Chronic effect of insulin-like growth factor I on renin synthesis, secretion, and renal function in fetal sheep

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Marsh, Amanda C., Karen J. Gibson, June Wu, Phillip C. Owens, Julie A. Owens, and Eugenie R. Lumbers. Chronic effect of insulin-like growth factor I on renin synthesis, secretion, and renal function in fetal sheep. Am J Physiol Regulatory Integrative Comp Physiol 281: R318–R326, 2001.—In the adult, insulin-like growth factor I (IGF-I) increases glomerular filtration rate (GFR) and renal blood flow (RBF) during both acute and chronic treatment. To study its effects on the developing kidney, chronically catheterized fetal sheep (120 ± 1 days gestation) were infused intravenously for up to 10 days with 80 μg/h IGF-I (n = 5) or vehicle (0.1% BSA in saline, n = 6). In contrast to previous acute studies in adult rats and humans, after 4 h of IGF-I fetal GFR and RBF were unchanged. Fractional sodium reabsorption increased (P < 0.05). However, by 4 days, GFR per kilogram had risen by 35 ± 13% (P < 0.05), whereas RBF remained unchanged. Tubular growth and maturation may have occurred, as proximal tubular sodium reabsorption increased by ~35% (P < 0.005). Therefore, despite a marked increase in filtered sodium (~30%, P < 0.05), fractional sodium reabsorption did not change. Although the effects of IGF-I on renal function were delayed, plasma renin activity and concentration were both elevated after 4 h (as soon as 20 min after beginning administration) (12, 20) and in a sustained manner over several days of treatment (14, 33). These observations have led others to postulate that during adult life, endogenous IGF-I may contribute to the physiological regulation and maintenance of glomerular filtration (8).

Despite the well-characterized response of the adult kidney to IGF-I, there have been no reports of the effects of prolonged IGF-I treatment on fetal renal function. During fetal life, IGF-I is produced by a wide variety of tissues, including the fetal kidney (5, 18), and the kidney expresses the type 1 IGF receptor (13). In fetal sheep, circulating levels of IGF-I increase over the latter half of gestation, from ~25 to almost 100% of adult levels by term (4). In the developing kidney, IGF-I is a potent stimulus for growth, both in late-gestation fetal sheep (25) and in the neonatal rat (41). This means that IGF-I may affect renal function in the fetus not only via direct actions, as occurs in the adult, but also by stimulating renal growth. Our study was carried out to determine whether the function and/or growth of the fetal kidney were altered during prolonged infusions of IGF-I into late-gestation fetal sheep. We wanted to compare any acute effects with those that occurred after 4 days of IGF-I infusion, by which time any growth-related changes in renal function were likely to be apparent.

The second aim of this study was to determine whether the fetal renin-angiotensin system was affected by prolonged IGF-I infusion. The renin-angiotensin system makes an important contribution to the functional development of the kidney. During fetal life, ANG II maintains fetal GFR through its effects on efferent arteriolar tone, so that in fetal sheep, GFR falls during pharmacological blockade of the fetal renin-angiotensin system and is restored by an infusion of ANG II (28). Not only does the renin-angiotensin system support fetal renal function, but normal renal development depends on the integrity of this system. Disruption of the renin-angiotensin system by a variety of methods during development results in severe, persistent abnormalities in renal morphology and function (15, 19, 34, 43). Recent evidence that the renin-angiotensin system and IGF-I might interact to influence renal development is that in neonatal rats...
METHODS

Experiments were approved by the Animal Care & Ethics Committee of the University of New South Wales. They were carried out in 11 chronically catheterized pregnant ewes and their fetuses.

Surgical preparation and animal care. At 109–117 days gestation (term 150 days), ewes were anesthetized with an intravenous injection of 1–2 g thiopentone sodium (Pentothal; Abbott, Kurnell, New South Wales, Australia) and following tracheal intubation were maintained by 2–3% halothane (Fluothane; Clifford Hallam Pharmaceuticals, Riverwood, New South Wales, Australia) in oxygen. As described previously (31), catheters were placed in the fetus into a femoral artery, both tarsal veins, and the bladder. An amniotic catheter was sutured to the fetal skin over the flank for measurement of intra-amniotic pressure and injection of antibiotics. The left renal artery was located through a para-vertebral incision, and the cuff of a 20-MHz directional pulsed Doppler flow probe (1.6- or 1.8-mm internal diameter cuff; Iowa Doppler Products, Iowa City, IA) was tied around it for measurement of fetal RBF. A maternal femoral artery and vein were also catheterized. All maternal wounds were infiltrated with 0.5% bupivacaine HCl (Marcan; Astra Pharmaceuticals, North Ryde, New South Wales, Australia). Six-hundred milligrams procaine penicillin and 750 mg dihydrostreptomycin (3 ml Illium Penstrept; Troy Laboratories, Smithfield, New South Wales, Australia) were given intramuscularly to the ewe and into the amniotic cavity. The same dose was injected daily into the amniotic cavity for 2 days following surgery.

