Angiotensin II infusion to the midgestation ovine fetus: effects on the fetal kidney

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THE RENIN-ANGIOTENSIN SYSTEM (RAS) is thought to have an important role in fetal kidney function by maintaining glomerular filtration rate (GFR) and ensuring a high urine flow rate (11). Production of large amounts of fetal urine is thought to be necessary for normal fetal fluid volumes (amniotic and allantoic in the sheep; Ref. 27). Long-term infusions of ANG I or ANG II to the ovine fetus after 110 days of gestation can cause an increase in fetal urine flow rate without increasing plasma concentrations of factors known to be diuretic such as cortisol (14, 21). When ANG I is infused to the fetus for a prolonged period, large volumes of fetal fluids may accumulate (1), which in turn may have detrimental effects on fetal growth and development. Inhibitors of the RAS, such as captopril, an angiotensin-converting enzyme inhibitor, cause a decrease in fetal GFR and urine flow rate when infused to the ovine fetus during the last third of gestation (12). Hypertensive women when treated with captopril during pregnancy often had severely deformed babies due to lack of amniotic fluid resulting from reduced fetal urine flow (16).

Most of these effects, however, have been observed in the late-gestation fetus. The experimental studies in the sheep have been performed between 110 days and term (150 days). In the sheep, as in the human, nephrogenesis is completed before birth (27). In the sheep nephrogenesis is completed by day 130, although there is tubular growth after this period. Thus most of the experiments in the fetal sheep have been done near the completion of kidney development. Very little investigation has been done earlier in gestation during the period of active nephrogenesis. Evidence from gene knockout studies and inhibition of the RAS indicate a role for ANG II in the normal morphological development of the kidney (for reviews see Refs. 1 and 27), and thus ANG II may be very important during early kidney development. It is known that all components of the RAS, including the ANG II receptors, are present from as early as 40 days of gestation in the sheep (3, 25). This means that ANG II can be produced and may be able to act within the kidney from very early in gestation. However, it is not known whether the fetal kidney is responsive to changes in ANG II concentrations at this early age. This study was designed to examine the effects of a 3-day infusion of ANG II on fetal renal function at approximately midgestation (75–85 days). It was hypothesized that the fetus may be able to respond to increased levels of ANG II by increasing urine flow rate and altering composition.

At midgestation there is an abundance of both angiotensin receptor types present in the ovine fetal kidney (3). The AT1 receptor is located within developing glomeruli and within cells of the medulla and medullary rays. The AT2 receptor is expressed within interstitial cells of the renal cortex and also in the macula densa (3). Although the AT1 receptor is well known to mediate most of the known effects of ANG II (13), the AT2 receptor, being present at high levels during development, may have roles in apoptosis and inhibiting cell proliferation (15). In the fetal kidney the AT2 receptor may be important for the normal growth of the kidneys and maintaining fetal renal function.
kidney and urinary tract (29). Little is known about the regulation of these receptors during early stages of development. In late gestation, renal AT<sub>1</sub>-receptor gene expression can be decreased by cortisol infusion to the ovine fetus (20) but not by renal denervation (18). In one study, ANG II at a relatively high dose was able to downregulate both the AT<sub>1</sub> and AT<sub>2</sub> receptors in the late gestation ovine fetus (19). In this study we examined whether infusion of ANG II for 3 days would alter the mRNA levels of the angiotensin receptors within the kidney at midgestation. As receptor gene expression is relatively high in the fetal kidney at this age (3), we hypothesized that there may be a downregulation of these receptors in the kidney after a 3-day infusion of ANG II.

Renin concentrations in fetal plasma have been documented to be significantly higher than in the adult animal, although concentrations of ANG II are usually similar (11). This may be due to lack of a sensitive feedback system in the fetus. In this study we measured the plasma concentrations and gene expression of renin after ANG II or saline infusion. This would allow us to determine whether the kidney can decrease renin production when plasma concentrations of ANG II are high.

