Effects of infusions of ACTH in the chronically catheterized pregnant ewe and her fetus

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Lumbers, Eugenie R., C. Bernasconi, and J. H. Burrell. Effects of infusions of ACTH in the chronically catheterized pregnant ewe and her fetus. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R445–R452, 1998.—To study the effects of elevated maternal levels of adrenocorticotropic hormone (ACTH) on the fetus, nine chronically catheterized pregnant ewes (132 ± 0.9 days of gestation) were infused intravenously for 3 days with Synacthen (5 μg·kg⁻¹·day⁻¹). Four ewes were given 0.15 M saline intravenously over the same period. ACTH induced hypertension in the ewe. Mean arterial pressure (MAP) increased from 101 ± 4.4 to 114 ± 3.9 mmHg at 48 h (P < 0.05); cardiac output increased from 8.6 ± 0.5 to 10.4 ± 1.0 l/min after 24 h (P < 0.05). Within 2–4 h, maternal cortisol levels increased from 24.6 ± 6.3 to 287 ± 30 nmol (P < 0.05) and remained high. Fetal plasma cortisol levels increased from 20.4 ± 4.5 to 45.6 ± 9.5 nmol (P < 0.05) within 2–4 h and then increased further. Fetal MAP was increased at 24 h. There was no effect on fetal blood gases or pH. Ewes became hyperglycemic and lactacidemic by 24 h (P < 0.05), and the fetuses were also hyperglycemic and lactacidemic (P < 0.05) at this time. There were no changes in fetuses carried by saline-infused ewes. Both ewes and fetuses had raised plasma osmolalities and, since hematocrit fell, retained fluid. Ewes became hypokalemic; the fetuses did not, but there was an increase in fetal K excretion. Thus ACTH-induced hypertension in the ewe had minimal effects on fetal MAP, fetal blood gas status, and pH. The fetus, however, did show many of the other effects of maternal glucocorticoid and mineralocorticoid excess, partly because its cortisol levels were increased but also as a consequence of metabolic and endocrine changes in the ewe.

adrenocorticotropic hormone; cortisol; blood pressure; fetal; glucose; lactate

HYPERTENSION IN PREGNANCY is associated with increased fetal morbidity and mortality (see Ref. 8). We have recently developed animal models of pregnancy-associated hypertension to study the effects of various forms of maternal hypertension on fetal health. Our hypothesis is that, regardless of the mechanisms by which maternal hypertension is induced, a high maternal blood pressure indicates a breakdown in control of cardiovascular function. This may lead to impaired control of uteroplacental blood flow, which places the fetus “at risk,” as occurred in one of our models, one-kidney maternal hypertension (8).

We wanted to develop other models of maternal hypertension in pregnancy. We therefore studied the effects on mother and fetus of hypertension induced in the mother by adrenocorticotropic hormone (ACTH) (12). Only one other study has examined the effects of this form of hypertension in the pregnant animal, and its effects on the fetus have not been investigated (2). In this paper, we describe the effects of maternal infusions of ACTH for 72 h on the mother and fetus compared with the effects of infusing 0.15 M saline to the ewe for the same period of time.

METHODS

These experiments were approved by the Animal Care and Ethics Committee of the University of New South Wales.

Thirteen Merino cross ewes were studied. Of these, nine ewes were infused with ACTH (Synacthen, Ciba-Geigy Australia, Pendle Hill, NSW, Australia), and four were infused with saline (0.15 M sodium chloride, Baxter Healthcare, Toongabbie, NSW, Australia). On the 1st day of the experiment, the average gestational age of fetuses was 132 ± 0.9 days.

Surgical Preparation

Ewes. After 3–5 days in the laboratory, animals were fasted overnight and anesthetized with thiopental sodium (1.0 g iv, Pentathol, Abbott Australasial, Sydney, Australia). Anesthesia was maintained with 1–3% halothane in oxygen (Fluothane, ICI, Macclesfield, Cheshire, UK).

Arterial and venous polyvinyl catheters (2.7 mm OD, 1.5 mm ID) were inserted into a maternal femoral artery and vein and exteriorized via a subcutaneous tunnel onto the ewe’s back. An Edwards Swan-Ganz (Edwards Critical Care Division, Irvine, CA) catheter was advanced into the pulmonary artery to measure cardiac output (CO). Its location was verified by recording pressure at the tip of the catheter. This catheter was sutured to the skin of the animal.

Fetuses. The fetus was partially exteriorized through a hysterotomy, and polyvinyl catheters (1.5 mm OD, 1.0 mm ID) were inserted into a maternal femoral artery and vein and suprapubically into the urinary bladder. A catheter was sutured to the fetal skin for recording intra-amniotic pressure. The fetus was returned to the uterus, and the incisions were closed (10).

After surgery, ewes were given procaine penicillin (600 mg im) and dihydrostreptomycin sulfate (750 mg im, Hydronen, Bomac Laboratories, Asquith, NSW, Australia). These doses of antibiotics were also given into the amniotic cavity at the end of surgery and for the following 2 days. No experiments were carried out for at least 5 days after surgery.