Ewes were housed in individual metabolic cages in a room maintained between 18 and 22°C. They had free access to tap water and were fed 1,200 g lucerne chaff, 300 g oats, and 6 g NaCl each day. Maternal and fetal vascular catheters were flushed daily with heparinized 0.15 M saline (100 IU heparin/ml, Heparin Injection BP; David Bull Laboratories, Mulgrave, Victoria, Australia). A recovery period of at least 5 days was allowed before beginning experiments.

Study outline. Five fetuses aged 120 ± 1 days gestation (mean ± SE) were given an intravenous infusion of 80 μg/h recombinant human IGF-I (25). This dose was chosen because when infused for 10 days into fetal sheep of the same gestational age as those used in the present study, it led to an approximate threefold increase in circulating IGF-I levels and stimulated renal growth (25). The IGF-I (GroPep, Adelaide, South Australia, Australia) was dissolved at a concentration of 0.05 mg/ml in 0.15 M saline containing 0.1% BSA (Sigma Chemical, St. Louis, MO). On the first day of the study (day 0) following a 2-h control period, fetuses were given a bolus injection of 80 μg IGF-I, and then IGF-I was infused at a rate of 1.65 ml/h for 4–5 h. The infusion rate was then changed so that fetuses received 0.42 mg/ml IGF-I at a rate of 0.19 ml/h for the next 6–10 days. Six control fetuses aged 120 ± 0.4 days gestation received an infusion of vehicle for 9–10 days at the same rates as the treated group. Infusions were prepared immediately before use and delivered through a filter (0.2-μm pore size, Minisart; Sartorius, Göttingen, Germany).

Experiments were carried out on the first and fourth day of infusion (days 0 and 4, respectively) to compare the acute and chronic effects of IGF-I infusion on fetal renal function and the activity of the circulating renin-angiotensin system. At the end of the study, we also measured renal weights and the levels of renin within the fetal kidney.

At the start of each experimental day, the fetal bladder was opened and drained under gravity for at least 45 min. The ewe was given a loading dose of lithium chloride (150 μmol/kg iv). The fetus was given intravenous loading doses of lithium chloride (250 μmol/kg) and 125I-sodium iohalumate (1.8 μCi/kg; Amersham), followed by a continuous intravenous infusion for the duration of the experiment of 10 μmol·kg⁻¹·h⁻¹ and 0.3 μCi·kg⁻¹·h⁻¹, respectively, in 0.15 M saline at 0.95 ml/min.

On day 0, 12 consecutive 30-min urine collections were made; four control periods followed by eight infusion periods covering the first 4 h of infusion, during which time the initial infusion rate of 1.65 ml/min was used. Urine was collected anaerobically under a layer of mineral oil for acid-base measurements. Blood samples were taken at the midpoint of the second, fourth, eighth, and final collection period. On day 4, four 30-min urine collections were made, with blood sampled at the midpoint of the second and final period. Data were averaged to obtain a single value for each variable for the 2-h control period on day 0 and for the day 4 experiment. Results from days 0 and 4 are reported for the control period, the final 30-min period on day 0 (4 h), and the experiment on day 4 (4 days).

Arterial pressure, heart rate, and RBF. Arterial pressure, heart rate, and intra-amniotic pressure were monitored continuously during each experiment using pressure transducers (EasyVent Deadender Cap; Ohmeda, BOC) and a polygraph (model 79D; Grass Instrument, Quincy, MA). RBF was also monitored continuously using the flow probe placed during surgery and a 545C-4 Directional Pulsed Doppler Flowmeter (Bioengineering, University of Iowa) connected to the polygraph. Output from the polygraph was interfaced to an IBM compatible computer using a Metrabyte DAS16 interface card (Keithley, MA). Intra-amniotic pressure was subtracted from the recorded blood pressure to obtain true fetal arterial pressure. Mean values for fetal arterial pressure, heart rate, and RBF were obtained for the control period and for each of the infusion periods on days 0 and 4. As the probe measured relative blood flow only, mean values for RBF during the final infusion period on day 0 and during the day 4 experiment were expressed as a percentage of control RBF.

