheep plasma) and enzyme inhibitor (phenylmethylsulfonyl fluoride in maleic acid) supplied with a commercially available RIA Kit (Iso-Tex Angiotensin I Radioimmunoassay Kit #BL680, Iso-Tex Diagnostics Laboratories, Friendswood, TX). The ANG I generated was measured with this same kit. Plasma PRC was calculated as the difference between plasma ARC and PRC. Samples from both intact and denervated animals were analyzed simultaneously and in duplicate.

Measurement of Renal Tissue Renin

Approximately 200 mg of renal cortical tissue (previously harvested and stored at −80°C) were minced and submerged in 3 ml of ice-cold saline. The minces were homogenized on ice for ~1 min with the use of a Teflon pestle. The homogenate was centrifuged at 3,000 g for 10 min, and the supernatant was collected and subsequently diluted to 0.25 mg/ml with saline containing 5.2 mM British Anti-Lewisite (2,3-dimercapto-1-propanol), 0.59 mM 8-hydroxyquinoline, and 10 mM disodium EDTA. Tissue TRC and ARC were determined by incubating aliquots of this mixture at 37°C with nephrectomized sheep plasma, with and without previous trypsin activation. The concentration of ANG I generated was then measured, and the tissue ARC was expressed as nanograms of ANG I generated per milligram wet tissue weight per hour of incubation. Tissue PRC was determined by subtracting the tissue ARC from the tissue TRC.

Measurement of Blood Chemistries

Serum electrolytes were measured with the use of an automated flame photometer.

Determination of Renal Renin mRNA

Extraction of total RNA. Samples of kidney cortical tissue (100 mg) or isolated renin-containing cells from both intact and denervated fetuses 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, 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 rRNasin ribonuclease inhibitor, 20 μl of ATP, CTP, GTP, and UTP, 2 μl Clia I-linearized renin template DNA, 2 μl T7 RNA polymerase, and 41 μl nuclelease-free H2O. At the end of the incubation, five units of RQ1 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 quantitation of renin mRNA.

Labeling of antisense RNA probe. The in vitro transcription reaction was performed with linearized template and SP6 RNA polymerase with the use of the procedure described by Melton et al. (19) with minor modifications. Antisense renin probe (specific activity 6–9 × 108 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 rRNasin ribonuclease inhibitor, 4 μl each of 2.5 mM ATP, GTP, and UTP, 2.4 μl of 100 mM CTP, 1 μl EcoR I-linearized renin template DNA, 5 μl (50 μCi) of [α-32P]CTP, and 1 μl SP6 RNA polymerase. The in vitro transcription mixture was then 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 counts per min per 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 five 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 with the use of a scanning densitometer, and the resulting signal was quantitated with the use of 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.

Immunocytochemistry

Tissue preparation. A slice (~1.0 × 0.5 × 0.5 cm) of kidney cortex was fixed in Bouin’s solution for 8 h, dehydrated, and embedded in paraffin. Five-micrometer, serial sagittal sec-
tions were obtained throughout the tissue; every fifth section was collected on a microscope slide. Each slide contained five sections that represented ~100 μm of tissue (the estimated thickness of a typical glomerulus). Each tissue block yielded ~20 microscope slides, and every fourth slide was used for immunocytochemistry.

Immunocytochemistry for renin-containing cells. All slides from a single kidney cortex were processed and immunostained simultaneously. Sections were rehydrated and then incubated (overnight at room temperature) with renin antiserum (1:1,000 in Tris/NaCl buffer containing 1.0% bovine serum albumin). After this step, the slides were washed and incubated for 2 h with secondary antiserum (1:250; goat anti-rabbit IgG-horse radish peroxidase conjugate). Reaction product was visualized with diaminobenzidine/H2O2; sections were counterstained with hematoxylin and eosin and mounted.

Tissue sampling and analysis. A single photomicrograph of a selected region within a paraffin section was obtained with the use of a ×10 objective and sampling the same region for each section. All glomeruli showing both parietal and visceral layers were included in the survey and classified as either immunopositive (i.e., staining for renin cells) or immunonegative. Data were expressed as a percentage of immunopositive glomeruli in the total population, which was typically 200–300 glomeruli.