The ewes were housed in metabolic cages. They were fed a diet of lucerne chaff and oats, and water was available ad libitum. Ewes were given between 6 and 12 g sodium chloride daily with their feed. The Na content of lucerne chaff is ~33 mmol/kg (4). Vascular catheters were flushed daily with 0.15 M saline containing heparin (100 U/ml, CSL, Parkville, VIC, Australia).

Experimental Protocol

Experiments were carried out over 4 days. Water intake, food intake, and urine output were recorded over 24-h periods. Samples of maternal urine were kept for analysis.

Day 1. Before the start of an experiment, ewes were given lithium chloride (6.36 mg/kg iv, Fisons, Homebush, NSW,
Australia. Fetuses were given lithium chloride (10.6 mg/kg iv) and sodium \(^{125}\)I othalamate (1.8 µCi/kg estimated body wt, Amersham, Buckinghamshire, UK). Fetuses were then infused with lithium chloride (0.43 mg·kg\(^{-1}\)·h\(^{-1}\)) and sodium \(^{125}\)I othalamate (0.3 µCi·kg\(^{-1}\)·h\(^{-1}\)) for the remainder of the experiment.

During the experiment, maternal and fetal arterial pressures and heart rates (HR) and intra-amniotic pressures were recorded continuously using pressure transducers (Bell & Howell, Pasadena, CA) connected to a Grass polygraph (model 7 or 7D, Grass Instruments, Quincy, MA) and an IBM-compatible PC. Pressures and HR were averaged over 30-min periods. Fetal urine was collected and measured at the end of each 30 min. Maternal CO was measured by thermodilution, using a Swan-Ganz catheter connected to a CO computer (model COM-1, American Edwards Laboratories); 10 ml of ice-cold saline were injected rapidly. Each estimate of CO was the mean of at least three determinations, taken when the ewe was standing quietly.

Two arterial blood samples (4–5 ml each from mother and fetus) were taken in the control period. After 2 h of control measurements, nine ewes were then given an intravenous infusion of 5 µg·kg\(^{-1}\)·day\(^{-1}\) ACTH (ACTH treated) for 72 h. Four ewes were given 0.15 M saline (saline infused, at same rate, 0.66 ml/h) for 72 h. Fetal urine was collected each 30 min for 4 h, and blood samples were taken at 3 and 4 h after the infusion began.

Days 2 and 3. Arterial pressures and HR were measured 24 and 48 h later for 1 h. Two 30-min collections of fetal urine were measured, and samples were stored for later analysis. Fetal and maternal arterial blood samples were also taken.

Day 4. The control 2-h protocol as described for day 1 was used. After completion of the experiment, the ewe was given a lethal dose of pentobarbital sodium (Euthatal, 350 mg/ml).

Biochemical Analysis

Hematocrits were measured using a microcentrifuge (Hettich, Tuttingen, Germany). Blood gases and arterial pH were measured on a Ciba-Corning blood gas system (model 288, Medfield, MA). Maternal and fetal plasma and urinary Na, K, Cl, and creatinine levels were measured using an automated analyzer (Beckman Synchron CX3, Beckman Instruments, Fullerton, CA), and creatinine levels were measured using an automated analyzer (Perkin-Elmer atomic absorption spectrophotometer (model PE272). Fetal and maternal plasma glucose and lactate levels were measured using a YSI Stat glucose and lactate analyzer (model 2300, Yellow Springs Instrument, Yellow Springs, OH).

Plasma renin activity (PRA) was measured as the rate of generation of angiotensin (ANG) I by endogenous renin acting on its endogenous substrate at 37°C. ANG I was measured by radiimmunoassay (11). Fetal and maternal plasma cortisol concentrations were measured using a competitive binding radiimmunoassay (Johnson and Johnson Clinical Diagnostics, Amersham, UK).

Analysis of Data

Data collected during an experiment have been averaged into 30-min intervals and analyzed for the following periods: 1) the 2-h period before infusion of ACTH or saline to the ewe, i.e., control; 2) the first 2 h after the infusion of ACTH or saline, i.e., 0–2 h; and 3) the next 2 h after infusion, i.e., 2–4 h. Data were collected for 1 h at 24 h, at 48 h, and for 2 h at 72 h, respectively.

Maternal total peripheral resistance (TPR, expressed as arbitrary units) was calculated from the mean arterial pressure (MAP) and CO (mmHg·1\(^{-1}\)·min\(^{-1}\)). Transplacental gradients (TP) were calculated as the differences between maternal and fetal concentrations; i.e., TP = maternal concentration – fetal concentration.

HCO\(_3\) levels were calculated using the formula HCO\(_3\) = 0.0294·PCO\(_2\)·10\(^{-4}\)·0.9911 + 0.6578·pH + 0.0262·pH\(^2\). Body weight was estimated using a formula derived from the postmortem weights and ages of 84 fetuses in this laboratory (5).