Blood samples. Arterial blood samples (5 ml) were withdrawn anaerobically into syringes containing 50 IU heparin and replaced with equivalent volumes of heparinized saline. Arterial Po2, PcO2, and pH were measured at 37°C and corrected to 39.5°C using a Ciba-Corning Blood Gas System (model 288; Medfield). Hematocrit was determined in duplicate using a microhematocrit centrifuge and reader (Hettich, Tuttingen, Germany). Plasma was separated by centrifuging for 10 min at 2,500 rpm, 4°C in tubes containing an additional 50 IU heparin, and stored at −20°C for biochemical analysis.

Ewes were killed at the end of the study by intravenous injection of 3–3.5 g pentobarbitone sodium (Ilium Pentabar; Troy Laboratories). Fetuses were removed, and their body
weights were recorded. Fetal kidneys were decapsulated and weighed. Slices of kidney were frozen in liquid nitrogen and stored at –80°C for measurement of renal renin levels.

Biochemical analysis. Concentrations of IGF-I in plasma were measured by specific RIAs (10) calibrated with ovine IGF-I (9) after removal of IGF-binding proteins by size-exclusion HPLC of plasma at pH 2.5 (36). The intra- and interassay coefficients of variation were 3 and 2.9%, respectively. Plasma glucose and lactate were measured using a Glucose/l-Lactate Analyzer 2300 Stat (J Morris Scientific, Chatswood, New South Wales, Australia). Osmolality was measured using a Fiske One-Ten osmometer (Needham Heights, MA). Concentrations of sodium, chloride, potassium, and phosphate were measured on a Beckman Synchro CX3 Clinical System (Beckman Instruments, Gladesville, New South Wales, Australia). GFR was measured as the renal clearance of 125I-sodium iothalamate in plasma and urine was determined from the activity of 125I using a Packard Auto Gamma counter (model 5650; Downers Grove, IL). Lithium concentrations in plasma and urine were measured using a Perkin-Elmer 272 Atomic Absorption Spectrophotometer (Norwalk, CT). Fractional reabsorption of sodium by the proximal (FRNaP) and distal tubules was calculated from the renal clearance of lithium, where the FRNaP was calculated from the formula

\[ \text{FRNaP} = \left(1 - \text{clearance of lithium} \div \text{GFR}\right) \times 100\% \ldots 1 \]  

Fetal renal function and renin release was calculated from the Henderson-Hasselbach equation (2). Filtration fraction (FF) was determined as the rate of formation of ANG I in nanograms per milliliter per hour when 200 μl of plasma were incubated for 2 h at pH 7.5 and 37°C. Because the ANG I measured is formed by endogenous renin acting on endogenous substrate, PRA gives a measure of the capacity of plasma to generate ANG I. Plasma renin concentration (PRC) was determined as the rate of formation of ANG I in nanograms per milliliter per hour when 100 μl of fetal plasma were incubated with an excess of sheep angiotensinogen [100 μl of nephrectomized sheep plasma (NSP)] at pH 7.5 and 37°C for 2 h. In this assay, the activity of renin is not limited by substrate availability, and so PRC is a measure of the amount of active renin in the circulation. The concentration of angiotensinogen in fetal plasma was measured by determining how much ANG I had been generated in 20 μl of plasma by an excess of human renin (0.5 mU; Calbiochem-Novabiochem, Alexandria, New South Wales, Australia) during a 1-h incubation at pH 7.5 and 37°C. The incubation time and total reaction volume (200 μl) were determined in preliminary experiments. All measurements were made in duplicate and averaged to give a single result for each plasma sample. ANG I levels were measured by RIA using methods previously described (30).

Renal renin was measured following the same principles that were used to measure PRC. Homogenates of renal cortex were incubated with NSP, and the concentration of ANG I formed by the end of the incubation was measured by RIA. Preliminary experiments were carried out to determine how much to dilute the homogenate, so that the generation of ANG I in the presence of 100 μl NSP at 37°C and pH 7.5 occurred in a linear manner depending on the amount of renin present, and so that the maximum rate of ANG I formation was reached by the end of 1 h of incubation. To ensure that the assay measured only the ANG I that was generated by renal renin acting on NSP, a pair of duplicates from each sample was placed on ice, whereas a second pair of duplicates was incubated at 37°C and pH 7.5 for 1 h. Samples were then treated in the same way as plasma samples (30). The protein concentration of each renal homogenate was determined in triplicate following the method of Lowry et al. (26). Renal renin levels were calculated by dividing the ANG I formed during the 1-h incubation by renal protein concentration and are expressed as micrograms ANG I per milligram of protein.

