Regulation of maternal ACTH in ovine pregnancy: does progesterone play a role?

Maureen Keller-Wood and Charles E. Wood

Department of Pharmacodynamics and Physiology and Functional Genomics, University of Florida, Gainesville, Florida

Submitted 28 April 2008; accepted in final form 11 August 2008

Keller-Wood M, Wood CE. Regulation of maternal ACTH in ovine pregnancy: does progesterone play a role? Am J Physiol Endocrinol Metab 295: E913–E920, 2008. First published August 12, 2008; doi:10.1152/ajpendo.90399.2008.—Pregnancy is characterized by increased plasma adrenocorticotrophic hormone (ACTH) and cortisol. Studies suggest that progesterone acts as an antagonist at mineralocorticoid receptors. Therefore, we tested the hypothesis that chronic progesterone, produced by treatment of nonpregnant ewes or during pregnancy, will result in increased plasma ACTH relative to the plasma cortisol concentrations. We studied three groups of ewes: ovariectomized nonpregnant, nonpregnant treated with progesterone, and pregnant ewes. In two series of studies, ewes were adrenalectomized and replaced with 0.35 mg/kg/H18528 cortisol over 24 h were used to increase daily replacement doses to 0.5, 1, or 1.5 mg/kg/H18528 cortisol. In the first study, additional infusions of cortisol over 24 h were used to increase daily replacement doses to 0.5, 1, or 1.5 mg/kg/H18528 cortisol, and intact pregnant and nonpregnant ewes were studied with infusions of cortisol at 0, 0.5, and 1 mg/kg/H18528 cortisol. In adrenalectomized ewes chronically treated with 0.35 mg/kg/H18528 cortisol, plasma ACTH concentrations were decreased significantly in the nonpregnant progesterone-treated ewes compared with the ovariectomized nonpregnant ewes. With 0.5 mg/kg/H18528 cortisol, plasma ACTH levels were greater in pregnant ewes than in nonpregnant ewes with or without progesterone. Overall plasma ACTH levels at 0.35 mg/kg/H18528 cortisol were significantly related to the plasma protein concentration, suggesting that the ACTH levels in the hypocorticotoid ewes are most closely related to plasma volume. Across all steroid doses, ACTH was positively related to plasma proteins and progesterone, and negatively related to cortisol. We conclude that increased progesterone does not alter the feedback relation of cortisol to ACTH, but may modulate ACTH indirectly through plasma volume.

Changes in feedback effectiveness of cortisol and/or glucocorticoids have been suggested to be a mechanism driving increased adrenocorticotrophic hormone (ACTH) secretion. In humans, a larger dose of dexamethasone is required to suppress morning ACTH concentrations in pregnant women (20). In previous studies, we found that that, although there is no difference in the efficacy of increased cortisol to suppress ACTH in the pregnant ewe, decreasing plasma cortisol in pregnant ewes from pregnant to nonpregnant levels resulted in greater increases in ACTH in ewes during pregnancy than postpartum. We found that this was true for both basal ACTH and ACTH stimulated by acute hypotension (13, 16). This suggested that the set point for regulation of cortisol was altered in pregnancy; the fact that the difference occurred at concentration of cortisol <10 ng/ml (estimated as 2.5 nM free cortisol) suggested that this reset involves altered action at the high-affinity mineralocorticoid receptors (MRs). Previous studies in our laboratory suggested that mineralocorticoid availability is also increased in ovine pregnancy in hippocampus (25) and that progesterone binds to the sheep MR with an affinity similar to that of cortisol (24). Increases in plasma progesterone have been demonstrated in the rat to inhibit cortisol action at MR, as reflected by increased cytosolic MR availability with progesterone treatment (3), suggesting that increased progesterone may mediate the greater ACTH responses in the pregnant state.

An alternative explanation for greater plasma ACTH with lower cortisol is a greater sensitivity to a cortisol-mediated signal in the pregnant state, for example, if reduced cortisol sensitizes the system to stimulatory inputs. There is little evidence that increased responsiveness to physiological stresses such as hypotension result in augmented ACTH secretion during pregnancy (12); conversely, maternal baroreceptor function is thought to reset to a lower resting blood pressure in the pregnant state (2, 12, 29). However, it has been suggested that regulation of blood volume changes in human and rodent pregnancy (27). This “underfill” hypothesis assumes a reset of the volume control reflex so as to create a constant error signal driving prolonged volume expansion in pregnancy. Such a change in reflex responsiveness to input from the volume receptors would also be expected to result in an increased drive to ACTH, although this possibility has not been studied.