RESULTS

Ewes

Saline-infused ewes. In the four ewes that received 0.15 M saline for 72 h, there were no changes in any of the following variables: plasma cortisol (control values were 16.5 ± 5.9 nM; Fig. 1), MAP (91.3 ± 3.5 mmHg), CO (7.7 ± 1.2 l/min, n = 3), arterial PO\(_2\) (106 ± 3.5 mmHg), PCO\(_2\) (40.9 ± 0.9 mmHg), pH (7.44 ± 0.01), plasma HCO\(_3\) (27.1 ± 0.75 mM), Cl (111 ± 2.1 mM), creatinine (0.06 ± 0.00 mM), Na (PNa, 148 ± 1.3 mM), K (PK, 4.1 ± 0.18 mM), Na-to-K ratio (PNa/K, 36.2 ± 1.3), osmolality (291 ± 2.2 mosmol/kg), lactate (0.49 ± 0.08 mM), glucose (2.8 ± 0.3 mM), PRA (5.5 ± 1.8 ng·ml\(^{-1}\)·h\(^{-1}\)), hematocrit (28.7 ± 0.5%), water intake (109 ± 34 ml/h), and urinary osmolality (937 ± 84 mosmol/kg).

ACTH-infused ewes. Maternal plasma cortisol levels were significantly increased by 2–4 h of ACTH infusion (P < 0.001, Fig. 1). Control values for maternal plasma cortisol were 24.6 ± 6.3 nM; during ACTH infusion cortisol levels exceeded 280 nM (Fig. 1). Maternal MAP and CO were only measured in eight of these nine ewes. Maternal MAP (which was similar in saline-infused ewes in control) increased within 1–2 h (P < 0.05) and remained high (Fig. 2). CO fell (P < 0.05, Table 1).

Table 1 details only those variables that changed significantly in the ewes. Arterial pH had increased by 24 h of ACTH infusion and stayed high for the rest of the experiment (P < 0.05). By 24 h of infusion of ACTH, hematocrit had decreased when compared with values measured at 2–4 h. At 48 h of infusion, hematocrit was lower than both control levels and levels measured within 2–4 h of beginning the infusion (Table 1). Plasma osmolality had increased by 24 h. At 48 h, P\(_K\) levels were significantly reduced compared with all
other values, and $P_{\text{Na/K}}$ was increased. Plasma $\text{HCO}_3^-$ levels increased and Cl levels decreased (Table 1).

Plasma glucose and lactate levels increased (Figs. 3 and 4).

There were no changes in the following variables; arterial $\text{PO}_2$ (110 ± 6 mmHg, $n = 9$), $\text{PCO}_2$ (40.2 ± 1.0 mmHg), plasma creatinine (0.05 ± 0.003 mM, $n = 9$), $P_{\text{Na}}$ (150 ± 0.8 mM, $n = 9$), PRA (2.27 ± 0.59 ng·ml$^{-1}$·h$^{-1}$, $n = 8$), and urinary osmolality (679 ± 123 mosmol/kg, $n = 7$).

**Fetuses**

Fetuses carried by ewes that received 0.15 M saline for 72 h. In four control fetal sheep, plasma cortisol levels progressed over the experimental period (Fig. 1). In one fetus, this increase occurred within 2–4 h of beginning of the saline infusion. Levels increased from control values of 24.6 nM to 54.4 (2–4 h) and then to 82.4 (24 h), 136 (48 h), and 239 nM (72 h). In the other three fetuses, levels were 16.2 ± 4.4, 17.7 ± 5.6, 16.8 ± 2.1, 19.9 ± 5.6, and 69.7 ± 23.3 nM at the same times. Fetal MAP was different when measured by ANOVA ($P < 0.05$), but no individual mean was different (Fig. 2).

Table 2 describes only those variables that changed significantly. $P_{K}$ changed according to ANOVA ($P = 0.002$), but no individual mean was different. Fetal $P_{\text{Na/K}}$ decreased from 24 h until the end of the study (Table 2).

The following variables did not change: fetal HR (189 ± 16 beats/min), hematocrit (34.2 ± 0.94%), arterial $\text{PO}_2$ (25 ± 1.6 mmHg), $\text{PCO}_2$ (52 ± 1.6 mmHg), pH (7.34 ± 0.02), plasma $\text{HCO}_3^-$ (28.3 ± 0.5 mM), glucose (0.81 ± 0.11 mM), lactate (1.36 ± 0.08 mM), osmolality (283 ± 1.5 mosmol/kg), $P_{\text{Na}}$ (147 ± 0.6 mM), creatinine (0.12 ± 0.02 mM), urine flow (0.75 ± 0.03 ml/min), glomerular filtration rate (GFR; 4.9 ± 0.7 ml/min), urinary osmolality (128 ± 12.9 mosmol/kg), urinary Na (26.6 ± 5.8 µmol/min) and K excretion (4.1 ± 2.1 µmol/min), urinary Na-to-K ratio (12.9 ± 6.6), total amount of Na (686 ± 97.8 µmol/min) and K (15.2 ± 2.6 µmol/min) reabsorbed, total fractional Na (0.96 ± 0.007) and K (0.82 ± 0.08) reabsorption, proximal and distal Na reabsorption [359 ± 64 and 327 ± 34 (µmol/min)], proximal and distal fractional Na reabsorption (0.53 ± 0.02 and 0.47 ± 0.02), and PRA (2.88 ± 0.8 ng·ml$^{-1}$·h$^{-1}$).