**METHODS**

**Animals.** All experiments were approved by the Howard Florey Institute Animal Ethics Committee in accordance with guidelines of the National Health and Medical Research Council of Australia. Fetuses of known mating date were cannulated under general anesthesia between 72 and 77 days of gestation as described previously (7). Because of the small size of the fetuses, particularly the carotid artery, Silastic cannulas were modified to allow a narrow diameter tip to be placed into the artery. A fine Silastic cannula (ID 0.51 mm, OD 0.94 mm) was inserted ~2 cm into a cannula of larger diameter (ID 0.76 mm, OD 1.65 mm) and sealed at the point of joining. The narrow-diameter cannula was cut to a length of 1.5–2 cm, which was placed into the fetal carotid artery. A similar cannula was placed in the jugular vein. The fetal bladder was cannulated (ID 0.76 mm, OD 1.65 mm), and a cannula was placed in the amniotic fluid as the uterus was closed. A single cannula (ID 0.76 mm, OD 1.65 mm) was placed in a maternal jugular vein.

Animals were allowed to recover for 4–5 days before commencement of any experimental protocol. Vascular cannulas were flushed daily with heparinized saline to maintain patency. Ewes had free access to food (oaten and lucerne chaff) and water at all times.

**Basal measurements.** On day 1 of the protocol, urine flow rate, GFR, and blood pressure were measured for a 3-h period. Basal samples (6 ml) were taken for blood gases, hematocrit, and plasma renin concentrations. To measure GFR, basal samples of urine and plasma were taken, and then [51Cr]EDTA was infused intravenously at a constant rate of 10 μCi/h via a Braun perfusor pump (Melsungen, Hassen, Germany). After a 1-h equilibration period, urine and blood samples (2 ml) were taken hourly, the blood sample being taken at the midpoint of each urine collection. Duplicate aliquots (500 μl) of urine and plasma were counted on a gamma counter (Packard Cobra 5010; Packard Instruments, Downers Grove, IL). Fetal blood pressure was measured using a Gould chart recorder and was also collected on a personal computer 486 data-acquisition system using custom software. This collects a 10-s sample every 10 min. Amniotic fluid pressure was subtracted from the carotid artery pressure to give mean arterial blood pressure (MAP).

**Infusions protocols.** Fetuses were infused with isotonic saline (n = 8) or ANG II (1 μg/h, n = 9) for 3 days. On each day urine flow rate was measured for 2 h and a fetal blood sample was taken for blood gas analysis (0.8 ml). On the final day of infusion, fetal GFR was measured for 3 h. At completion of the infusion, fetal blood samples were taken for plasma renin concentrations. Ewes and fetuses were killed with an injection of pentobarbitone (100 mg/kg, Lethabarb; Arnolds, Reading, UK). Samples of amniotic and allantoic fluid were taken for analysis, and volumes of these fluids were measured. Fetuses were weighed, kidneys were dissected, and the wet weight was obtained. Cotyledons were removed from the uterus, and total placental weight was obtained.

A separate group of four fetuses was infused with ANG II at a dose of 5 μg/h. Measurements were taken as described previously. Two of the fetuses in this group died before completion of the protocol, and thus no further animals were included in this group. Thus only partial results from this group are included below.

**Sample analysis.** Fetal arterial blood gases were measured using a Ciba Corning 278 blood gas analyzer (Australian Diagnostics, Melbourne, Australia). Hematocrit was measured in duplicate. Electrolytes in plasma, urine, and amniotic and allantoic fluids were measured using a Beckman synchron CX-5 clinical system (Beckman Instruments, Brea, CA). Coefficients of variation for the measurement of each electrolyte using this system have been reported previously (26) and are generally <5% (except for creatinine (8%) and CO<sub>2</sub> (19%)). Osmolality was measured by freezing-point depression using an Advanced Osmometer (Advanced Instruments, Needham Heights, MA).

Plasma renin concentrations were measured by a previously described assay (6). This measures the generation of ANG I and has a sensitivity of 0.2 pmol·ml⁻¹·h⁻¹ with an interassay variation of 9%.

**Analysis of data.** All values are means ± SE. For analysis of blood pressure, a mean value for each 24-h period after commencement of the ANG II or saline infusion was obtained for each animal. The 4-h period before infusion (including the equilibration hour and 3 h of GFR measurement) were used to calculate basal arterial pressure.

**Statistics.** Values are reported as means ± SE. A repeated measures ANOVA was used to assess changes in blood pressure and renal parameters. A Tukey-Kramer post hoc test was used to ascertain significance on specific days. Fluids taken at postmortem were compared by t-test. Linear regression analysis was performed to assess whether urine flow rate and allantoic fluid volumes were related. A similar analysis was used to examine if there was a relationship between fetal age and basal blood pressure. A nonparametric test (Mann-Whitney rank test) was used to compare results from the real-time PCR analysis.

