Pregnancy alters cortisol feedback inhibition of stimulated ACTH: studies in adrenalectomized ewes

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Received 9 November 2000; accepted in final form 31 January 2001

Keller-Wood, Maureen, and Charles E. Wood. Pregnancy alters cortisol feedback inhibition of stimulated ACTH: studies in adrenalectomized ewes. Am J Physiol Regulatory Integrative Comp Physiol 280: R1790–R1798, 2001.—These studies test the hypothesis that pregnancy alters the feedback effects of cortisol on stimulated ACTH secretion. Ewes were sham-operated (Sham), or adrenalectomized (ADX) at ~108 days gestation and replaced with aldosterone (3 μg·kg⁻¹·day⁻¹) and with cortisol at either of two doses (ADX + 0.6 and ADX + 1 mg·kg⁻¹·day⁻¹); ewes were studied during pregnancy and postpartum. Mean cortisol levels produced in ADX ewes were similar to normal pregnant ewes (ADX+1) or nonpregnant ewes (ADX+0.6), respectively. Plasma ACTH concentrations in response to infusion of nitroprusside were significantly increased in the pregnant ADX+0.6 ewes (1,159 ± 258 pg/ml) relative to pregnant Sham ewes (461 ± 117 pg/ml) or the ADX+1 ewes (442 ± 215 pg/ml) or the same ewes postpartum (151 ± 69 pg/ml). Plasma ACTH concentrations were not significantly different among the groups postpartum. Increasing plasma cortisol to 20–30 ng/ml for 24 h before hypotension produced similar inhibition of ACTH in all groups. Pregnancy appears to decrease the effectiveness of low concentrations of cortisol to inhibit ACTH responses to hypotension.

corticotropin; glucocorticoid; mineralocorticoid; feedback; arginine vasopressin

IN BOTH OVINE AND HUMAN PREGNANCY, maternal plasma cortisol concentrations increase (2, 8, 22). Neither the mechanism nor the physiological significance of this chronic increase in maternal plasma cortisol concentration is understood.

In previous studies, we demonstrated that the elevated cortisol results from changes in negative feedback control. We used maternal adrenalectomy combined with replacement of cortisol and aldosterone to demonstrate that if maternal plasma cortisol concentrations are maintained at the level appropriate for nonpregnant ewes, then basal ACTH is markedly increased in ewes during pregnancy, but not postpartum (16). From this result we concluded that the set point for cortisol is reset to higher levels during pregnancy.

However, our experiments have also shown that increases in cortisol in the pregnant ewe cause a suppression of basal ACTH concentrations (15, 16). We found that both pregnant and nonpregnant ewes responded to high physiological concentrations of cortisol (namely, such as those produced endogenously after stress) with equivalent inhibition of stimulus-induced secretion of ACTH (15, 16). To reconcile these results, we proposed that pregnancy results in a change in sensitivity to low (basal or unstimulated) concentrations of cortisol while not altering the sensitivity to high (stress induced) concentrations of cortisol.

In the present study we further test the hypothesis that the feedback action of low, basal cortisol is selectively decreased during pregnancy. We test the effect of both reduced and elevated plasma cortisol levels on stress-induced ACTH secretion. Although increases in basal cortisol levels are known to inhibit ACTH responses to stimuli, it is not clear whether small changes in basal plasma cortisol, such as occur during pregnancy, are physiologically relevant in the context of feedback suppression of stimulated ACTH secretion. The model of adrenalectomy and adrenal steroid replacement (19) in chronically catheterized ewes allows the comparison of the feedback effects of small differences in steady-state cortisol concentrations during pregnancy and postpartum.

For this study, ewes were studied after sham adrenalectomy (Sham) or after adrenalectomy (ADX) with replacement of cortisol to levels that are normal either for a pregnant ewe or for a nonpregnant ewe. ACTH and arginine vasopressin (AVP) responses to hypotension were measured in control experiments in these three groups and in experiments in which cortisol was increased to stress levels for the preceding 24 h. AVP was measured because posterior pituitary secretion of vasopressin is also stimulated by hypotension, but is not inhibited by glucocorticoids in other experiments in ewes or in fetal sheep (1, 9, 41). The relative AVP response is therefore an index of the intensity of the hypotension between steroid treatment groups.