Fetuses carried by ewes treated with ACTH. One fetus died between 48 and 72 h. Arterial pressure could not be measured in another fetus at 72 h, although fetal
arterial PO2 was 15 mmHg. Therefore no data were collected from this fetus at 72 h. Within 2–4 h of maternal ACTH infusion, fetal plasma cortisol levels were increased. They remained high for the remainder of the experiment (Fig. 1). Levels in these fetuses were higher at 2–4 and 24 h than levels in fetuses carried by saline-infused ewes (P < 0.006, Fig. 1). The transplacental cortisol gradient increased within 2–4 h and remained elevated for the remainder of the experiment (Table 3). At 24 h, MAP was increased (Fig. 2), HR did not change (167 ± 6 9.9 beats/min, n = 8), arterial PO2 (23.4 ± 0.9 mmHg, n = 9), and pH (7.35 ± 0.005, n = 9) did not change.

Table 1. Effects of infusion of 5 μg·kg⁻¹·day⁻¹ of ACTH for 72 h into pregnant ewes

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>2–4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
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</thead>
<tbody>
<tr>
<td>CO</td>
<td>8.6 ± 0.5 (8)</td>
<td>8.5 ± 0.6 (8)</td>
<td>10.4 ± 1.0*† (6)</td>
<td>9.6 ± 1.0 (5)</td>
<td>8.6 ± 0.4 (5)</td>
</tr>
<tr>
<td>pH</td>
<td>7.45 ± 0.007 (9)</td>
<td>7.45 ± 0.009 (9)</td>
<td>7.49 ± 0.007*† (9)</td>
<td>7.50 ± 0.016*† (5)</td>
<td>7.51 ± 0.009*† (7)</td>
</tr>
<tr>
<td>Hct</td>
<td>28.3 ± 0.5 (9)</td>
<td>29.4 ± 0.9 (9)</td>
<td>26.7 ± 0.7 (9)</td>
<td>25.6 ± 1.3*† (5)</td>
<td>26.6 ± 1.0*† (7)</td>
</tr>
<tr>
<td>P HCO3</td>
<td>27.3 ± 0.8 (9)</td>
<td>27 ± 0.8 (9)</td>
<td>28.7 ± 0.7 (9)</td>
<td>29.9 ± 1.4*† (5)</td>
<td>30.9 ± 0.7**† (7)</td>
</tr>
<tr>
<td>P Na</td>
<td>296 ± 2.6 (9)</td>
<td>294 ± 2.3 (9)</td>
<td>302 ± 2.9*† (9)</td>
<td>300 ± 3*† (6)</td>
<td>298 ± 1.6 (7)</td>
</tr>
<tr>
<td>P K</td>
<td>111 ± 0.7 (9)</td>
<td>112 ± 0.6 (9)</td>
<td>111 ± 0.7 (8)</td>
<td>109 ± 0.6 (6)</td>
<td>106 ± 2.5*† (6)</td>
</tr>
<tr>
<td>P Cl</td>
<td>4.1 ± 0.1 (9)</td>
<td>4.0 ± 0.1 (9)</td>
<td>4.0 ± 0.1 (8)</td>
<td>3.5 ± 0.3*† (8)</td>
<td>3.7 ± 0.3 (6)</td>
</tr>
<tr>
<td>P Na/K</td>
<td>36.7 ± 0.5 (9)</td>
<td>38.1 ± 0.7 (9)</td>
<td>37.9 ± 1.3 (8)</td>
<td>44.4 ± 3.4*† (6)</td>
<td>40.8 ± 2.9 (6)</td>
</tr>
</tbody>
</table>

Values are means ± SE for no. of animals in parentheses. Con, control; CO, cardiac output (l/min); Hct, hematocrit; P HCO3, plasma HCO3 (mM); P Na, plasma Na (mM); P K, plasma K (mM); P Na/K, plasma Na-to-K ratio. *P < 0.05 from control. †P < 0.05 from 2 to 4 h. ‡P < 0.05 from 24 h.

Arterial PCO2 changed according to ANOVA, but no individual mean was different (P < 0.05, Table 2). Hematocrit was decreased at 72 h relative to 24 h (P < 0.05, Table 2). Plasma HCO3 (28.3 ± 0.4 mM, n = 9), P Na (148 ± 0.2 mM, n = 9), P K (4.2 ± 0.15 mM, n = 9), creatinine (0.13 ± 0.01 mM, n = 9), P Na/K (36 ± 1.0, n = 9), and PRA (2.6 ± 1.3 ng·ml⁻¹·h⁻¹, n = 8) did not change. Plasma Cl levels were different using ANOVA (P < 0.05, Table 2), but no individual mean was different.