Determination of Renal Catecholamines

Tissue (100–200 mg) was homogenized with an ultrasonic homogenizer in 300 μl of ice chilled 0.1 M perchloric acid with dihydroxybenzylamine at a 125-pg/50-μl concentration as an internal standard. The homogenates were centrifuged at 10,000 × g for 15 min at 4°C, and then the catecholamines in the supernatant were adsorbed on alumina. After elution with the use of a mobile phase of 0.1 M sodium phosphate, 600 μg/ml 1-octanesulfonic acid, 0.1 mM EDTA, and 8% methanol with a pH of 7.3. Quantitation was accomplished with electrochemical detection (28).

Data Analysis

Data were expressed as means ± SE and were analyzed with two-way ANOVA with significant effects analyzed by Newman-Keuls test assessing specific differences. Dunnett’s t-tests were used to compare appropriate data. Differences were considered significant at the level of P < 0.05.

### Table 1. Comparison data for physiological parameters in intact and denervated fetuses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intact</th>
<th>Denervated</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>167 ± 8.1</td>
<td>180 ± 10.8</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>54.4 ± 3.3</td>
<td>49.9 ± 2.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.32 ± 0.03</td>
<td>7.31 ± 0.05</td>
</tr>
<tr>
<td>PO2, mmHg</td>
<td>46.1 ± 3.4</td>
<td>46.5 ± 6.5</td>
</tr>
<tr>
<td>PCO2, mmHg</td>
<td>17.6 ± 1.1</td>
<td>19.2 ± 7.0</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>36.5 ± 1.7</td>
<td>34.8 ± 4.6</td>
</tr>
<tr>
<td>Na+, mmol/l</td>
<td>142.1 ± 1.1</td>
<td>140.3 ± 1.3</td>
</tr>
<tr>
<td>K+, mmol/l</td>
<td>4.1 ± 0.4</td>
<td>4.0 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. HR, heart rate; MAP, mean arterial pressure.

Fig. 1. Heart rates in fetuses with denervated kidneys (▲, n = 8) and intact kidneys (●, n = 7) at rest and in response to isoproterenol stimulation. *P < 0.05 (in both intact and denervated groups) for each dose of isoproterenol that differs from resting heart rate. Points are means ± SE. When symbols are not shown, data points overlap.

### RESULTS

There were no statistically significant differences in arterial blood pressure, blood gases, pH, plasma electrolyte concentrations, or hematocrit between denervated and intact animals before the experiment (Table 1), and all were within normal limits. These variables did not change significantly in response to isoproterenol. Resting heart rates were equivalent in both intact and denervated animals, and there were similar dose-related increases in heart rate in response to isoproterenol in both groups (Fig. 1). Mean arterial pressure did not change significantly in response to any dose of isoproterenol (Table 2).

The adequacy of renal denervation was confirmed by renal norepinephrine levels, which were reduced by >90% in fetuses with denervated kidneys (1,655 ± 423 vs. 30,244 ± 12,016 pg/g wet tissue wt of cortex).

When the effects of isoproterenol on plasma ARC were analyzed, there were significant group effects, dose effects, and groups by dose interactions. Plasma ARC was significantly lower in denervated animals than in intact animals under basal conditions and at each dose of isoproterenol (Fig. 2). Although there were dose-related increases in plasma ARC in both groups after isoproterenol, the sensitivity to β-adrenergic

### Table 2. Comparison data for MAP in intact and denervated fetuses before and at varying doses of isoproterenol stimulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intact</th>
<th>Denervated</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP before stimulation</td>
<td>54.4 ± 3.3</td>
<td>49.9 ± 2.1</td>
</tr>
<tr>
<td>MAP during 0.03 μg · kg⁻¹ · min⁻¹</td>
<td>55.2 ± 3.8</td>
<td>48.5 ± 2.5</td>
</tr>
<tr>
<td>MAP during 0.06 μg · kg⁻¹ · min⁻¹</td>
<td>51.1 ± 2.5</td>
<td>50.4 ± 2.6</td>
</tr>
<tr>
<td>MAP during 0.12 μg · kg⁻¹ · min⁻¹</td>
<td>51.4 ± 3.6</td>
<td>47.9 ± 3.5</td>
</tr>
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</table>

Values are means ± SE.
stimulation (defined as the lowest dose producing a detectable effect) was greater in the fetuses with intact renal nerves. Thus in the intact animals, even the lowest dose of isoproterenol increased plasma ARC, but in the denervated fetuses only the highest dose had a significant effect on plasma ARC. Isoproterenol caused an approximate doubling of plasma ARC in both denervated and intact animals at the maximum dose used.