METHODS

Animal preparation. Fifteen time-dated pregnant ewes of mixed Western breeds underwent surgery on days 106–114

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CONTROL OF STIMULATED ACTH DURING OVINE PREGNANCY

of gestation (normal term: 148–150 days) as previously described (2, 19). Each ewe had catheters placed in both femoral arteries and veins and was subjected to ADX or to sham ADX. In ADX, aldosterone and cortisol were replaced by intravenous infusion (syringe infusion pumps; Razel Scientific Instruments, Stamford, CT, or Orion Research, Cambridge MA) of aldosterone hemisuccinate and cortisol hemisuccinate (Solucortef, Upjohn, Kalamazoo, MI) or by infusion of aldosterone with placement of subcutaneous implants containing cortisol hemisuccinate (Innovative Research, Sarasota, FL) at the end of the adrenalectomy procedure.

Postoperatively, ADX ewes were also infused with cortisol at 2 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \) and aldosterone at 2 \( \text{ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) for the first 16–20 h and with cortisol at 1.0 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) and aldosterone at 2 \( \text{ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) for the next 24 h. Thereafter, aldosterone was replaced by infusion, and the maintenance dose of cortisol was administered by continued infusion or via the implants for the remainder of the experiment (Fig. 1). All ewes were treated with Polyflex (ampicillin; 750 mg im) twice a day for 5 days postoperatively and once a day thereafter. Ewes were also treated with Banamine (flunixin meglumine, 1 mg/kg im once or twice a day) for 1–3 days as necessary for relief of postoperative pain. Ewes were monitored daily, and plasma samples were collected to analyze plasma electrolytes, plasma proteins, and hematocrit every 1–2 days.

**Experimental protocol.** At surgery, ewes were divided into three study groups: sham ADX (Sham), ADX to 0.6 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \) (ADX+0.6), or ADX and replaced to 1 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \) (ADX+1) (Fig. 1). Steady-state replacement of cortisol was achieved in ADX ewes using subcutaneous implants containing cortisol hemisuccinate placed in the midscapular region (4–8 pellets of 200 mg cortisol each, released over 21 days to produce release rates of \( \sim 0.6 \) or 1 mg cortisol-\( \text{kg}^{-1} \cdot \text{day}^{-1} \)). The implants were replaced at 21-day intervals throughout the study; experiments were not performed on the 2 days before or after implant placement. The first two ewes were replaced using infusion of cortisol (1 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \)). One of the two ewes died as the result of infusion pump failure; as a result of this, we adopted the technique of implanting subcutaneous pellets as a more reliable method of delivering cortisol to the ewe. Aldosterone was replaced in all ADX ewes by infusion of 2 \( \text{ng} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \) of aldosterone hemisuccinate (3 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \); Sigma, St. Louis, MO). The aldosterone treatment dose was chosen on the basis of the estimated production rate in pregnant and nonpregnant ewes; cortisol treatment doses were chosen on the basis of estimated production rates in pregnant and nonpregnant ewes of \( \sim 1 \) and 0.6 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \), respectively.

After at least 5 days of recovery from surgery, ewes were then studied in three experiments each. Experiments were separated by a minimum of 72 h, and all experiments were started between 8:30 and 9:30 AM. The three experiments were designed to test the negative feedback relationship between plasma cortisol and ACTH. These experiments consisted of 1) control replacement at 0.6 or 1 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \), as described above (or no replacement in the case of Sham ewes); 2) additional infusion of 1.4 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1} \) cortisol over 24 h; and 3) additional infusion of 2.0 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) cortisol over 24 h. In each of these three experiments, a period of hypotension was induced at 24 h to measure ACTH responses to a stimulus. Hypotension was induced by infusion of nitroprusside (Nitropress, Abbot, North Chicago, IL) at a rate of 10 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) for 10 min. The relationship between cortisol and unstimulated ACTH during the first 8 h of cortisol infusion has been previously reported (18). The order of the three experiments was varied among ewes in each experimental treatment group. Not all of the experiments were valid or otherwise completed because of fetal death, impending abortion, or problems with the infusion of cortisol.

In addition to the experiments in pregnant ewes, we also performed experiments on the ewes (5–32 days) postpartum. The time interval of study postpartum was necessary to allow for recovery from delivery or abortion and for the three experiments (cortisol infusion rates) per ewe; no differences were noted in responses between ewes studied with a given cortisol dose at the beginning vs. the end of this interval. In all ewes, postpartum plasma progesterone concentrations were \( < 0.2 \) ng/ml. A total of 15 ewes were studied during pregnancy: 4 in the Sham treatment group, 5 in the ADX+0.6 group, and 6 in the ADX+1 group. A total of 12 ewes, 4 per treatment group, were studied postpartum at the control cortisol replacement doses. However, loss of catheters or animals resulted in inclusion of only three ewes in some groups infused with 1.4 and/or 2 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) postpartum.