**Gene expression of the angiotensin receptors and renin.** Tissues were frozen in liquid nitrogen immediately after removal from the fetus and stored at ~80°C until extraction. The method of Chirgwin et al. (5) was used to extract total RNA from the fetal kidney. mRNA expression for the AT<sub>1</sub> and AT<sub>2</sub> receptors was determined using two methods. First, a solution hybridization nuclease assay was used. This assay has been described previously (3). In brief, 10 μg RNA were annealed to each probe (AT<sub>1</sub>, AT<sub>2</sub>, and GAPDH) for 70 min and then digested with S1 nuclease. The digested RNA was
precipitated with isopropanol and analyzed by gel electrophoresis. Quantitation was performed using a Fuji BAS 2000 Biomaging analyzer (Bertold, Australia). Kidneys from eight midgestation fetuses were used (4 ANG II infused and 4 saline infused).

Second, a real-time PCR method was used. This system has the advantage that only a very small amount of starting material (cDNA) is required (50 ng) and can be performed relatively quickly (<3 h). This assay was used to examine gene expression for renin, AT₁, and AT₂ receptors in the kidneys of nearly all fetuses infused with ANG II (n = 7) or saline (n = 8).

For this method, all samples were first reverse transcribed. For each total RNA sample, 1 μg was reverse transcribed in a 10-μl reaction containing 1 × TaqMan reverse transcriptase buffer, 5.5 mM MgCl₂, 500 μM each 2’-deoxyoxynucleoside 5’-triphosphate, 2.5 μM random hexamers, 0.4 U/μl RNase inhibitor, and 1.25 U/μl MultiScribe reverse transcriptase (PE Biosystems). To assess contaminating genomic DNA contamination, controls were set up where no reverse transcriptase was included in reverse transcription reactions with all total RNA samples. The reverse transcription reactions were carried out in a GeneAmp PCR System 9600 (PE Applied Biosystems) at 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min. Each reaction was then diluted 1/10 in 0.01 M EDTA, pH 8.0, and stored at −80°C.

Real-time PCR. For the relative quantitation of renin, AT₁ and AT₂ receptors, and the endogenous reference 18S ribosomal RNA (18S), real-time quantitative PCR was performed (8) using an Applied Biosystems PRISM 7700 Sequence Detector (PE Biosystems). A multiplex comparative C T method was employed in this study, where a C T value reflects the cycle number at which DNA amplification is first detected. In the multiplex reactions, renin, AT₁ receptors, or AT₂ receptors were detected in the one tube with 18S, where primers were limited for 18S. This was possible because of the different reporter dyes attached to each target and reference TaqMan probes, both of which fluoresce at different emission wavelength maxima. In preliminary experiments, we demonstrated no effect on C T values when we compared multiplex to non-multiplex renin and AT₁ and AT₂ receptor reactions as well as primer-limited multiplex to non-primer-limited 18S single tube reactions. For the comparative C T method, a validation experiment was performed where we demonstrated approximately equal efficiencies of renin and AT₁ and AT₂ receptor amplifications together with the amplification of 18S over a range of template concentrations (50 ng-5 pg).

For real-time PCR all primers and TaqMan probes were designed using Primer Express Version 1.0 (PE Biosystems). Primer and TaqMan probe sequences for the renin and AT₁ and AT₂ receptors are presented in Table 1. The TaqMan primer and probes for 18S were supplied by PE Biosystems in a control reagents kit. PCR reactions were carried out in 25-μl volumes consisting of 1 × TaqMan Universal PCR Master Mix (including passive reference), 50 nM TaqMan 18S probe, 20 nM 18S forward primer, 80 nM 18S reverse primer, and the appropriate concentration of primers and TaqMan probe for renin and AT₁ and AT₂ receptors as described in Table 1. cDNA (50 ng) and no reverse transcriptase preparations were amplified using the following conditions: 50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Calculations for real-time PCR. In each assay the C T value of one sample from a saline-infused fetus was determined six times. The mean ± SE of these six measurements was used to determine an intra-assay coefficient of variation. This mean value was used as a “calibrator” to which all other samples were compared. Thus comparative C T calculations for the expression of renin and AT₁ and AT₂ receptors were all relative to an internal control. First, 18S C T values were subtracted from renin and AT₁ and AT₂ receptor values for each well to give a ΔC T value. ΔC T values were achieved by subtracting the calibrator ΔC T value from each ΔC T value. The expression of renin and AT₁ and AT₂ receptors relative to the calibrator was evaluated using the expression 2 −ΔΔC T.