Derived variables and statistical methods. For purposes of calculating doses, fetal body weight at the time of each experiment was estimated from gestational age using a formula derived from the body weights and ages of 84 fetuses in this laboratory.

\[ \text{Weight} = \text{age}^{0.4155} + 530.055 \ldots 2 \]  

where weight is in grams and age is in days. A more accurate estimation of fetal body weight at the time of each experiment was made using the following equation

\[ \text{BWE} = \frac{\text{BWD} + \text{estBWD} \times \text{estBWE}}{3} \]  

where BWE is body weight at the time of the experiment; estBWE is body weight at the experiment, estimated from equation 2. BWD is body weight at postmortem; estBWD is body weight at post mortem, estimated from equation 2.

Although fetal visceral growth is stimulated by exogenous IGF-I treatment, body weight does not increase (25). Equation 2 should therefore estimate the weight of IGF-I-treated fetuses with similar accuracy as it does for control fetuses. To test this, the mean deviation of actual from estimated fetal body weight at postmortem (estBWD – BWD) was compared between the two groups using a nonpaired t-test. The mean deviation of each group was found to be similar (vehicle: 47 ± 206 g; IGF-I: −13 ± 195 g; means ± SE). Also, when estBWD and BWD were compared within each group using Student’s paired t-tests, no difference was found.

Plasma bicarbonate concentrations were calculated from the measured arterial Pco2 and pH using a formula derived from the Henderson-Hasselbach equation (2). Filtration fraction relative to control was calculated from the formula

\[ \text{Filtration Fraction} = \frac{\text{GFR}}{\text{RBF}} \times \cdots \]  

where GFR and RBF, are expressed as a percentage of their respective control values. Filtration fraction therefore had a value of 1 during the control period.

Data are reported as means ± SE. Within each treatment group, means were compared using ANOVA for repeated measures and a statistical software package (SPSS/PC; SPSS, Chicago, IL). When differences were detected by ANOVA, a Student-Newman-Keuls test was used to determine which period means were different. Differences between treatment groups were examined using an independent t-test. Statistical significance was set at P < 0.05.

RESULTS

Fetal plasma IGF-I levels. Infusion of recombinant human IGF-I increased fetal plasma IGF-I levels from preinfusion concentrations of 154 ± 14 to 325 ± 16 ng/ml at 4 h (P < 0.001, n = 5), and they remained high at 4 days (277 ± 33 ng/ml, P < 0.001, n = 5). In the
Table 1. Fetal plasma composition

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>4 h</th>
<th>4 days</th>
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</thead>
<tbody>
<tr>
<td>Osmolality, mosmol/kgH2O</td>
<td>284 ± 1</td>
<td>282 ± 0.4</td>
<td>288 ± 1†§</td>
</tr>
<tr>
<td>Sodium, mmol/l</td>
<td>142 ± 1</td>
<td>143 ± 1</td>
<td>144 ± 1*‡</td>
</tr>
<tr>
<td>Chloride, mmol/l</td>
<td>103 ± 0.4</td>
<td>106 ± 1*</td>
<td>107 ± 1†</td>
</tr>
<tr>
<td>Potassium, mmol/l</td>
<td>3.8 ± 0.3</td>
<td>3.6 ± 0.2</td>
<td>4.2 ± 0.3‡</td>
</tr>
<tr>
<td>Phosphate, mmol/l</td>
<td>2.65 ± 0.15</td>
<td>2.69 ± 0.07</td>
<td>2.63 ± 0.21</td>
</tr>
<tr>
<td>Bicarbonate, mmol/l</td>
<td>28.9 ± 0.6</td>
<td>27.3 ± 0.7†§</td>
<td>29.4 ± 0.4§</td>
</tr>
</tbody>
</table>

Values are means ± SE for the control period and after 4 h and 4 days of insulin-like growth factor I (IGF-I) infusion. Fetuses (n = 5) were infused intravenously with 80 μg/h IGF-I for 6–10 days. *P < 0.05, †P < 0.005 compared with control; ‡P < 0.05, §P < 0.005 compared with 4 h (Student-Newman-Keuls test).

Vehicle group, plasma IGF-I concentrations did not change from control values of 118 ± 36 ng/ml (n = 4).

Maternal responses. No change was apparent in maternal variables of either treatment group.

Fetal arterial blood gases, pH, and plasma composition. Fetal arterial oxygen tension (P O2) fell from control to 4.3 ± 0.19 mmHg and 7.4 ± 0.01, respectively, n = 4). Hematocrit did not change in either group (control values IGF-I: 29 ± 1%, n = 5; vehicle: 32 ± 1%, n = 5).