Fetal plasma glucose and lactate levels were both increased by 24 h (P < 0.001, Figs. 3 and 4) and remained high relative to control and 2–4 h. Plasma

Fig. 3. Effects of infusing ACTH (filled bars) or 0.15 M saline (open bars) into pregnant ewes on maternal (A) and fetal (B) plasma glucose levels. *P < 0.05 compared with control; #P < 0.05 compared with 2–4 h. Bars and brackets, means ± SE; no. of ewes in which measurements were made = 9, 9, 9, 4, 7, respectively; no. of fetuses = 9, 9, 9, 6, 7, respectively.

Fig. 4. Effects of infusing ACTH (filled bars) or 0.15 M saline (open bars) into pregnant ewes on maternal (A) and fetal (B) plasma lactate levels. *P < 0.05 compared with control; #P < 0.05 compared with 2–4 h. Bars and brackets: means ± SE; no. of ewes in which measurements were made = 9, 9, 9, 4, 7, respectively; no. of fetuses = 9, 9, 9, 6, 7, respectively.
osmolality was also elevated at this time relative to control and 2–4 h; it was still high at 48 h (Table 2).

Urinary flow decreased so that at 24 h it was less than the mean of values measured at 2–4 h (Table 2). Urinary osmolality increased over the first 4 h of maternal ACTH infusion and was further increased from 24 h on (Table 2). GFR did not change; control values were 3.76 ± 0.02 ml/min (n = 8), at 2–4 h they were 3.8 ± 0.4 ml/min (n = 8), and at 72 h they were 4.3 ± 0.6 ml/min (P = 0.07, n = 7). Urinary Na excretion (30.5 ± 6 μmol/min, n = 9) did not change; the total amount of Na (533 ± 36.4 μmol/min, n = 8) and Cl (392 ± 26.2 μmol/min, n = 4) reabsorbed did not change nor did total fractional Na reabsorption (0.96 ± 0.005, n = 8). The fractional reabsorption of Na by the proximal tubule tended to fall (P = 0.07; values were 0.53 ± 0.04 (n = 7), 0.47 ± 0.19 (n = 7) at 2–4 h, and 0.41 ± 0.21 at 72 h (n = 6), and the fractional reabsorption of Na by distal tubules tended to increase (P = 0.06, 0.44 ± 0.03, 0.5 ± 0.05, and 0.56 ± 0.08). The amount of Na reabsorbed by the proximal tubule did not change (296 ± 31.3 μmol/min, n = 7), but the amount reabsorbed distally did change so that it was increased after 72 h of maternal ACTH relative to control and to 2–4 h (Table 2).

K excretion increased at 48 h compared with all earlier samples and it increased further by 72 h (Table 2). Urinary Na-to-K ratio changed according to ANOVA (P = 0.05), but no individual mean was different (Table 2). K reabsorption decreased so that by 72 h of maternal

Table 2. Changes in fetal values for fetuses carried by saline-infused ewes and those carried by ACTH-treated ewes

<table>
<thead>
<tr>
<th></th>
<th>1–2 h</th>
<th>2–4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fetuses carried by saline-infused ewes</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PK</td>
<td>3.88 ± 0.13 (4)</td>
<td>3.99 ± 0.17 (4)</td>
<td>4.25 ± 0.11 (3)</td>
<td>4.27 ± 0.13 (3)</td>
<td>4.26 ± 0.08 (4)</td>
</tr>
<tr>
<td>PNaK</td>
<td>38.1 ± 1.4 (4)</td>
<td>37.3 ± 1.5 (4)</td>
<td>34.2 ± 0.9b (3)</td>
<td>34 ± 1.0b (3)</td>
<td>34 ± 0.29b (4)</td>
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<tr>
<td><strong>Fetuses carried by ACTH-treated ewes</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCO2</td>
<td>52.9 ± 1.3 (9)</td>
<td>53.9 ± 1.1 (9)</td>
<td>53.8 ± 1.0 (9)</td>
<td>58.3 ± 1.3 (4)</td>
<td>55.4 ± 2.3 (7)</td>
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<tr>
<td>PCl</td>
<td>109 ± 2.6 (9)</td>
<td>108 ± 1.0 (9)</td>
<td>107 ± 1.6 (6)</td>
<td>105 ± 1.6 (6)</td>
<td>104 ± 1.3 (7)</td>
</tr>
<tr>
<td>HCO3</td>
<td>35 ± 1.4 (9)</td>
<td>34.2 ± 1.3 (9)</td>
<td>36.1 ± 1.9 (9)</td>
<td>35.2 ± 1.6 (4)</td>
<td>33.7 ± 2c (7)</td>
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<td>POMer</td>
<td>284 ± 2.1 (9)</td>
<td>286 ± 2.7 (9)</td>
<td>293 ± 2.0a (9)</td>
<td>293 ± 2.3b (6)</td>
<td>287 ± 3.0 (7)</td>
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<tr>
<td>Vmin</td>
<td>0.74 ± 0.12 (9)</td>
<td>0.62 ± 0.12 (8)</td>
<td>0.79 ± 0.13a (9)</td>
<td>0.49 ± 0.11 (9)</td>
<td>0.66 ± 0.11 (6)</td>
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<tr>
<td>UrO2</td>
<td>6.8 ± 1.3 (9)</td>
<td>6.1 ± 1.3 (8)</td>
<td>6.2 ± 1.4 (9)</td>
<td>6.3 ± 1.5 (9)</td>
<td>12.2 ± 3.8 (6)</td>
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<tr>
<td>UNa</td>
<td>7.2 ± 1.8 (9)</td>
<td>7.0 ± 2.1 (8)</td>
<td>9.4 ± 3.0 (9)</td>
<td>6.3 ± 2.0 (9)</td>
<td>6.3 ± 3.5 (6)</td>
</tr>
<tr>
<td>UCl</td>
<td>142 ± 23 (9)</td>
<td>151 ± 30a (8)</td>
<td>147 ± 27 (9)</td>
<td>227 ± 33a (9)</td>
<td>224 ± 36 (6)</td>
</tr>
<tr>
<td>TRNaK</td>
<td>255 ± 26 (7)</td>
<td>288 ± 25 (7)</td>
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</table>