The purpose of this series of experiments is to further investigate the regulation of ACTH during ovine pregnancy. Two hypotheses are tested: first, that decreased cortisol will result in more dramatic increases in plasma ACTH in pregnant...
ewes than in nonpregnant ewes; second, that increases in circulating progesterone in both pregnant or progesterone-treated nonpregnant ewes inhibit corticosteroid feedback effects and result in increased plasma ACTH concentrations. These studies were conducted in an ovine model in which we have manipulated cortisol by removal of the maternal adrenals and chronic replacement of cortisol using subcutaneous pellets; this model eliminates adrenal secretion of epinephrine, which is not believed to be involved in the physiological regulation of ACTH, and adrenal secretion of aldosterone, which was equivalently replaced in all adrenalectomized ewes. We tested the effect of cortisol on ACTH at two chronic and relatively low replacement doses and at three higher levels of cortisol infusion over a 24-h period. These somewhat higher doses of cortisol are still well within the physiological range for the sheep, and would not be expected to fully activate glucocorticoid receptors. To test the second hypothesis regarding progesterone effects on cortisol inhibition of ACTH, we used chronic progesterone treatment in a group of nonpregnant adrenalectomized ewes to mimic the chronic increase in progesterone produced in ovine pregnancy.

**METHODS**

**Animal Use**

Adult female pregnant and nonpregnant sheep of mixed breeds were used in two separate studies. Each study was approved by the University of Florida Institutional Care and Use Committee. All ewes were housed in the University of Florida Health Sciences Center Animal Care facility in rooms with controlled light (12:12), humidity, and temperature throughout the period of study. All ewes had free access to food, salt blocks, and water except for the 24-h period before surgery, during which food, but not water, was withheld. All surgical procedures were performed under halothane anesthesia; in all ewes, femoral artery and venous catheters were placed as previously described (1), and some ewes were subjected to adrenalectomy and/or ovariohysterectomy to alter endogenous cortisol and/or progesterone secretion. All pregnant ewes were studied in late gestation between 110 and 133 days.

In study I, ewes were assigned to one of five experimental groups: adrenal-intact, nonpregnant (n = 6) and adrenal-intact pregnant (n = 6) in which the adrenals were visualized but left undisturbed and adrenalectomized nonpregnant (n = 5), adrenalectomized pregnant (n = 10), and adrenalectomized nonpregnant treated with progesterone (n = 7). Additional pregnant adrenalectomized ewes were studied because of preterm delivery or maternal death in four of these ewes. Because the studies were conducted during the time of year when ewes would be expected to have ovarian cycles, both the adrenal-intact and adrenalectomized nonpregnant ewes were ovariohysterectomized at the time of surgery to eliminate variations in progesterone and estradiol during the experiments. The pregnant and nonpregnant progesterone-treated ewes were not ovariohysterectomized. Bilateral adrenalectomy was performed as previously described (14); pellets containing cortisol hemisuccinate (Innovative Research, Sarasota, FL) were placed subcutaneously at the time of surgery to provide chronic replacement. The number of pellets placed was calculated to produce release of ~0.35 mg·kg⁻¹·day⁻¹ of cortisol in study I.

In study II, adrenalectomized, ovariohysterectomized nonpregnant (n = 6), adrenalectomized pregnant (n = 5), and adrenalectomized nonpregnant treated with progesterone (n = 4) were also studied. In this study, the cortisol hemisuccinate pellets were placed to produce release of ~0.5 mg·kg⁻¹·day⁻¹ of cortisol. In both studies, all of the adrenalectomized ewes were infused with 3 mg·kg⁻¹·min⁻¹ of aldosterone hemisuccinate beginning after the end of surgery and continuing throughout the duration of the study.