Plasma osmolality had increased after 4 days of IGF-I infusion, largely due to increases in plasma concentrations of sodium and chloride ions (Table 1). Plasma glucose and lactate concentrations did not change from control values of 1.43 ± 0.01 and 1.24 ± 0.04 mmol/l, respectively. In vehicle-infused fetuses, plasma potassium levels increased from 3.8 ± 0.2 mmol/l during control to 4.3 ± 0.2 mmol/l at 4 days (P < 0.05, n = 5). There were no changes during vehicle infusion in plasma osmolality nor in the plasma levels of sodium, chloride, phosphate, glucose, or lactate (control levels: 284 ± 2 mosmol/kgH2O, 143 ± 1 mmol/l, 107 ± 2 mmol/l, 2.52 ± 0.14 mmol/l, 1.35 ± 0.19 mmol/l, and 1.20 ± 0.07 mmol/l, respectively, n = 5).

Fetal arterial pressure and heart rate. In IGF-I-infused fetuses, systolic, diastolic, and mean arterial pressures and heart rate remained similar to control values (53.6 ± 1.2 mmHg, 32.1 ± 1.0 mmHg, 41.1 ± 1.0 mmHg, and 189 ± 4 beats/min, respectively, n = 5). In vehicle-infused fetuses, these variables also remained similar to control values of 50.9 ± 1.1 mmHg, 30.9 ± 1.7 mmHg, 39.0 ± 1.6 mmHg (n = 4), and 184 ± 4 beats/min (n = 6), respectively.

Fetal renal function. Although there was no change in fetal GFR after 4 h of IGF-I infusion, in absolute terms it had risen 60 ± 16% above control by day 4 (P < 0.005, n = 5) and 35 ± 13% above control after correction for fetal body weight (P < 0.05; Fig. 1A). RBF tended to fall during infusion, although this did not reach significance (Fig. 1B). The increased GFR at 4 days meant that there was a large increase in filtration fraction relative to control on day 4 (Fig. 1C). GFR and RBF did not change significantly with vehicle infusion, although there was a small increase in filtration fraction by day 4 (Fig. 1).

Apart from an increase in the fractional reabsorption of sodium (P < 0.05) and chloride (not significant), electrolyte reabsorption did not change at 4 h. On day 4, however, the increase in GFR, combined with increases in plasma electrolyte concentrations, meant that the filtered loads of sodium, chloride, phosphate, and potassium were all increased (Fig. 2A). There was a corresponding increase in the tubular reabsorption of these solutes (although the increase in potassium reabsorption did not reach significance; Fig. 2B), so that their fractional reabsorption rates on day 4 were similar to control, despite the elevated GFR (Fig. 2C).

Fig. 1. Renal hemodynamics. A: fetal glomerular filtration rate (GFR) per kilogram fetal body weight; B: renal blood flow (RBF) relative to control; and C: filtration fraction (FF) relative to control during the control period and after 4 h and 4 days of an infusion of vehicle (open bars, n = 4–5) or insulin-like growth factor I (IGF-I) (hatched bars, n = 4–5). Values are means ± SE. *P < 0.05 compared with control period; †P < 0.05, ††P < 0.01 compared with 4 h (Student-Newman-Keuls test).
Apart from a very small increase in fractional sodium reabsorption on day 4 (from 96.3 ± 0.7% on day 0 to 97.7 ± 0.5%, P < 0.05, n = 4), these variables did not change in fetuses infused with vehicle.

The increases in tubular sodium reabsorption following 4 days of IGF-I infusion were due to increases in sodium reabsorption by the proximal tubule, and hence the FRNaP did not change (Table 2). Distal sodium reabsorption (absolute and fractional) did not change, although the delivery of sodium to the distal tubule tended to be higher at 4 days (not significant), and a greater percentage of the sodium that reached the distal tubule was reabsorbed (P < 0.01, Table 2).