**Assays.** Blood collected for measurement of plasma hormones was collected into tubes containing 0.015 M EDTA; samples for measurement of plasma electrolytes and hematocrit were collected in tubes containing heparin. Samples were placed on ice and then spun for 20 min at 2,000 rpm in a refrigerated centrifuge. Aliquots of plasma were frozen for analysis of hormones by radioimmunoassay.

One milliliter of each blood sample was placed in a heparinized tube for determination of plasma sodium and potas-
sium concentration (Nova 1, Nova Biomedical, Waltham, MA). Plasma protein concentrations were measured using a refractometer. Hematocrit measurements (% packed cell volume) were performed on duplicate samples of blood collected in microcapillary tubes and spun for 3 min at 12,000 rpm (Damon Division, International Equipment, Needham Heights, MA). Hematocrits were read to the nearest one-half percent. Plasma proteins were read using a refractometer to the nearest one-tenth of a percent.

ACTH, AVP, and cortisol assays were performed as previously described (2, 23, 38) using antibodies produced in this laboratory. The cortisol antibody has <5% cross-reactivity with cortisol hemisuccinate; the cortisol hemisuccinate released by the pellet would not influence cortisol measurements. For assay of ACTH, plasma was extracted using glass (Corning, Corning, NY), and ACTH was eluted with 1:1 0.25 N HCl and acetone (2); for AVP assay, plasma was extracted with bentonite, and AVP was eluted with 1:4 N HCl and acetone (23). For assay of cortisol, plasma was extracted with ethanol (38). All extracts were dried in a Savant evaporator (Holbrook, NY) and reconstituted in assay buffer. Aliquots of standard were also extracted and used in each assay to correct for recovery. Each ACTH or AVP extraction and assay included samples from all groups studied.

The percentage of free plasma cortisol was estimated by ultrafiltration by a modification of the technique of Ham- mond et al. (13). An aliquot (0.4 ml) of plasma collected at the 8 h of cortisol infusion was incubated with [1,2,6,7-3H]cortisol, Amersham Pharmacia Biotech, Piscataway, NJ; ~50,000 dpm) and [14C]glucose (Amersham Pharmacia Biotech; ~12,000 dpm) for 1 h at 39°C. An aliquot (0.300 ml) was transferred to an ultrafiltration chamber (Cen trifree Micropartition device, 30,000 molecular weight exclusion, Millipore) and spun for 3 min at 800 rpm at ~39°C. Approximately 25 µl of fluid was filtered to the lower chamber during centrifugation. At the end of the centrifugation, aliquots of ultrafiltrate from the lower chamber and plasma from the upper chamber were counted. The percentage of free cortisol was calculated as the ratio of 3H to 14C in the ultrafiltrate (lower chamber) divided by the ratio of 3H to 14C in the plasma incubate (upper chamber). This method does not violate the principle of equilibrium dialysis, because the volume of fluid filtered is small relative to the original volume. Percentages of free cortisol estimated by this method ranged from 3 to 30% with total cortisol concentrations of 1–50 ng/ml. The free cortisol concentration was estimated by multiplying the percentage of free cortisol by the total cortisol concentration measured by radioimmunoassay.

Mean arterial pressure data were collected at 10 Hz using a Keithley data-acquisition system and Asyst Software (Asyst Technologies, Stamford, CT). One-minute averages were calculated and used for analysis. In the case of several experiments in which the data were not saved to the computer hard drive, pressures were read at 10-s intervals from the chart output of the Grass polygraph (Astro-Med, West Warwick, RI) and 1-min means were calculated.

**Analyses.** Mean hormone, electrolyte, protein, glucose, and hematocrit data were analyzed by analysis of variance (36). The data were compared among groups by analysis of variance, corrected for repeated measures across time. The ACTH and AVP data were log transformed before analysis. Differences among means were compared by Duncan’s multiple-range test. For all statistical analysis, the criterion for significance was \( P < 0.05 \).

The relationship between total and free cortisol concentrations and plasma ACTH responses were analyzed by linear regression analysis after logarithmic transformation of ACTH values. Slopes and elevations of the relationships in pregnant and postpartum states were compared by \( t \)-test (42).