RESULTS

In vivo studies: effects of saline or ANG II. Fetal body weights at postmortem were 325 ± 17 g (n = 8) in the saline-infused control fetuses and 335 ± 28 g (n = 9) in the ANG II-infused fetuses (1 μg/h, P > 0.05). There was also no difference in total kidney weight (4.65 ± 0.33 g saline infused, 4.67 ± 0.37 g ANG II infused) or placental weights (490 ± 40 g saline infused, 506 ± 41 g ANG II infused). There were three sets of twins in the control group and two in the ANG II infusion group. Because of cannula failure in some animals, not all parameters could be measured in every animal. In three animals, no blood could be obtained from the vascular cannula although the animals had urine. These animals were infused intravenously with saline and used for urine flow and excretion rates. At postmortem it was determined that venous cannulas were in place. In some animals the arterial cannula was not patent over the entire 3 days, so arterial blood gas

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Table 1. Primer and probe sequences used in real-time PCR

<table>
<thead>
<tr>
<th>Forward primer</th>
<th>Sequence (5’-3’)</th>
<th>Final Concentration, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin</td>
<td>TGGCCAGGACCTGTGACT</td>
<td>900</td>
</tr>
<tr>
<td>AT₁, R</td>
<td>GGGCTGTCACAGCTGGAA</td>
<td>900</td>
</tr>
<tr>
<td>AT₂, R</td>
<td>TGGTCTGGGTCATGATTTG</td>
<td>300</td>
</tr>
<tr>
<td>Reverse primer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renin</td>
<td>TCCGTGACCTGCCAAAG</td>
<td>50</td>
</tr>
<tr>
<td>AT₁, R</td>
<td>CGGAAGGACTCTACATAGGT</td>
<td>900</td>
</tr>
<tr>
<td>AT₂, R</td>
<td>CCATCCAAGGTAGACATCCA</td>
<td>300</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TaqMan probe</th>
<th>Sequence (5’-3’)</th>
<th>Final Concentration, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin</td>
<td>TCTGTGTGACTGTGATCC</td>
<td>≥225</td>
</tr>
<tr>
<td>AT₁, R</td>
<td>ACCGTTGCCCCCTGGCA</td>
<td>150</td>
</tr>
<tr>
<td>AT₂, R</td>
<td>TGGCTTCCTCCATGGACCT</td>
<td>75</td>
</tr>
</tbody>
</table>

Nucleotide position is shown in parentheses. AT₁, R and AT₂, R, angiotensin receptors. For each TaqMan probe, Fam (6-carboxyfluorescein) was attached at the 5’ end and TAMRA (6-carboxytetramethylrhodamine) was attached at the 3’ end. Accession numbers (GenBank/EMBL) are as follows; renin-L43524, AT₁R-AF056308, and AT₂R-S81979.

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values were not obtained each day. Thus for each parameter, actual numbers used for each analysis are shown in parentheses.

**Blood gases.** Fetal blood gases were similar between the groups and did not vary over the course of the experimental protocol. Before infusion the pH, P\textsubscript{CO\textsubscript{2}}, and P\textsubscript{O\textsubscript{2}} were, respectively, 7.45 ± 0.02, 37.7 ± 2.6 mmHg, and 29.1 ± 1.2 mmHg in control fetuses (n = 5) and 7.49 ± 0.02, 38.4 ± 1.4 mmHg, and 28.5 ± 1.8 mmHg in fetuses that were to receive ANG II (n = 8). After 3 days of infusion values were 7.44 ± 0.01, 40.8 ± 1.9 mmHg, and 25.2 ± 2.0 mmHg in control (saline-infused) fetuses (n = 5) and 7.45 ± 0.03, 42.1 ± 2.6 mmHg, and 24.3 ± 1.4 mmHg, respectively, in fetuses that had been infused with ANG II (n = 6).

**Blood pressure.** Fetal blood pressure was significantly elevated by ANG II infusion as shown in Fig. 1 (n = 5 saline, n = 6 ANG II, P < 0.01). In fetuses infused with ANG II, MAP increased from a basal of 36 ± 2 mmHg to be 41 ± 3, 44 ± 3, and 43 ± 3 mmHg at 24, 48, and 72 h, respectively. The MAP in fetuses receiving saline was 32 ± 1, 31 ± 1, 32 ± 1, and 32 ± 1 mmHg at 0, 24, 48, and 72 h, respectively. There was no change in fetal heart rate over this period (data not shown). Although there was no increase in blood pressure in animals receiving saline over 3 days, there was a significant correlation between fetal age and blood pressure (Fig. 2). When the basal blood pressure from all fetuses was plotted against fetal age, there was a significant increase in blood pressure with increasing gestational age between 74 and 84 days of gestation (P < 0.01).