Additional cortisol was also infused for the first two postsurgical days in all adrenalectomized ewes; this infusion rate was 2 µg·kg⁻¹·min⁻¹ for the first 15–18 h and 1 µg·kg⁻¹·min⁻¹ for the next 24 h. All adrenalectomized ewes were also infused with 1 liter of 0.9% sodium chloride (Baxter Healthcare) for the first 12–15 h postsurgery through the intravenous catheter. These postoperative replacement doses have been used in our previous studies in adrenalectomized ewes, and this replacement dose of aldosterone normalizes plasma aldosterone concentrations to those of pregnant ewes. In contrast, normal production rate of cortisol in pregnant ewes is estimated to be ~1 mg·kg⁻¹·day⁻¹, whereas normal production of cortisol in nonpregnant ewes is ~0.5 mg·kg⁻¹·day⁻¹. In previous studies in adrenalectomized pregnant and nonpregnant ewes, we have used these replacement doses to produce cortisol concentrations similar to those in intact pregnant and nonpregnant ewes (13, 16). Thus the replacement dose of 0.35 mg·kg⁻¹·day⁻¹ in study I produces concentrations of cortisol that are on average below those of an intact nonpregnant ewe, and the dose of 0.5 mg·kg⁻¹·day⁻¹ in study II produces levels similar to a normal nonpregnant ewe. Although previous studies in our laboratory showed no circadian rhythm in ACTH or cortisol in pregnant or nonpregnant ewes (1), all adrenalectomized ewes are unable to increase plasma cortisol in response to stimuli such as feeding and thus lack the normal pattern of ultradian and stimulus-induced secretion expected in adrenal-intact ewes.

In the progesterone-treated groups, progesterone treatment was begun ~60 days before surgery. Progesterone implants (several of 300 mg each) were placed subcutaneously (Innovative Research); for this procedure, ewes were sedated with ketamine, and a region between the scapulas was anesthetized using intradermal and subcutaneous lidocaine. Progesterone delivery using the implants was ~25 mg/day for the first 30 days; additional implants were then placed to deliver 65 mg/day for the next 30 days. At surgery, additional progesterone implants were placed to achieve a delivery rate of 115 mg/day throughout the time of the experiments, achieving plasma progesterone concentrations similar to those in pregnant ewes.

**Experimental Protocols**

*Study I.* Ewes were studied in three to four experiments each. Adrenal-intact ewes were studied with no infusion of corticosteroids or with infusions of 0.35 and 0.70 µg·kg⁻¹·min⁻¹ of cortisol (equivalent to 0.5 and 1.0 mg·kg⁻¹·day⁻¹; Solucortef; Upjohn; Kalama-zoo, MI) for 24 h. Adrenalectomized ewes were studied with additional infusion of 0.15, 0.5, or 0.85 µg·kg⁻¹·min⁻¹ of cortisol. These infusions are equivalent to 0.2, 0.7, and 1.2 mg·kg⁻¹·day⁻¹ of cortisol, respectively, and thus achieved total daily replacement of ~0.5, 1, or 1.5 µg·kg⁻¹·day⁻¹, similar to the adrenal-intact nonpregnant ewes with no replacement, and infused with 0.5 or 1 mg·kg⁻¹·day⁻¹. In the adrenalectomized ewes, the cortisol was added to the infusion of aldosterone. In the adrenalectomized ewes, the aldosterone infusion volumes were recorded daily; all cortisol infusion experiments were done following a period of at least 24 h in which there were no interruptions of the infusion (i.e., with kinks in the catheter or pump errors). In each experiment, samples for measurement of plasma ACTH, cortisol, progesterone, electrolytes, plasma protein, and packed cell volume were withdrawn at 0, 1, 2, 3, 4, 6, 8, and 24 h after the start of the cortisol infusions; the total volume of blood removed was ~80 ml. Arterial blood pressure was also measured in the ewes using the arterial catheters. Mean arterial pressure was calculated in 1-min intervals for the first 4 h of saline or cortisol infusion and for 1 h at 24 h of infusion.

*Study II.* Ewes were studied in a single experiment in which blood samples were collected as in study I. No additional cortisol infusions were used, but aldosterone was replaced as in study I.

In both studies, blood samples were collected from an arterial catheter without entering the ewes’ pens. Catheters were routed out of the animals’ pen using a tether system that allowed the free movement...
of the ewes in the pen. In study I, the order of experiments was randomized among ewes. Some experiments were repeated because of interruption of the intravenous cortisol or aldosterone infusion. At least 24 h elapsed between the end of one experiment and the start of the next experiment.