**RESULTS**

Cortisol levels produced by cortisol replacement and cortisol infusion. The initial values of plasma cortisol before infusion of cortisol on the first experimental day are shown in Table 1. The cortisol values in ADX+0.6 were significantly lower than the values in either Sham and ADX+1.0 during pregnancy; the values in the ADX+1.0 group were not different from the Sham animals. The values in the three groups are similar to the average mean values over the 8 h of infusion on the day before nitroprusside (16) and the average daily values (18) that we have previously reported. In Sham ewes, postpartum cortisol values were lower than during pregnancy; there were no differences between values during pregnancy and postpartum in ADX+0.6 or ADX+1 ewes.

At the start of infusion of nitroprusside, mean total cortisol concentrations in control experiments (without additional cortisol infusion) were 5.4 ± 1.2 ng/ml in the Sham ewes, 5.2 ± 2.1 ng/ml in the ADX+0.6 ewes, and 10.8 ± 2.7 ng/ml in the ADX+1 ewes during pregnancy. Postpartum, the cortisol values at the start of the nitroprusside infusion were 4.1 ± 1.9 ng/ml in the Sham ewes, 8.4 ± 2.4 ng/ml in the ADX+0.6 ewes, and 8.8 ± 1.0 ng/ml in the ADX+1 at the start of nitroprusside; the mean values in the ADX+0.6 ewes postpartum are high as a result of one ewe with increased cortisol relative to during pregnancy. Plasma cortisol concentrations produced by the infusion of 1.4 and 2 µg·kg\(^{-1}\)·min\(^{-1}\) of cortisol were not different among the treatment groups nor were they different between pregnant and nonpregnant ewes. The total plasma

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**Table 1. Mean values of plasma cortisol, electrolyte, protein, and PCV**

<table>
<thead>
<tr>
<th>Plasma Cortisol, ng/ml</th>
<th>Plasma Na(^{+}), mEq/l</th>
<th>Plasma K(^{+}), mEq/l</th>
<th>Plasma Protein, mg/100 ml</th>
<th>PCV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant Sham</td>
<td>8.8 ± 1.1</td>
<td>147.4 ± 0.6</td>
<td>4.88 ± 0.06</td>
<td>7.6 ± 0.2</td>
</tr>
<tr>
<td>Pregnant ADX + 0.6</td>
<td>4.8 ± 0.8</td>
<td>148.3 ± 1.4</td>
<td>4.41 ± 0.26</td>
<td>7.3 ± 0.1</td>
</tr>
<tr>
<td>Pregnant ADX + 1</td>
<td>9.6 ± 1.6</td>
<td>145.7 ± 0.7</td>
<td>5.13 ± 0.42</td>
<td>8.3 ± 0.4</td>
</tr>
<tr>
<td>Postpartum Sham</td>
<td>5.4 ± 1.4</td>
<td>148.0 ± 0.8</td>
<td>4.93 ± 0.31</td>
<td>8.1 ± 0.4</td>
</tr>
<tr>
<td>Postpartum ADX + 0.6</td>
<td>6.3 ± 0.8</td>
<td>150.0 ± 0.8</td>
<td>3.74 ± 0.19</td>
<td>7.7 ± 0.1</td>
</tr>
<tr>
<td>Postpartum ADX + 1</td>
<td>9.0 ± 2.1</td>
<td>151.0 ± 3.9</td>
<td>4.01 ± 0.31</td>
<td>8.5 ± 0.2</td>
</tr>
</tbody>
</table>

Plasma cortisol values are expressed as means ± SE of the average value of cortisol in each ewe at the start of 24-h cortisol infusions as previously reported (18). Other values are means ± SE at 0 min before nitroprusside in control experiments. ADX, adrenalectomized; Sham, sham operated; PCV, packed cell volume.
cortisol concentrations at the start of nitroprusside averaged across all groups at 24 h of infusion were 24.7 ± 2.0 ng/ml during 1.4 μg·kg⁻¹·min⁻¹ and 30.0 ± 2.0 ng/ml during 2 μg·kg⁻¹·min⁻¹.

Mean arterial pressure, plasma electrolytes, and protein concentration. Mean arterial pressure, plasma electrolytes, plasma proteins, and packed cell volume (PCV) were also measured to determine whether the altered cortisol levels altered the degree of hypotension or the recovery from hypotension. Overall there was no significant effect of dose of chronic cortisol treatment (Sham, ADX+0.6, or ADX+1) or the 24-h infusion of cortisol (1.4 or 2 μg·kg⁻¹·min⁻¹) on the mean arterial pressure in the 10 min before nitroprusside (Fig. 2).