** Urine flow rates and GFR.** Details of the urine flow in the two experimental groups can be seen in Fig. 3 (n = 8 saline, n = 9 ANG II). ANOVA showed there was a significant difference in fetal urine flow rate between the two groups over time (P < 0.05). A post hoc test indicated urine flow rate was significantly elevated on day 2 of infusion in fetuses receiving ANG II. The flow rate on this day was 18 ± 3 ml/h in ANG II-infused fetuses and 10 ± 2 ml/h in those fetuses receiving an infusion of saline. GFR (when measured before and on day 3 of infusion) was significantly different in fetuses receiving ANG II (22 ± 5 to 35 ± 4 ml/h, n = 5) compared with those receiving saline (15 ± 4 to 16 ± 4 ml/h, n = 4, P < 0.05). When fetal weight (measured at postmortem) was taken into account, the GFR on day 3 was 45 ml·kg\textsuperscript{-1}·h\textsuperscript{-1} in saline-infused fetuses and 93 ml·kg\textsuperscript{-1}·h\textsuperscript{-1} in ANG II-infused fetuses.

Urinary concentrations of sodium, chloride, and potassium were not different between the groups at any stage (P > 0.05 for all 3 electrolytes). However, urinary excretion rates of sodium, chloride, and potassium were all elevated on day 2 of the ANG II infusion (P < 0.05 for all 3 electrolytes) due to the increase in urine flow. Fractional sodium excretion rates were not different when calculated prior to and after 3 days of infusion of saline or ANG II (P > 0.05).

Fetal urine osmolality was similar between the groups and did not alter over the course of the 3-day protocol. Values were 180 ± 12, 158 ± 11, 155 ± 11, and 153 ± 13 mosmol/kg water in control fetuses over the 3 days and 166 ± 9, 162 ± 11, 174 ± 10, and 179 ± 7 mosmol/kg water in ANG II-infused fetuses.

**Plasma renin levels.** Plasma concentrations of renin can be seen in Fig. 4. Plasma renin concentrations were
significantly decreased by the infusion of ANG II when measured on day 3 of infusion (P < 0.05).

Fetal fluids. The volumes and composition of the amniotic and allantoic fluids are shown in Table 2. There was no difference between the groups in the volume or composition of either fluid from samples taken at postmortem. Volume of allantoic fluid at postmortem for each individual animal was closely correlated with the average fetal urine flow rate for that animal over days 2 and 3 of infusion (Fig. 5, P < 0.001).

Effects of ANG II. Of the four fetuses that received 5 μg/h ANG II, one fetus was dead on the second day of infusion while one was dead on the third day of infusion. At postmortem, the fetus that died on the third day was found to have a partially fluid-filled sac replacing the brain. One of the two remaining fetuses that survived the duration of the protocol was also found to have this condition at postmortem. Thus only one fetus successfully completed the entire protocol without gross abnormalities. The basal blood pressure was 35 ± 2 mmHg in this group (n = 4) and was 38 ± 1 and

Table 2. Fluid volumes and compositions after a 3-day infusion of saline or ANG II

<table>
<thead>
<tr>
<th></th>
<th>Amniotic Fluid</th>
<th>Allantoic Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>ANG II</td>
</tr>
<tr>
<td>Volume</td>
<td>66 ± 46</td>
<td>49 ± 34</td>
</tr>
<tr>
<td>Sodium</td>
<td>133 ± 3</td>
<td>134 ± 2</td>
</tr>
<tr>
<td>Potassium</td>
<td>6.1 ± 0.4</td>
<td>6.4 ± 0.3</td>
</tr>
<tr>
<td>Chloride</td>
<td>118 ± 1</td>
<td>116 ± 2</td>
</tr>
<tr>
<td>CO₂</td>
<td>20 ± 1</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Osmolality</td>
<td>288 ± 1</td>
<td>287 ± 3</td>
</tr>
<tr>
<td>Urea</td>
<td>6.1 ± 0.2</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td>Creatinine</td>
<td>45 ± 5</td>
<td>52 ± 4</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.6 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Phosphaté</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Protein</td>
<td>3.7 ± 0.9</td>
<td>4.7 ± 0.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.2 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Fructose</td>
<td>9.7 ± 0.5</td>
<td>9.1 ± 0.8</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.6 ± 0.2</td>
<td>2.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; no. = 9 saline and 8 ANG II infusions. All parameters in mmol/l except volume (ml), osmolality (mosmol/kg water), creatinine (μmol/l), and protein (g/l).