Figure 3 shows the corresponding information for plasma prorenin, demonstrating a group effect (plasma prorenin significantly lower in denervated animals) but no dose effect and no dose by group interaction.

Total and active renal renin content as well as renal renin mRNA were unaffected by renal denervation. In denervated animals, active renal renin content was 1,206 ± 179 ng ANG I · g protein⁻¹ · h⁻¹, whereas it was 1,264 ± 126 ng ANG I · g protein⁻¹ · h⁻¹ in intact animals. Total renal renin content was 2,681 ± 448 and 2,799 ± 387 ng ANG I · g protein⁻¹ · h⁻¹, respectively, in denervated and intact animals. For renal renin mRNA, the corresponding figures were 14.00 ± 2.59 vs. 11.23 ± 0.07 pg/20 μg total RNA, respectively.

Immunohistochemistry showed no difference in the proportion of glomeruli staining positive for renin between intact vs. denervated animals (32.7 ± 3.3 vs. 30.2 ± 2.5%).

After dispersion and culture for 24 h, the renin mRNA concentrations were similar in juxtaglomerular cells obtained from intact (5.8 ± 2.2 pg/35 μg total RNA, n = 7) and denervated (3.9 ± 0.8 pg/μg total RNA, n = 5) kidneys. Isoproterenol (10⁻⁵ M) increased the renin mRNA in cells from intact kidneys, but not in cells from denervated kidneys (Fig. 4).

**DISCUSSION**

The results of this study show a marked dichotomy in the effects of renal denervation on renal renin and renin mRNA levels in the fetal kidney compared with the effects on renin secretion. The data indicate that renal denervation does not reduce steady-state levels of renin mRNA nor renin in the kidney. However, denervation markedly lowers the plasma levels of renin and prorenin under basal conditions and attenuates the renin response to an acute β-adrenergic stimulus of physiological intensity. Thus in the fetus, both basal- and β-adrenergic-mediated renin secretions appear dependent on the presence of the renal nerves, whereas expression of normal concentrations of renin mRNA and protein does not.

The lack of an effect of bilateral denervation on the concentrations of renin mRNA and renin in fetal kidneys differs significantly from what has been reported in adult animals. For example, both renal renin content and renin mRNA are reduced by 50% or more in adult mice and rats subjected to kidney denervation (13, 38, 41). In contrast, and consistent with the present observation, the renal nerves do not appear necessary for the expression of basal levels of renin mRNA in the newborn rat (6), and we have
found no differences in renin, renin mRNA, and the percentage of renin-positive glomeruli in fetal lambs with unilateral renal denervation when comparing the denervated kidneys with the intact kidneys (27). These observations all suggest that there is some functional support for the expression of renin (unrelated to the renal nerves), which is absent or replaced by the renal nerves in the adult, present in the perinatal period. What provides this trophic support is not known at this time, but some of the hormonal changes occurring close to term could be important in this respect because the renin gene contains hormone-responsive elements (5).

Alternatively, changes in the feedback efficacy of ANG II on renin expression in the perinatal period could be involved. There is good evidence in adult animals that ANG II can inhibit renin expression (9, 16), and removal of this inhibition allows an increase in renin mRNA levels, even in the absence of intact renal nerves (38). If the feedback effect of ANG II is reduced in the perinatal period, this could allow maintenance of renin expression independent of any neural contribution. There is some evidence for reduced feedback of ANG II on renin in late gestation (7).

Another potential explanation for our results is related to evidence that some populations of cells in the kidney are more responsive to β-adrenergic stimulation than others. For example, Baumbach and Skott (2) have shown that cells located near the glomerulus are less sensitive to isoproterenol than those located further from the glomerulus (2). In the fetus with renal denervation, renin gene expression would be more easily maintained if the cells most responsible for renin production at a given gestational age were those less dependent on the renal nerves for gene expression. This would account for the observed ability of the fetus to maintain renin gene expression in the face of denervation when the adult does not.