Although the mean arterial pressure was greater in the ADX+1 group than in Sham or ADX+0.6 group at most times after the end of the infusion of nitroprusside, the mean arterial pressure response during nitroprusside also was not significantly different between the treatment groups during pregnancy or postpartum. The mean arterial pressure response was not altered by prior infusion of cortisol over 24 h.

In response to hypotension, PCV rapidly increased, followed by a decrease from 20 to 60 min; plasma proteins also decreased from 20 to 60 min (data not shown). These reductions reflect increased vascular volume in response to reduced pressure. Ewes in the ADX+0.6 group showed a smaller decrease in PCV after hypotension; however, the decrease in plasma proteins during hypotension was similar in all treatment groups in the ewes during pregnancy. There were no significant effects of the 24-h infusion of cortisol on the plasma protein or PCV response to hypotension.

Plasma potassium concentrations tended to be lower and plasma sodium levels were higher in the ewes postpartum than during pregnancy, so that there was a significant effect of pregnancy, although there were no significant differences among individual means (Table 1). Neither the potassium nor the sodium response to hypotension was significantly altered by the different treatments or by the 24-h infusion of cortisol.

ACTH and cortisol. When the ewes were studied in control experiments, basal ACTH levels were significantly greater prenitroprusside in the ADX+0.6 ewes during pregnancy compared with postpartum or compared with the ADX+1 or Sham ewes during pregnancy. This is consistent with the results over the previous day of study (16). ACTH responses to hypotension were significantly increased in the ewes in the ADX+0.6 group (1,159 ± 258 pg/ml at 10 min, n = 4) during pregnancy compared with the same ewes postpartum (151 ± 69 pg/ml at 10 min, n = 4) (Fig. 3, top). Similarly, ACTH responses were significantly increased in the ewes in the ADX+1.0 group (442 ± 215 pg/ml at 10 min) during pregnancy compared with the same ewes postpartum (76 ± 17 pg/ml at 10 min, n = 3). However, ACTH responses to hypotension in the

![Graph of mean arterial pressure during pregnancy (left) or postpartum (right) in the 3 treatment groups: Sham ADX (●) or ADX+0.6 (○) or ADX+1 (●). Nitroprusside was infused from 0 to 10 min. Data at top are from ewes with the basal replacement doses of cortisol; data at bottom are after 24 h of infusion of 1.4 μg·kg⁻¹·min⁻¹ of cortisol. Data are shown as means ± SE. n Values are: pregnant: Sham, n = 4, n = 3 for 1.4 μg·kg⁻¹·min⁻¹ of cortisol; ADX+0.6, n = 4; ADX+1, n = 6; postpartum: Sham, n = 4 with 0 cortisol, n = 3 for 1.4 μg·kg⁻¹·min⁻¹ of cortisol; ADX+0.6, n = 3; ADX+1, n = 3.](image)

![Graph of plasma ACTH and cortisol concentrations in Sham (●), ADX+0.6 (○), and ADX+1.0 (●) ewes during control experiments. Data are shown as means ± SE. Data from ewes during pregnancy are shown on the left; postpartum data are shown on the right. n Values are: pregnant: Sham, n = 4; ADX+0.6, n = 4; ADX+1, n = 6; postpartum: Sham, n = 4; ADX+0.6, n = 4; ADX+1, n = 3. *Differ from 0 min in same group; †Significantly different from postpartum in the same group, ‡significantly different from Sham group, †significantly different from ADX+0.6, ‡significantly different from in ADX+1.](image)
ADX+1 ewes were similar to responses in Sham ewes (461 ± 117 pg/ml at 10 min, n = 4) during pregnancy. The responses in the Sham ewes, a group of animals that self-regulates plasma cortisol, were not different during pregnancy (461 ± 117 pg/ml at 10 min, n = 4) compared with postpartum (447 ± 81 pg/ml at 10 min; n = 4). Thus reduction of cortisol in pregnancy caused greater ACTH responses relative to either the same cortisol levels postpartum (as in the ADX+0.6 group) or normal pregnant cortisol levels (Sham), whereas normalization of cortisol levels in pregnancy (ADX+1) resulted in normalization of the ACTH response.