37 ± 5 on days 1 and 2 of infusion, respectively (n = 3). In the two fetuses surviving until day 3, the blood pressure was 40 and 48 mmHg. At postmortem, the allantoic fluid volumes were 300, 500, 740, and 1,450 ml in the fetuses that died on days 2 and 3 or were killed after 3 days, respectively.

Expression of the AT₁ and AT₂ receptors. The results of the RNase protection assay at midgestation can be seen in Fig. 6. The infusion of ANG II did not cause any change in the expression of mRNA for the AT₁ or AT₂ receptor in fetuses at midgestation when measured by this method. For the real time PCR method, the intra-assay coefficient of variation was determined to be 7% for the AT₁ receptor and 5% for the AT₂ receptor. Interestingly, when this method was used, it was determined that there was a significant decrease in expression levels of the AT₁ receptors (P < 0.01) in those fetuses that had been infused with ANG II (Table 3).

Renin gene expression. The coefficient of variation for renin gene expression in the real-time PCR assay was 14%. Renin gene expression was significantly decreased in the kidneys of fetuses that had been infused with ANG II (P < 0.05, Table 3). In one fetus that had been infused with saline, there was significantly less renin gene expression (>2 SD) than in all other saline-infused fetuses. The plasma renin in this fetus was similarly lower than all other fetuses in the saline group. For statistical analysis of plasma renin and

Fig. 4. Plasma renin levels in fetuses receiving saline (n = 5, solid bars) or ANG II (n = 6, open bars) for 3 days. **P < 0.01.

Fig. 5. Relationship between fetal urine flow rate (average of days 2 and 3) and allantoic fluid volume in fetuses infused with saline (●) and ANG II (○).
AT1 receptor is not present in the vascular smooth system (17), but it is not known whether this can occur arterial pressure by effects on the sympathetic nervous system. The human, umbilical artery resistance is considerably low. It is known that in the midgestation kidney, ANG II can lead to changes in gene expression of renin and the AT1 receptor in the midgestation kidney. The pressor effects of ANG II are probably mediated through the AT1 receptor. ANG II can increase mean arterial pressure by effects on the sympathetic nervous system (17), but it is not known whether this can occur at midgestation. It has been reported recently that the AT1 receptor is not present in the vascular smooth muscle of fetal blood vessels until very close to term (after about 140 days of gestation). The only site in the fetal circulation with large amounts of the AT1 receptor appears to be the umbilical cord (10). This would mean that the umbilical cord must constrict quite considerably to produce the observed changes in fetal arterial pressure. Although the umbilical circulation is considered a low-resistance vascular bed, it is known that in the human, umbilical artery resistance is considerably higher at midgestation and falls toward term probably due to growth of the placental vasculature tree with increasing gestational age (23). Thus at midgestation, there may only be a limited capacity for further increases in resistance. The inability of a higher dose of ANG II to further increase blood pressure may indicate that the AT1 receptors in the umbilical cord are fully saturated by the lower dose used in this study and therefore the pressor response is maximal. Administration of ANG II to later gestation fetuses increases umbilical vascular resistance but this does not result in changes in umbilical flow due to the rise in fetal blood pressure (9). A similar situation may have occurred in this study, as there was no change in fetal arterial Po2, which might be expected if umbilical blood flow had decreased.