As an index of maternal volume status, maternal electrolytes and plasma protein concentrations were also measured in each sample. Plasma protein was determined by refractometry. Plasma sodium and potassium concentrations were determined by ion-specific electrodes (NOVA1; NOVA Biomedical).

Radioimmunoassay

Plasma ACTH was extracted from plasma and measured by radioimmunoassay as previously described (1); the antibody used recognizes all forms of ACTH containing the 17–24 sequence, including proopiomelanocortin (POMC) and the 16-kDa precursor. Plasma POMC concentrations were determined using a commercial kit (Immunodiagnostic Systems) that recognizes POMC and 16-kDa precursor but not 1–39 ACTH. Plasma cortisol was measured after extraction of plasma with ethanol, as previously described (33). Plasma progesterone and aldosterone were measured using a commercial radioimmunoassay (Diagnostic Products); for assay of aldosterone, the kit was modified by addition of two times the sample volume and one-half the volume of radiolabeled aldosterone to increase the sensitivity of the assay to 12.5 pg/ml.

Statistics

Changes in mean arterial pressure, plasma ACTH, and cortisol over time in study I were analyzed by three-way analysis of variance (treatment group, cortisol infusion rate, time; SPSS) corrected for repeated measures. Differences in mean values for the hormones between 4 and 8 h of infusion were analyzed by two-way analysis of variance (SigmaStat). Differences in mean values for ACTH and cortisol among the groups in study II were analyzed by one-way analysis of variance (SigmaStat; Systat Software, San Jose, CA).

Relationships between various measured variables, including plasma progesterone, proteins and electrolytes, and plasma ACTH, were determined using backward regression analysis (SigmaStat).

RESULTS

Study I

Plasma progesterone, cortisol, and ACTH. Plasma progesterone was significantly greater in the adrenal-intact pregnant ewes than the adrenal-intact nonpregnant ewes, and in the adrenalectomized pregnant ewes or adrenalectomized nonpregnant ewes treated with progesterone than in the adrenalectomized nonpregnant ewes (Fig. 1). Plasma cortisol in the adrenal-intact pregnant ewes was significantly greater than in the adrenal-intact nonpregnant ewes (pregnant vs. nonpregnant, Fig. 1) or in ewes in any of the adrenalectomized groups replaced with 0.35 mg cortisol·kg⁻¹·day⁻¹ (adrenalectomized pregnant, adrenalectomized nonpregnant, adrenalectomized nonpregnant with progesterone; Fig. 1).

In the adrenalectomized ewes in which basal cortisol replacement consisted of 0.35 mg·kg⁻¹·day⁻¹, plasma ACTH was significantly and dramatically increased in all groups of adrenalectomized ewes compared with the intact groups (Fig. 1). There was a significant effect of treatment among the groups of adrenalectomized ewes replaced at 0.35 mg·kg⁻¹·day⁻¹. Mean ACTH over the 8 h of basal sampling was significantly greater in the nonpregnant ewes compared with the nonpregnant, progesterone-treated ewes; ACTH in the pregnant adrenalectomized ewes was not statistically different than in either the nonpregnant ewes or in the nonpregnant adrenalectomized ewes treated with progesterone (Fig. 1).

Infusion of cortisol produced cortisol dose-related increases in plasma cortisol in all groups of ewes. Increases in plasma cortisol produced by infusion of cortisol decreased plasma ACTH in all groups of adrenalectomized ewes (Fig. 2), except for the pregnant adrenalectomized ewes infused with 0.2 mg·kg⁻¹·day⁻¹ cortisol. Overall, there was a significant decrease in ACTH over the 24 h of cortisol infusion and an interaction between cortisol infusion dose and time on the circulating ACTH, but no significant effects of group on the changes in ACTH over time (data not shown). Mean ACTH concentrations at 4–24 h of cortisol infusion were greater in the adrenalectomized pregnant ewes than in the adrenalectomized nonpregnant ewes with or without progesterone treatment with the infusion of 0.2 mg·kg⁻¹·day⁻¹ cortisol (i.e., with total replacement of ~0.5 mg·kg⁻¹·day⁻¹), but not with higher rates of cortisol infusion.