When cortisol was increased for 24 h to levels similar to those resulting from stress, ACTH responses were suppressed in all groups. Infusion of cortisol at 1.4 (Fig. 4, bottom) or 2 μg·kg⁻¹·min⁻¹ (data not shown) similarly inhibited plasma ACTH response to hypotension in all treatment groups during pregnancy or postpartum. This demonstrates that adequate increases in cortisol can effectively inhibit ACTH responses in both pregnant and nonpregnant subjects.

Plasma cortisol concentrations increased during nitroprusside in the Sham ewes, but not in either group of ADX ewes, verifying the completeness of the adenectomy procedure. The cortisol response was absent during pregnancy and postpartum in the ADX ewes (Fig. 3, bottom). The plasma cortisol responses were reflective of the ACTH responses in the Sham group (Fig. 3, bottom). The cortisol response was more prolonged in the Sham ewes when pregnant than during the postpartum period, as expected for the more prolonged ACTH response during pregnancy. Also as expected, the suppression of the ACTH response in the Sham groups after 24 h of infusion of 1.4 or 2 μg·kg⁻¹·min⁻¹ of cortisol infusion suppressed the cortisol responses in Sham ewes (Fig. 4, top).

The relationship between plasma cortisol concentrations and the logarithm of the plasma ACTH concentrations in the pregnant ewes was significantly different compared with the postpartum ewes (Fig. 5). This difference was significant whether the cortisol was expressed as the cortisol levels at 0 time (data not shown) or the mean levels from 1 to 8 h of infusion on the previous day (Fig. 5, left). This difference is not accounted for by a change in the percentage of free cortisol during pregnancy. The percentages of free cortisol at the basal cortisol levels were 11 ± 4% in the Sham, 17 ± 4% 1 in the ADX+0.6, and 15 ± 3% in the ADX+1 ewes during pregnancy, and 4 ± 1, 11 ± 5, and 17 ± 9% in these groups, respectively, postpartum. The differences between pregnant and postpartum values are not significant. The changes in the percentage of free cortisol are opposite to those that would account.
for the differences in ACTH responses observed; an increased proportion of free cortisol should cause more effective feedback suppression in the ADX + 0.6 group. The difference in the relationship between cortisol and ACTH during pregnant and postpartum states is also significant if the estimated free plasma cortisol levels on the previous day are used in the analysis (Fig. 5, right). The difference between pregnant and nonpregnant ewes is revealed by comparison of the plasma ACTH levels at plasma cortisol levels <8–10 ng/ml (the plasma concentration in intact pregnant ewes). ACTH responses were increased in the ADX ewes during pregnancy compared with postpartum when total plasma cortisol levels were decreased to concentrations of <5 ng/ml or free levels were <1 ng/ml, as in the ADX + 0.6 group. On the other hand, ACTH values were similar when total cortisol concentrations were >10 ng/ml or free concentrations were >2 ng/ml (as during infusion of 1.4 or 2 μg·kg⁻¹·min⁻¹).

AVP. Plasma AVP concentrations were significantly increased in response to infusion of nitroprusside. Although the basal plasma AVP and the response to hypotension appear to be greater in the ADX + 0.6 group (3.8 ± 1.5 pg/ml at 0 min and 116 ± 38 pg/ml at 10 min) than in the Sham (1.7 ± 0.2 pg/ml at 0 min and 42 ± 20 pg/ml at 10 min) or ADX + 1 (3.0 ± 1.1 pg/ml at 0 min and 47 ± 22 pg/ml at 10 min) groups during pregnancy, the effect of treatment was not significant. The increase in AVP during hypotension was also not altered by the 24 h of infusion of cortisol (data not shown).

DISCUSSION

This study demonstrates a significant influence of pregnancy on the regulation of stimulated ACTH by basal plasma cortisol but no significant influence of pregnancy on regulation of ACTH by cortisol levels greater than normal basal values. When plasma cortisol concentrations in pregnant ewes are maintained at levels that are lower than normal for pregnant ewes (although within the normal range for nonpregnant ewes), plasma ACTH concentrations during hypotension are significantly increased. This is illustrated by the increased response in the pregnant ADX + 0.6 group and increased elevation of the relationship between total or free cortisol and ACTH in the pregnant ewes compared with postpartum ewes.

The reduction of plasma cortisol in the ADX + 0.6 group of ewes could, theoretically, alter ACTH secretion in several ways: 1) reducing cortisol and removing the adrenal secretory response may impair the ability of the animal to compensate for the hypotension, resulting in a greater stimulus intensity, 2) removing the adrenal secretory response may eliminate a rapid feedback effect of cortisol on the ACTH response to stress, and 3) a decrease in basal cortisol and variability in cortisol together with the change in set point could increase activity in central pathways or hypothalamic neurons controlling stimulus-induced secretion of ACTH.