### Table 2. Renal handling of sodium and water

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>4 h</th>
<th>4 days</th>
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<tbody>
<tr>
<td>RNaP, µmol·min⁻¹·kg⁻¹</td>
<td>109 ± 13</td>
<td>102 ± 17</td>
<td>148 ± 19*</td>
</tr>
<tr>
<td>FRNaP, %</td>
<td>56.6 ± 1.8</td>
<td>60.4 ± 3.3</td>
<td>59.2 ± 6.9</td>
</tr>
<tr>
<td>DDNa, µmol·min⁻¹·kg⁻¹</td>
<td>86 ± 13</td>
<td>63 ± 4</td>
<td>106 ± 23</td>
</tr>
<tr>
<td>RNaD, µmol·min⁻¹·kg⁻¹</td>
<td>80 ± 12</td>
<td>60 ± 4</td>
<td>101 ± 21</td>
</tr>
<tr>
<td>%DDNaR, %</td>
<td>40.9 ± 1.8</td>
<td>37.4 ± 2.8</td>
<td>38.8 ± 6.3</td>
</tr>
<tr>
<td>V, ml·min⁻¹·kg⁻¹</td>
<td>0.23 ± 0.04</td>
<td>0.10 ± 0.02*</td>
<td>0.19 ± 0.02$</td>
</tr>
<tr>
<td>CH3O, ml·min⁻¹·kg⁻¹</td>
<td>0.13 ± 0.03</td>
<td>0.03 ± 0.01*</td>
<td>0.10 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE in 5 fetuses during the control period and after 4 h and 4 days of an intravenous infusion of 80 µg/h IGF-I. RNaP, FRNaP, absolute and fractional reabsorption of sodium by the proximal tubule; RNaD, FRNaD, absolute and fractional reabsorption of sodium by the distal tubule; DDNa, distal delivery of sodium; %DDNaR, percentage of distal delivery of sodium reabsorbed by the distal tubule; V, urine flow rate; CH3O, free water clearance. Absolute rates are expressed per kilogram fetal body weight. * P < 0.05, † P < 0.01, ‡ P < 0.005 compared with control; § P < 0.05, # P < 0.005 compared with 4 h (Student-Newman-Keuls test).

Urine flow rate and free water clearance were both greatly decreased by 4 h, but by 4 days they had returned to control levels (Table 2). Renal osmolar excretion and excretion rates of sodium, chloride, potassium, and phosphate did not change from control values of 29.5 ± 5.0 mosmol·min⁻¹·kg⁻¹ and 5.1 ± 4.1, 2.6 ± 1.9, and 0.28 ± 0.18 µmol·min⁻¹·kg⁻¹, respectively. These variables did not change in vehicle-infused fetuses. Urinary osmolality did not change from control values in either treatment group (IGF-I: 128 ± 5.3 and 33.1 ± 13 mosmol/kg H2O, n = 5; vehicle: 129 ± 8 mosmol/kg H2O, n = 6).

**Renal acid-base handling.** The filtered load of bicarbonate was increased after 4 days of IGF-I infusion (from 39.1 ± 5.1 µmol·min⁻¹·kg⁻¹ during control to 52.0 ± 4.9 µmol·min⁻¹·kg⁻¹, P < 0.05, n = 5). Bicarbonate reabsorption was higher on day 4 compared with 4 h (53.8 ± 5.1 and 33.1 ± 5.3 µmol·min⁻¹·kg⁻¹, respectively, P < 0.05, n = 4). Urinary pH, fractional bicarbonate reabsorption, and excretion rates of bicarbonate, titratable acid, and ammonium ions did not change (control values: 6.6 ± 0.1, 96.5 ± 0.7%, 1.5 ± 0.3 µmol·min⁻¹·kg⁻¹, 0.2 ± 0.1 µmol·min⁻¹·kg⁻¹, and 0.9 ± 0.2 µmol·min⁻¹·kg⁻¹, respectively, n = 4). Renal acid-base handling was measured in four control animals, and there were no changes with vehicle infusion.

**Fetal renin-angiotensin system.** Over the first 4 h of IGF-I infusion, PRA (Fig. 3) rose almost threefold (to 397 ± 119% of control, P < 0.05), and on day 4 it was still elevated (385 ± 67% of control, P < 0.05). There was a very large, sustained increase in PRC in the IGF-I-treated fetuses (Fig. 3), to 438 ± 117% of control at 4 h (P < 0.05) and 543 ± 119% of control at 4 days (P < 0.01). Plasma angiotensinogen levels tended to fall (not significant; Fig. 3). In fetuses infused with vehicle, PRA had increased by day 4 to 9.2 ± 2.1 ng ANG I·ml⁻¹·h⁻¹ (control: 5.1 ± 1.9 ng ANG I·ml⁻¹·h⁻¹; P < 0.05, n = 4). This was an increase to 247 ± 78% of control (not significant), but neither PRC
nor plasma angiotensinogen concentrations changed significantly (control: 7.3 ± 2.1 ng ANG I ml⁻¹ h⁻¹ and 1.8 ± 0.2 μg ANG I/ml, respectively, n = 4). Renal renin levels in IGF-I fetuses on day 10 were ~75% higher than those of vehicle-infused animals (Fig. 4).

Fetal body weight and kidney weight. Fetuses infused with IGF-I had a larger total kidney mass than control animals (27.4 ± 2.6 g, n = 5 compared with 20.9 ± 1.5 g, n = 6; P = 0.05). Kidney weight was also higher when expressed as a percentage of body weight (1.0 ± 0.1 compared with 0.8 ± 0.04%, P = 0.05). Fetal body weights were similar between groups (IGF-I: 2,636 ± 141 g; vehicle: 2,522 ± 227 g).