The fetal urine flow rate was highly variable among animals at this age with basal urine flows ranging from 3 to 9 ml. There was a significant increase in GFR observed on day 3 of infusion in fetuses receiving ANG II that may have been even larger if measured on day 2 when urine flow was maximal. This change in GFR occurred without any alteration in fetal urine osmolality. The mechanism by which ANG II increases urine flow and GFR is unknown but may be a direct action of ANG II on the fetal kidney. Urinary concentrations of sodium, potassium, and chloride were unaltered by the ANG II infusion, but the increase in urine flow rate in these animals resulted in a transient increase in excretion rates of these ions. This increase was modest, however, as there was no difference between the groups in terms of volume or composition of amniotic or allantoic fluid when measured at postmortem. Fetal urine at this age enters the allantoic fluid via the urachus (22). Thus it is most likely that allantoic fluid would be affected if fetal urine has been altered. The observation that the composition of allantoic fluid was not different between the groups indicates that the increased excretion rate of some ions by the fetuses infused with ANG II was not sufficient to have an effect on the fluid. This may be due in part to large variations between animals or may reflect the ability of the placenta to remove some of the ions and fluid that enters the allantoic compartment. It was observed that allantoic volume was closely correlated to fetal urine flow rate, and thus the fetuses receiving ANG II tended to have larger volumes of allantoic fluid. It may be that high levels of ANG II for an extended period (>3 days) could alter fluid volumes and composition.

The ability of ANG II to decrease plasma renin concentrations suggests that a negative feedback system exists in the fetus at midgestation, such that high levels of ANG II inhibit renin secretion by the kidney. Concentrations of renin in the fetus are significantly higher than in the adult, although levels of circulating ANG II are similar (11). It has been speculated that a possible reason for this may be lack of negative feedback of ANG II on renin secretion or that the system is less sensitive in the fetus. Infusions of ANG II to fetuses around 120 days suppress plasma renin activ-
ity (11, 12), suggesting that negative feedback operates at least late in gestation. Other negative regulators of renin, such as cortisol which can decrease renin mRNA in late gestation, do not have this effect on fetuses earlier in gestation (20). No studies have examined renin regulation at in vivo at midgestation, and in this study we demonstrate that ANG II is a potent negative regulator of renin gene expression even at this early stage of development.

The mechanism by which ANG II is able to suppress renin is an area of intensive investigation. There is increasing evidence that cyclooxygenase-2 (COX-2) may be involved. This form of COX is expressed in the macula densa, and levels increase when rats are treated with the angiotensin-converting enzyme inhibitor captopril, which causes an increase in renin production (4). Most interesting though was the observation that the increase in renin production with captopril treatment could be significantly inhibited by administration of a specific COX-2 inhibitor (4). This suggests that COX-2 may be a mediator of increased renin production by the kidney at least in the adult. It is of interest that there are more cells expressing COX-2 in immature and young adult rats than in mature adult rats (30). Little is known about COX-2 expression in the ovine fetus, and thus further investigation is needed to elucidate whether COX-2 is important in regulation of renin expression in the fetus.

Regulation of renal angiotensin receptors has been studied in the late gestation ovine fetus (around 130 days) where it has been shown that the AT₁ receptor is downregulated by ANG II infusion (at a dose of 10 μg/h, Ref. 19) and cortisol infusions (20). In this study we show that infusions of ANG II are able to downregulate mRNA levels for the AT₁ receptor at midgestation when measured by a very sensitive PCR method. This was not observed using a RNase protection assay. This may reflect the fact that the sample size was considerably larger in the PCR method. This was not observed using a RNase protection assay. This may reflect the fact that the sample size was considerably larger in the PCR method or that the “housekeeping” gene was different in the two methods. However, the varying results most likely reflect the increased sensitivity of the PCR technique compared with other methods. We did not see altered expression of the AT₂ receptor in the fetal kidney with ANG II infusion by either technique. Other investigators have observed a downregulation of the AT₂ receptor with ANG II infusion in the late gestation fetus (19), but by that stage, gene expression of the AT₂ receptor is already very low. In the adult rat, the renal AT₂ receptor was not altered by ANG II infusion but was significantly downregulated by ischemia (24), indicating that two receptors may be regulated by independent mechanisms in response to different stimuli.

No protein measurements were made in this study so we do not know whether the changes seen in mRNA levels were also reflected by changes in protein levels. However, we have shown previously that in the fetal kidney before 100 days of gestation (3) changes in mRNA expression for the AT₁ receptor are closely related to changes in protein levels, and we would hypothesize that protein levels also may have declined with ANG II infusion in this study.

Perspectives

The RAS has been shown to be important for the normal morphological development of the fetal kidney (1), and this study demonstrates that from at least midgestation ANG II can regulate many functional aspects of the developing kidney. Elevated concentrations of ANG II may have long-term consequences for the fetus, including alterations in fetal urine production and renin secretion. If maintained, this may eventually lead to fluid abnormalities and altered kidney development.

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REFERENCES