Our results are collectively consistent with the third alternative. Rapid feedback effects of cortisol have not been demonstrated in ewes (37), suggesting that this mechanism does not play an important role in this species. Furthermore, if differences in fast feedback (14) resulting from the normal secretion of adrenal cortisol were important, then the magnitude or duration of the ACTH response in the pregnant ADX + 1.0 group and nonpregnant ADX + 0.6 group should be increased compared with Sham pregnant and Sham nonpregnant ewes, respectively. We also found no evidence of impaired cardiovascular compensation for hypotension in the ADX ewes. Without replacement of cortisol in the ADX ewes, we would have expected alterations in cardiovascular function. For example, we observed that complete steroid withdrawal in ewes markedly decreases arterial pressure; experiments in ADX rats or dogs without steroid replacement (6, 7, 11, 12, 30, 33) and clinical reports in humans with adrenal insufficiency (20, 35) also indicate that corticosteroids are essential for normal blood pressure, vascular reactivity, and return of pressure after hemorrhage. The observation that the resting blood pressure and the blood pressure response to hypotension was not altered in the ADX + 0.6 group relative to the Sham group suggests that the level of replacement of cortisol and aldosterone is adequate to normalize mean arterial pressure and electrolytes and produces only small changes in PCV or plasma proteins. Interestingly, the results also suggest that acute cortisol or aldosterone responses to hypotension (above baseline concentrations) are not required for normal vascular reactivity. Nevertheless, it is possible that the effects of steroid withdrawal on vascular reactivity are nitric oxide (NO) dependent (40); in that case, differences in vascular responses between steroid treatment groups might not be expected in the presence of high concentrations of NO donors such as nitroprusside.

It is possible that the increased ACTH response to hypotension (in ADX + 0.6 vs. ADX + 1.0) might reflect an interaction between the blood volume and the magnitude of hypotension. One might expect that blood volume might be reduced as a consequence of reduced cortisol in the pregnant ewes; this could result in a greater response to the same degree of hypotension. The smaller decrease in PCV during hypotension in the ADX + 0.6 (vs. ADX + 1.0) ewes during pregnancy suggests that there is reduced influx of fluid into the vascular space, an effect that has been proposed to be mediated by cortisol (12). We conclude, however, that it is unlikely that this effect completely explains the increased ACTH response in the ADX + 0.6 (vs. ADX + 1.0 or Sham) group during pregnancy because there is no change in the dilution of plasma protein after hypotension and there is no significant difference in AVP responses among groups.

As expected based on previous studies in adrenal-intact ewes (15), pregnancy did not alter the suppression of stimulated ACTH that occurs when cortisol is increased to concentrations above the normal range of basal levels. During 24 h of infusion of cortisol to levels...
similar to those produced by hypotension (1.4 or 2 μg·kg⁻¹·min⁻¹), ACTH was suppressed in both pregnant and nonpregnant ewes. This result also agrees with the effect of cortisol infusions on basal ACTH that we have previously reported (16). These results suggest that the increase in ACTH in pregnant under-replaced ewes during hypotension is not simply the result of reduction of all glucocorticoid feedback effects during pregnancy.

The feedback effects of adrenal steroids on ACTH are believed to be mediated by both subtypes of corticosteroid receptor, mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). The relatively high affinity, but lower capacity, MR have been proposed as the mediator of the feedback effects of low levels of corticosteroids on basal ACTH, whereas the higher capacity GR are proposed as the mediators of feedback effects due to higher levels of corticosteroids or synthetic glucocorticoids (5, 25). These two receptors may also interact to control activity in this system (3, 31). The infusions of cortisol that suppressed ACTH in both pregnant and nonpregnant ewes elevated plasma cortisol concentration to levels within the range expected to increase GR occupancy (MR are fully saturated at these cortisol concentrations). On the other hand, the cortisol concentrations over which we found significant differences between pregnant and nonpregnant ewes are within the range of steeply increasing MR occupancy. This analysis is based on estimates of cortisol binding affinity to GR and MR in dogs (24), a species with similar circulating plasma cortisol concentrations to the ewes. These data therefore suggest that there may be a difference in MR occupancy or action in pregnancy. In normal nonpregnant ewes, more MR in hippocampus are available than in pregnant ewes (26), suggesting that the increase in sensitivity to feedback effects of cortisol in pregnancy might result from a change in MR activation and therefore MR-mediated feedback effects. The present data are consistent with the importance of the MR in regulating both basal and stimulus-induced ACTH.