Values are means ± SE for no. of animals in parentheses. Vmin, urine flow rate (ml/min); UrO2, urinary K excretion (μmol/min); UNaK, urinary Na-to-K ratio; UCl, urinary osmolality (mosmol/kg); TRNaK, distal Na reabsorption (μmol/min). *P < 0.05 from control. †P < 0.05 from 2 to 4 h. ‡P < 0.05 from 24 h. §P < 0.05 from all earlier values. ‡P < 0.05 from control 1–to-2- and 2–to-4 h samples.

Table 3. Transplacental gradients in animals treated with ACTH and those receiving saline infusions

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>2–4 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
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<tbody>
<tr>
<td>TPACE</td>
<td>3.98 ± 8.3 (9)</td>
<td>227 ± 33.6a (9)</td>
<td>221 ± 31.2a (9)</td>
<td>183.1 ± 18.4a (5)</td>
<td>180 ± 14.1a (7)</td>
</tr>
<tr>
<td>Saline</td>
<td>-4.18 ± 5.4</td>
<td>-10.7 ± 10.9</td>
<td>-19.6 ± 17.1</td>
<td>-21.9 ± 32.5</td>
<td>-37.5 ± 36.8</td>
</tr>
<tr>
<td>TPACE</td>
<td>2.4 ± 0.17 (9)</td>
<td>2.9 ± 0.26 (9)</td>
<td>3.4 ± 0.43a (9)</td>
<td>3.4 ± 0.34 (4)</td>
<td>4.2 ± 0.49a (7)</td>
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<tr>
<td>Saline</td>
<td>2.0 ± 0.19</td>
<td>2.0 ± 0.18</td>
<td>1.7 ± 0.07</td>
<td>2.0 ± 0.3</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>TPACE</td>
<td>-0.88 ± 0.08 (9)</td>
<td>-0.99 ± 0.08 (9)</td>
<td>-1.62 ± 0.39a† (9)</td>
<td>-1.69 ± 0.28 (4)</td>
<td>-0.99 ± 0.23 (9)</td>
</tr>
<tr>
<td>Saline</td>
<td>-0.87 ± 0.05</td>
<td>-0.92 ± 0.06</td>
<td>-1.06 ± 0.1</td>
<td>-1.05 ± 0.13</td>
<td>-2.3 ± 1.3</td>
</tr>
<tr>
<td>TPACE</td>
<td>-6.6 ± 1.4 (9)</td>
<td>-4.8 ± 1.3 (9)</td>
<td>-9.3 ± 2.3 (9)</td>
<td>-9.0 ± 3 (4)</td>
<td>-7.1 ± 2.6 (7)</td>
</tr>
<tr>
<td>Saline</td>
<td>-5.7 ± 0.95</td>
<td>-5.9 ± 0.69</td>
<td>-6.9 ± 0.93</td>
<td>-7.5 ± 0.33</td>
<td>-5.6 ± 1.1</td>
</tr>
<tr>
<td>TPACE</td>
<td>-1.1 ± 0.7 (9)</td>
<td>-0.9 ± 0.8 (9)</td>
<td>-1.1 ± 1a† (9)</td>
<td>2 ± 0.5† (8)</td>
<td>2.7 ± 0.9† (7)</td>
</tr>
<tr>
<td>Saline</td>
<td>-1.3 ± 0.59</td>
<td>-1.1 ± 0.7</td>
<td>-1.2 ± 0.5</td>
<td>-1.3 ± 0.84</td>
<td>-1.5 ± 1.1</td>
</tr>
<tr>
<td>TPACE</td>
<td>0.1 ± 0.01 (9)</td>
<td>0.11 ± 0.01 (9)</td>
<td>0.16 ± 0.01a† (9)</td>
<td>0.19 ± 0.02a† (8)</td>
<td>0.19 ± 0.02a† (7)</td>
</tr>
<tr>
<td>Saline</td>
<td>0.1 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.06 ± 0.02</td>
<td>0.08 ± 0.04</td>
</tr>
<tr>
<td>TPACE</td>
<td>0.72 ± 1.1 (9)</td>
<td>-0.25 ± 1.2 (9)</td>
<td>0.8 ± 1.8 (8)</td>
<td>8.2 ± 4.3a† (6)</td>
<td>5.9 ± 4.3 (6)</td>
</tr>
<tr>
<td>Saline</td>
<td>-1.9 ± 1.4</td>
<td>-0.7 ± 1.3</td>
<td>0.1 ± 1.6</td>
<td>-0.8 ± 1.0</td>
<td>0.9 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE for no. of animals in parentheses for ACTH treated; n = 4 for saline infused. TP, transplacental gradients (maternal–fetal concentrations). TPACE, TPglucose, TP lactate, and TP NaK, are given in mM. Only gradients that showed any change during ACTH infusion into ewe and matching saline controls are shown. *P < 0.05 from control. †P < 0.05 from 2–4 h. ‡P < 0.05 from 24 h.
ACTH it was less than control and less than the 2- to 4-h sample because the fractional reabsorption of K decreased by 72 h (Fig. 5).