Fig. 1. Plasma progesterone, cortisol, and adrenocorticotropic hormone (ACTH) concentrations in adrenal intact ewes (right) and in adrenalectomized ewes replaced with 0.35 mg·kg⁻¹·day⁻¹ cortisol and 3 μg·kg⁻¹·day⁻¹ aldosterone (left). Data are mean values ± SE in samples collected over 8 h. *Values in pregnant ewes (P, black bars) greater than in nonpregnant ewes (NP, open bars). aValues in adrenalectomized pregnant (aP, black bars) or adrenalectomized nonpregnant treated with progesterone (aNP4, gray bars) that were significantly different from in adrenalectomized nonpregnant (aNP, open bars), P < 0.05.

AJP-Endocrinol Metab • VOL 295 • OCTOBER 2008 • www.ajpendo.org
Plasma levels of the ACTH precursors (POMC) were not significantly different between intact pregnant and nonpregnant ewes but were significantly greater in the adrenalectomized pregnant ewes than in adrenalectomized nonpregnant ewes treated with progesterone. The infusion of cortisol for 4 h did not significantly decrease plasma POMC concentrations in any of the groups of ewes (Table 1).

Blood pressure. As expected, maternal arterial pressure was lower in the adrenal-intact pregnant ewes than in the adrenal-intact nonpregnant ewes (Fig. 3). Adrenalectomized pregnant ewes also had lower mean arterial pressure than adrenalectomized nonpregnant ewes. The reduction in cortisol did not significantly decrease mean arterial pressure in either the adrenal-intact pregnant or nonpregnant ewes compared with their respective adrenal-intact control group (Fig. 3). Infusion of higher doses of cortisol over 24 h also did not significantly increase mean arterial pressure in any of the groups (Fig. 3).

However, overall, adrenalectomized ewes treated with progesterone had significantly increased mean arterial pressure relative to the other groups (Fig. 3); mean arterial pressure at 24 h was greater in the adrenalectomized progesterone-treated ewes than in adrenalectomized pregnant ewes without any additional infused cortisol (basal rate of 0.35 mg·kg⁻¹·day⁻¹) and after 0.2 or 0.7 mg·kg⁻¹·day⁻¹ of infusion of cortisol. The mean arterial pressure at 24 h was also significantly different between the two adrenalectomized nonpregnant groups after infusion of 0.7 mg·kg⁻¹·day⁻¹.

Plasma electrolytes, glucose, and plasma protein. Mean plasma sodium, potassium, and protein were not significantly different between intact pregnant and nonpregnant ewes. Analysis of the electrolyte and protein concentrations during infusion of cortisol (Fig. 4, mean of values from hours 4–24) revealed that, although there was no overall effect of cortisol dose on plasma sodium, potassium, or proteins, there were overall group effects in the adrenalectomized ewes on both plasma proteins and sodium. At the basal dose of cortisol, plasma sodium concentrations were greater in the nonpregnant progesterone-treated ewes than in either the pregnant or ovariectomized nonpregnant ewes, whereas plasma protein was lower in the nonpregnant progesterone-treated and pregnant groups than in the nonpregnant ovariectomized groups (Fig. 4, A and B); plasma potassium was not different across the groups, although potassium tended to be decreased in the nonpregnant progesterone-treated ewes (Fig. 4C). During infusion of 0.2 mg·kg⁻¹·day⁻¹ cortisol, the sodium concentrations in the progesterone-treated group were also higher than in the pregnant group.