We believe that the present experiments in sheep are also relevant to women, although there is some inconsistency in the data and their interpretation. It has been suggested based on clinical data in pregnant women that all glucocorticoid feedback is altered in pregnancy. Dexamethasone is less effective as an inhibitor of morning ACTH levels during pregnancy (21), and betamethasone treatment has been shown to reduce both plasma ACTH and cortisol concentrations (32) in women, although this suppression is not complete during pregnancy. The reduced efficacy of high-dose glucocorticoids in women has suggested alterations in GR action. These results are in contrast to the ability to completely suppress ACTH by high doses of cortisol in the sheep. In our studies we have no evidence for desensitization of the glucocorticoid feedback effect of increased cortisol. One possible explanation for these species differences is that the human placenta secretes ACTH and CRF and that the secretion of these hormones is not fully suppressed by glucocorticoids (32), whereas the ovine placenta does not secrete ACTH or corticotropin-releasing factor in significant amounts (17, 18).

Experience in our laboratory with ADX pregnant ewes and clinical reports of hypoadrenal pregnant women suggest that increased cortisol secretion is important in both species. In women, adrenal insufficiency during pregnancy may result in crises during parturition or in the postpartal period (10, 21, 28, 29). In our laboratory we found that pregnant ADX ewes appear to develop symptoms of adrenal insufficiency such as hypotension, lethargy, and aphagia, more rapidly than do nonpregnant ewes. We also noted pronounced increase in abortion and in mortality in ADX ewes during labor or in the immediate postpartal period. In the present study and in the previous report (16), we did not note a difference in basal electrolytes, glucose, plasma proteins, or pressure between the ADX cortisol-replaced animals and the Sham ewes. This suggests that even the lower replacement dose, in the presence of normal aldosterone levels, is adequate to normalize these variables. In this study, the ability to recover from the acute hypotensive challenge is also normal. We suspect, however, that during more severe challenges, such as the volume loss and changes in cardiac output during and after delivery, it may be more difficult for the under-replaced ewes to compensate. In pregnant dogs with intact adrenals, the same volume loss as a percentage of initial volume resulted in a greater decrease in blood pressure than in the nonpregnant adrenal-intact dogs (4), suggesting that even adrenal-intact pregnant animals have reduced ability to compensate for volume loss. A compromised ability to compensate for volume loss in late gestation may be related to the increased mortality in late gestation we have observed in ADX, under-replaced ewes, and the peripartal presentation of hypoadrenocorticism in women (21).

**Perspectives**

Chronically elevated basal maternal cortisol levels during pregnancy result from a change in negative feedback action of cortisol in the maternal pituitary-adrenal axis. This decrease in sensitivity to feedback effectively resets the regulated level of basal cortisol and increases the response to stimuli when cortisol levels fall below the new set point. The alteration of stimulated ACTH secretion, as well as the basal secretion of ACTH, in response to changes in basal secretion of cortisol, in an adrenal-intact subject would help to ensure a adequate cortisol response to the stimulus. This regulatory function in the normal subject is important, because the inability to respond to hypocorticism appears to be associated with dire consequences in the peripartal period. We propose that the mechanism of the increase in set point is altered ability of cortisol to interact at MR in one or more corticosteroid feedback sites. Consistent with this hypothesis is our observation of an increase in available MR binding sites in hippocampus in pregnancy (26), possibly asso-
associated with decreased MR occupancy. A possible, and perhaps likely, mechanism for this effect is the known antimineralocorticoid action of progesterone in vitro and in vivo (27, 34). The increase in cortisol set point during pregnancy could therefore result from the antagonist action of progesterone. If so, the rate of placental steroidogenesis would indirectly influence the function of the maternal hypothalamus-pituitary-adrenal (HPA) axis. The compensatory increase in maternal HPA axis activity is likely necessary to maintain appropriate fluid balance and cardiovascular function, which would collapse in the absence of an appropriate cellular response to cortisol.

We thank S. Caldwell for excellent technical assistance in the care of these animals, E. Manlove for assistance in the measurement of electrolytes and plasma proteins, and R. Martin for assistance in the radioimmunoassays.

This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-38114.

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