Comparison of changes in fetuses carried by saline-infused ewes and those carried by ACTH-infused ewes. There was a striking difference between the changes in transplacental gradient of cortisol (TP_{cortisol}) in ACTH-infused animals compared with saline-infused animals. TP_{cortisol} fell in saline-infused animals, whereas it rose in ACTH-infused animals (Table 3). Transplantal gradients of glucose (TP_{glucose}) and lactate (TP_{lactate}) increased in ACTH-treated animals; there were no changes in these gradients in saline-infused animals (Table 3). The differences between maternal and fetal hematocrit, HCO_{3}, pH, and P_{Na/K} all increased in ACTH-treated animals. There were no changes in saline-infused animals (Table 3).

**DISCUSSION**

The aim of this project was to see whether ACTH-induced hypertension in the mother had any adverse effects on fetal health. Fetuses carried by ACTH-treated ewes showed changes characteristic of glucocorticoid and mineralocorticoid excess (discussed below). The changes in the ewes infused with ACTH were characteristic of glucocorticoid and mineralocorticoid excess (Table 1). In the adult sheep, cortisol alone cannot cause hypertension, although it does in humans (15). The ability of ACTH to cause a rise in arterial pressure is mimicked by a "cocktail" of steroids that are produced by the sheep adrenal in response to ACTH (12).

The lack of any effect of maternal ACTH-induced hypertension on fetal arterial blood gases and pH shows that this form of maternal hypertension was not associated with fetal "distress." This contrasts with the effects of one-clip one-kidney maternal hypertension, which caused marked falls in uteroplacental blood flow, fetal hypoxemia, fetal hypercapnia, and fetal death (8).

In the present study, only one fetus died during the experiment. Fetuses carried by ewes with renal hypertension also became hypertensive (8). Fetuses of ACTH-treated ewes did show a transient rise in MAP 24 h after the infusion of ACTH into the ewe began. However, fetuses carried by saline-infused ewes had a similar blood pressure pattern (Fig. 2), and this casts doubt on the significance of the changes in MAP seen in fetuses of treated ewes. In addition, Tangalakis et al. (13) showed that, in fetuses of this gestational age, intravenous cortisol did not cause a rise in resting arterial pressure, although it still caused an increased sensitivity to ANG II. Furthermore, because fetal arterial pressure increases with age (11), the small changes in arterial pressure seen in this study may not be treatment-induced effects.

Although fetal cortisol levels increased after ACTH, by the end of 72 h, they were similar to levels measured in control animals (Fig. 1) because there was a rise in cortisol levels in fetuses carried by saline-infused ewes. This rise in cortisol in control fetuses was almost certainly due to increased endogenous production of cortisol because TP_{cortisol} showed a progressive decline throughout the experiment (Table 3). It should be noted that, in one fetus, the rise occurred at the beginning of the experiment and was due to endogenous (i.e., fetal cortisol) production, since the transplacental gradient became more negative throughout the course of the experiment [falling from −17 nM (maternal – fetal) in control to −43.3, −70.4, −118.5, and −157.4 nM over experimental period]. In the other three fetuses, cortisol levels remained low, and the transplacental gradient was only significantly less (i.e., −53.5 nM) in the last 72 h. Clearly, in the fetus with the high plasma cortisol and the high endogenous production of cortisol, some other factor must have stimulated the hypothalamic-pituitary axis. We could not establish what the factor or factors might be. Because the fetus was healthy at the beginning of the experiment, we felt obliged to include it in the control group. In fetuses carried by ewes infused with ACTH, TP_{cortisol} was very low in control but became greater (maternal > fetal) within 4 h and remained high for the rest of the experiment (Table 3).