DISCUSSION

The present study compares the short- and long-term effects of infusions of IGF-I into fetal sheep on fetal renal function and the activity of the fetal renin-angiotensin system. We report that after 4 h of IGF-I infusion, there was a marked increase in renin secretion by the fetal kidney. There was a small increase in fractional sodium reabsorption (P < 0.05), without an increase in GFR. After 4 days, changes in renal function followed a very different pattern. There was a large increase in GFR, which was accompanied by a compensatory increase in tubular reabsorption that prevented excessive solute loss. Also, even after long-term IGF-I infusion, the renin-angiotensin system remained intensely activated. Renin secretion was still high, and, interestingly, at the end of the study, renal renin levels were increased.

In the adult, both short- and long-term administration of IGF-I causes marked increases in both GFR and RBF (12, 14, 20, 33). These effects are very well described, become apparent within as soon as 20 min of beginning IGF-I treatment, and have been attributed to decreased renal vascular resistance and an increase in the LpA (22). IGF-I causes renal vasodilatation; however, there have been conflicting reports as to whether the reduction in resistance was pre- or postglomerular or occurred at both sites (21, 22, 42). Generally, increases in GFR and RBF are not accompanied by changes in filtration fraction (12, 20).

The actions of IGF-I on the fetal kidney in the present study, however, were very different from those reported in the adult. RBF, if anything, fell, and the increase in GFR was delayed for 4 days. The increased filtration fraction on day 4 suggests that preferential vasoconstriction of the efferent arteriole may have occurred, an event that would increase GFR and reduce RBF. This efferent vasoconstriction is unlikely to have been a direct action of IGF-I, because it causes vasodilatation, but it might be expected as a consequence of the intense activation of the renin-angiotensin system that occurred. The fetal renin-angiotensin system is essential for maintenance of fetal GFR through an effect on efferent arteriolar tone; ACE inhibition causes RBF to increase, and GFR falls to such an extent that anuria results. These effects are reversed by infusion of ANG II (28). Increased circulating levels of ANG II in the present study may therefore have contributed to the rise in GFR by day 4. However, it is puzzling that GFR was not increased at 4 h, when the renin-angiotensin system was already intensely activated. Also, a greater reduction in RBF might be expected if efferent arteriolar constriction was alone responsible for such a large increase in GFR.

The rise in GFR may also have been due to an increase in the LpA. In addition to changes in arteriolar resistance, much of the increase in GFR in adult rats receiving IGF-I either as an acute intravenous infusion or for 6–7 days as subcutaneous injections has been attributed to large increases in the LpA (21, 22). If IGF-I acted similarly in the fetus, an increased LpA may have contributed to the rise in GFR at 4 days, and this would be consistent with the absence of a rise in RBF.

Although increases in the LpA and arteriolar resistance may partly underlie the rise in GFR that oc-
curred by day 4, they do not explain why the acute renal responses to IGF-I were so different to those following chronic treatment and why GFR only increased after several days of high circulating IGF-I levels. The stimulation of renal growth by IGF-I strongly suggests that the reason for this delay was that the rise in GFR was at least partly growth related. New nephrons are formed in the sheep fetus at the gestation age we studied and until −130 days gestation (38), and an increased number of glomeruli would result in an increase in total GFR in addition to any effects of IGF-I on glomerular hemodynamics or the LpA. As well, by stimulating the growth of existing glomeruli, IGF-I could increase glomerular surface area and therefore increase LpA over and above any direct actions. We did not measure the effects of IGF-I on glomerular size, number, nor its effects on renal tubular length, so we cannot determine the specific components of the kidney that were affected. However, there is evidence that IGF-I affects glomerular growth. It is a cell-cycle progression factor in adult human mesangial cells in culture (6), and mice transgenic for overexpression of human IGF-I have an increased glomerular volume (7), whereas IGF-I(−/−) mice have small kidneys with a reduced nephron number and glomerular volume (40). IGF-I has also been reported to increase renal mass in the neonatal rat (41) and in fetal sheep at the same gestational age as the present study (25).

The increase in tubular reabsorption on day 4 meant that fractional sodium reabsorption remained constant despite the increase in GFR, so that glomerulotubular balance was maintained. More importantly, from a developmental perspective, fractional reabsorption by the proximal tubule was maintained. This is an adult-like response. It is not unusual in the fetus for fractional sodium reabsorption to remain relatively constant when the filtered sodium load increases. However, the proximal tubule is immature, so that, in general, the compensatory increases in reabsorption occur in both the proximal and distal regions of the nephron (29). This is in contrast to the adult animal, in which glomerulotubular balance resides in the proximal tubule and there is no evidence of a relationship between distal tubular sodium reabsorption and GFR (29).