Although the fetus and the adult respond differently in regard to the effect of denervation on the concentrations of renin mRNA and renin in the kidney, the effects of denervation on renin secretion in fetal, neonatal, and adult animals seem to vary depending on the stimulus for secretion. Studies in neonatal lambs demonstrate that denervation attenuates the normal physiological increase in plasma renin activity seen at parturition (22, 33), and Nakamura (20) showed impaired renin secretion after renal denervation in the adult hypertensive rat. Other studies in adult sheep show decreased renin secretion in response to reductions in renal perfusion pressure after denervation (3). On the other hand, there is evidence that the renin secretory responses to hypoxia (17) and to furosemide (34) are unaffected by denervation, although the response to the latter remains controversial. Because the renal nerves are only one of the factors that can influence renin release, the responses to stimuli that do not act solely through these nerves may not be suppressed by denervation. This could explain the response to furosemide, which can act at the macula densa to influence renin secretion (4, 14).

It is of interest that denervation in late gestation resulted in low basal levels of plasma renin and decreased sensitivity to isoproterenol despite normal renin concentration. This mimics the pattern of renin secretion seen in early gestation fetuses (24) and may represent withdrawal of some sort of “priming” effect of the renal nerves to sensitize cells destined to secrete renin in late gestation. Exactly how this priming effect would occur is unknown, but Slotkin et al. (29) have suggested that early pioneer synapses provide a trophic signal that enables cells to increase their sensitivity to stimulation during the perinatal transition period (30). Moreover, β-agonist treatment of neonatal rats produces sensitization of the adenyl cyclase response to isoproterenol in cardiac tissue (8), and on the basis of studies using chemical denervation, innervation is important for signal transduction in β-adrenergic-mediated renin secretion (29). Thus denervation may result in functional desensitization of the β-receptor cyclic AMP signaling cascade. If this occurs, then responses mediated by increases in cyclic AMP may be impaired. Such a phenomena would also explain the lack of stimulation by isoproterenol of renin mRNA levels in cells obtained from denervated fetal kidneys. Of course, confirmation of this hypothesis awaits the measurement of cAMP in renin-containing cells after stimulation and the use of cell-permeable cAMP analogs to affect renin gene expression in vitro.

It seems unlikely that the changes in renin secretion observed in our study were secondary to denervation-related changes in renal hemodynamics. We did not observe effects on arterial pressure, and other investigators have also reported that renal denervation is not associated with significant changes in either systemic or renal hemodynamics (25, 26, 31, 32). Moreover, investigators have shown that although renal sympathetic nerve stimulation at a frequency equivalent to basal nerve activity increases renin release, the frequency is below the threshold for an effect on renal hemodynamics (1, 12, 21, 35). Finally, the work of Smith et al. (33) with the fetal lamb confirms that renal denervation does not change the renal hemodynamic response to parturition. Thus renal denervation probably decreases renin secretion by ablation of the basal nerve activity in the absence of changes in renal perfusion pressure.

Prorenin secretion is attenuated by renal denervation (similar to that which is seen with active renin), and our finding of no change in prorenin levels after isoproterenol stimulation is consistent with other studies that evaluate the effects of acute stimuli on prorenin secretion.

Our findings concerning fetal heart rate and mean arterial pressure responses to denervation confirm other findings in the literature (22, 25, 26, 31–33), showing no effect on resting heart rate, mean arterial pressure, and heart rate response to isoproterenol stimulation. The dose-related increments in heart rate in response to isoproterenol in both groups suggests that the dose of drug used was not maximal but pro-
duced responses in a physiological range. The same can be said for the renin response in the intact group.

**Perspectives**

It is important to recognize that the results of this study emphasize that it is not appropriate to view the fetus as a scaled-down version of the adult. The fetus frequently has mechanisms for regulating physiological functions that are very different from the adult. As we learn more about age-related alterations in physiological regulation, we are obliged to consider that how the developing organism reacts to physiological and pharmacological challenges may be controlled in part by maturation. This pertains not only to the fetus in utero but to the neonate and perhaps the adolescent as well. Given these differences, care should be taken when extrapolating the results of experimental manipulations in adult models and even more so when attempting to apply treatments that may be appropriate for adults to less mature subjects.

The authors thank Mike Dunlap and Harvey Boone for technical expertise and Dr. Eberhard Mueller-Heubach for continued support. We also thank Dr. Jeffery Schwartz for mindful evaluation and discussion of this manuscript. This study was supported by National Institutes of Health Grant HD-17644.

This work was presented in part in abstract form at the 45th and 46th annual meetings of the Society for Gynecologic Investigation, Atlanta, Georgia, March 11–14, 1998 and March 10–13, 1999.

**REFERENCES**