There were no changes in glucose and lactate levels in saline-infused ewes and their fetuses, whereas levels were increased in ACTH-treated ewes and fetuses (Figs. 3 and 4). Thus the increase in fetal glucose levels was clearly related to glucocorticoid excess in the ewe; i.e., high levels of glucose in the ewe caused a rise in TP_{glucose} resulting in increased glucose transfer to the fetal compartment (Table 3). Fowden et al. (3) showed that in fetal sheep there was a rise in hepatic and renal gluconeogenic enzymes which paralleled the gestation-dependent rise in cortisol levels and was abolished by adrenalectomy. Also, Wintour et al. (16) showed that infusions of dexamethasone at 0.76 mg/h for 48 h at 59–63 days of gestation caused a rise in both fetal plasma glucose and fructose (see also Ref. 14). Thus the hyperglycemia of fetuses carried by ACTH-treated ewes...
(Fig. 3) could be the result both of increased availability of glucose from the mother (due to her glucocorticoid excess) and induction of gluconeogenesis in the fetal compartments by the high levels of cortisol over the 72-h period.

The high fetal lactate levels may in part depend on increased metabolism of glucose within the fetoplacental compartment, since an increase in TP_{lactate} was only present at 24 h (Table 3), when maternal lactic acid levels were maximal (Fig. 4) and not at 48 or 72 h. Yet fetal lactic acid levels were high at these times (Fig. 4).

The rise in maternal and fetal plasma osmolality probably reflects the increase in plasma glucose and other solutes consequent on the glucocorticoid and mineralocorticoid effects induced in the mother by ACTH. The increase in fetal plasma osmolality probably caused an increase in fetal arginine vasopressin levels, which would have led to the small reduction in fetal urine flow and rise in fetal urinary osmolality (Table 2).

The fall in maternal and fetal hematocrit indicates that fluid retention occurred. The fall in fetal hematocrit was greater; i.e., the transplacental gradient widened (Table 3). If there was a rise in fetal fructose levels (16), this increase in solute in the fetoplacental compartment could account for the extra retention of water and the greater fall in fetal hematocrit relative to maternal, since fructose is not present in maternal plasma (16).

Changes in the maternal compartment did not always cause a corresponding change in the fetus. Thus fetal pH and HCO_{3}^{-} levels did not change, and the transplacental gradient between mother and fetus increased (Table 3) as the mother became alkalotic due to hypokalemia.

In fetuses carried by saline-infused ewes, there were no changes in the renal handling of Na and K. Yet in fetuses carried by ACTH-treated ewes, distal Na reabsorption increased, both total and fractional K reabsorption decreased, and K excretion increased (Table 2, Fig. 5). These changes in fetal renal K handling occurred in the absence of any changes in plasma K. GFR tended to increase (P = 0.07), fractional proximal Na reabsorption tended to decrease (P = 0.07), but fetal fractional reabsorption tended to increase (P = 0.06). These nonsignificant trends would have had an additive effect on distal Na delivery and so help explain why distal Na reabsorption increased significantly (Table 2). In chronically catheterized fetal sheep, there is endogenous mineralocorticoid activity, which is blocked by the specific aldosterone antagonist, spironolactone (7). Spironolactone caused a sharp fall in fractional excretion of K but had little effect on fractional Na excretion (7). Cortisol increases fetal GFR and depresses proximal fractional Na reabsorption (6, 17), and any kaliuretic effect of cortisol is most likely due to these effects on renin function. Thus the extent to which it has mineralocorticoid-like activity cannot be clearly defined. Although the changes in fetal renal K excretion could have been due to the high cortisol levels, the lack of any change in control fetuses and the fact that they occurred in the absence of any major changes in GFR or proximal tubular Na reabsorption could also mean that they occurred because there was transplacentinal transfer of a mineralocorticoid produced by the maternal adrenal as a result of ACTH stimulation. This may not be aldosterone, since ACTH only induces a transient rise in aldosterone secretion in the sheep (over 1st 24–48 h; Ref. 12), and the effects on fetal K excretion were evident at 72 h.

Perspectives

Glucocorticoids induce hypertension in the adult human being. This effect is mimicked in the sheep by administration of ACTH. In steroid-induced hypertension, there is also hyperglycemia, salt and water retention, and hypokalemia. In the present study, we have shown that the fetuses of ewes treated with ACTH develop many of these features, i.e., hyperglycemia, fluid retention, excess renal excretion of K, and possibly a raised arterial pressure. Fetal blood gas status and pH were unaffected. However, because many of the effects described in this study could also be mimicked by maternal dexamethasone treatment (14), these findings indicate that repeated injections of these steroids, as is often used in threatened premature labor, are not without metabolic consequences for the fetus.

We thank Dr. K. Gibson, Dr. A. D. Stevens, and Pamela Bode for their help and express our gratitude to Prof. Judy Whitworth for the measurements of plasma cortisol. We also thank Dr. D. Thomas, Dept. of Clinical Chemistry, Prince of Wales Hospital, for the measurement of plasma glucose and urinary electrolytes.

This work was supported by grants from the National Heart Foundation (Australia) and the National Health and Medical Research Council (Australia).

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Received 25 November 1996; accepted in final form 7 October 1997.

REFERENCES