We have found therefore that IGF-I increases the reabsorptive capacity of the fetal proximal tubule. Again, this could be related to growth of the kidney, and the proximal tubule and loops of Henle are still undergoing a great deal of growth and elongation at this stage in gestation (1). The synthesis of more transport proteins may also have increased tubular reabsorptive capacity. Regardless of the mechanism, maintenance of glomerulotubular balance by the proximal tubule in the face of a 60% increase in GFR, together with the increase in GFR itself, indicates that IGF-I treatment accelerated the functional maturation of the kidney. This raises another possible explanation for the delayed increase in GFR. It may be that the relative immaturity of the fetal kidney at the start of the treatment meant that IGF-I could not increase GFR acutely but that 4 days of high circulating IGF-I levels matured renal function to the extent that actions such as an increased LpA were possible.

There are considerable amounts of endogenous IGF-I in fetal plasma at the stage of gestation in the present study, and levels become increasingly elevated toward term (4). It has been suggested by others that because these plasma levels correlate with fetal body weight (24) and because infusions of IGF-I into the fetus in late gestation are associated with increased renal mass (25), IGF-I plays an important part in regulating both overall body growth and that of the fetal kidney. The functional maturation of the kidney is intimately associated with renal growth (27), and perhaps endogenous IGF-I, in addition to or by virtue of its influence on renal growth, participates in the normal growth-related maturation of renal function that occurs over this time.

The fetal kidney continued to secrete high levels of renin even following 4 days of stimulation by IGF-I. The resultant increase in circulating renin levels meant that the activity of renin in fetal plasma also remained high. Interestingly, by the end of the study, the renal stores of renin showed no sign of depletion, but, on the contrary, renal renin levels were increased. The sustained high levels of renin secretion were therefore supported by increased renin synthesis. Direct stimulation of renin secretion by IGF-I has been demonstrated in vitro in rat renal cortical slices (23), and it is likely that this also occurred in vivo in the present study throughout IGF-I infusion, because previously reported stimuli for renin secretion in the fetus do not appear to be responsible. Fetuses did not become hypotensive (32) nor was there any increase in heart rate or arterial pressure indicative of increased β-adrenergic stimulation (30). PRA was increased in the control animals on day 4, suggesting that blood sampling may have contributed to the activation of the renin-angiotensin system with chronic IGF-I infusion (3); however, the increase was small compared with that during IGF-I and was not accompanied by changes in PRC. The fall in PO2 by day 4 was probably too small to be a stimulus for renin release (39). Also, the delivery of sodium past the macula densa to the distal tubule was not reduced on day 4, and so it is unlikely that the macula densa was involved in the stimulation of renin release.

In summary, long-term infusions of IGF-I into late-gestation fetal sheep stimulated renal growth and function and resulted in an appreciable, sustained activation of the fetal renin-angiotensin system characterized by an increase in the synthesis and release of renin by the fetal kidney. The renal functional responses to this long-term infusion were very different to those observed both during short-term infusion into the fetus and in adult life. They indicate that IGF-I accelerated the maturation of renal function, possibly through its effects on renal growth. The degree of influence that the increased activity of the renin-an-
giotensin system had on the renal growth and these functional changes remains to be determined.

Perspectives

The finding that the fetal renin-angiotensin system underwent a sustained activation of such magnitude during long-term IGF-I treatment has exciting implications. The fetal renin-angiotensin system is highly active during late gestation (3, 45), and there is a large body of evidence that shows that an intact renin-angiotensin system is required for normal renal development. Disruption of the renin-angiotensin system during development by targeted gene disruption (19, 34), ACE inhibition (15), or angiotensin-receptor blockade (43) results in severe abnormalities in renal morphology and function. ANG II may act directly as a renal growth factor, as it causes proliferation of cultured human fetal mesangial cells (37) and hypertrophy of cultured murine proximal tubule cells (44). Also, because ANG II contributes to the maintenance of fetal glomerular filtration, it may facilitate renal development by supporting renal function (28). Because IGF-I not only stimulates renal growth in the fetus but also increases the activity of a system important for normal kidney growth and development, we speculate that the renin-angiotensin system contributes to the renotrophic effects of IGF-I during fetal life. This is supported by the finding that the gross renal structural and functional deficits caused by ACE inhibition in neonatal rats were not only associated with reduced renal IGF-I gene expression but were also prevented by co-administration of IGF-I (35), which suggests that IGF-I and the renin-angiotensin system might act in concert to promote normal renal development. Further experiments are required to see whether, conversely, blockade of the fetal renin-angiotensin system prevents the growth-stimulating effects of IGF-I on the kidney.

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