Table 1. Plasma POMC concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Cortisol Dose, mg·kg⁻¹·day⁻¹</th>
<th>Nonpregnant (n = 5)</th>
<th>Pregnant (n = 5)</th>
<th>Adrenalectomized Nonpregnant (n = 5)</th>
<th>Adrenalectomized Pregnant (n = 7)</th>
<th>Adrenalectomized Nonpregnant + Progesterone (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenalectomized ewes from study I</td>
<td>0.35</td>
<td>83±34</td>
<td>189±67</td>
<td>39±16</td>
<td>39±16</td>
<td>39±16</td>
</tr>
<tr>
<td></td>
<td>0.35±0.22</td>
<td>30±8</td>
<td>79±17</td>
<td>50±31</td>
<td>50±31</td>
<td>50±31</td>
</tr>
<tr>
<td></td>
<td>0.35±0.72</td>
<td>54±16</td>
<td>80±22</td>
<td>40±17</td>
<td>40±17</td>
<td>40±17</td>
</tr>
<tr>
<td></td>
<td>0.85±1.0</td>
<td>55±8</td>
<td>62±11</td>
<td>21±7</td>
<td>21±7</td>
<td>21±7</td>
</tr>
<tr>
<td>Adrenal-intact ewes</td>
<td>0</td>
<td>64±21</td>
<td>82±20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+0.5</td>
<td>50±13</td>
<td>69±28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenalectomized ewes from study II</td>
<td>0.5</td>
<td>35±9</td>
<td>46±11</td>
<td>40±11</td>
<td>40±11</td>
<td>40±11</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE of sample taken at 4 h; n no. of ewes. Units are pM. POMC, proopiomelanocortin.
ACTH was positively related to plasma progesterone (cortisol also did not alter plasma glucose (Table 2). Plasma concentrations remained unchanged by pregnancy or by treatment group. Infusion of cortisol in the adrenalectomized ewes (Fig. 5). Plasma ACTH concentrations were also greater in the pregnant adrenalectomized ewes than in the nonpregnant progesterone-treated adrenalectomized ewes (Fig. 5). Plasma ACTH concentrations were also greater in the pregnant adrenalectomized ewes than in the nonpregnant progesterone-treated adrenalectomized ewes (Fig. 5). As in study I, the lowest ACTH concentrations were measured in the adrenalectomized nonpregnant ewes treated with progesterone. There were no significant differences in plasma cortisol, plasma sodium, potassium, or protein concentrations among these three groups (Table 3). There were also no differences in POMC concentrations among the groups (Table 1). As expected, progesterone was greater in the pregnant and progesterone-treated ewes than in the nonpregnant ovariolectomized ewes (Table 3).

In the ewes replaced with 0.5 mg·kg\(^{-1}\)·day\(^{-1}\) of cortisol, plasma ACTH was not related to plasma progesterone concentration (\(P = 0.134\)). The logarithm of the plasma ACTH concentrations was positively related to plasma potassium concentrations and negatively related to plasma protein and cortisol (\(P < 0.004\); overall \(r = 0.831\)).

Overall, the ACTH concentrations in ewes at both basal cortisol replacement doses of 0.35 and 0.5 mg·kg\(^{-1}\)·day\(^{-1}\), and at 0.35 mg·kg\(^{-1}\)·day\(^{-1}\) during higher-dose cortisol infusions, were exponentially and positively correlated to plasma protein and plasma progesterone, but negatively correlated to plasma cortisol (\(P < 0.001\), \(r = 0.666\)).

**Study II**

To assess the effect of a slightly higher dose of chronic cortisol replacement, as used in previous studies (13), the effect of replacement at 0.5 mg·kg\(^{-1}\)·day\(^{-1}\) was examined. As in the previous study with this dose of cortisol, plasma ACTH concentrations were significantly greater in the adrenalectomized pregnant ewes than in the nonpregnant adrenalectomized ewes (Fig. 5). Plasma ACTH concentrations were also greater in the pregnant adrenalectomized ewes than in the nonpregnant progesterone-treated adrenalectomized ewes (Fig. 5). As in study I, the lowest ACTH concentrations were measured in the adrenalectomized nonpregnant ewes treated with progesterone. There were no significant differences in plasma cortisol, plasma sodium, potassium, or protein concentrations among these three groups (Table 3). There were also no differences in POMC concentrations among the groups (Table 1). As expected, progesterone was greater in the pregnant and progesterone-treated ewes than in the nonpregnant ovariolectomized ewes (Table 3).

Mean plasma glucose concentrations were not significantly changed by pregnancy or by treatment group. Infusion of cortisol also did not alter plasma glucose (Table 2).

**Regression analysis.** In the adrenalectomized ewes, plasma ACTH was positively related to plasma progesterone (\(r = 0.765\), \(P < 0.006\)). However, this relationship was not significant in the adrenalectomized groups (\(P = 0.53\), as indicated by the low ACTH values in the nonpregnant progesterone-treated ewes. Backward regression analysis was used to explore relationships between relevant variables reflecting hormonal influences (cortisol, progesterone) and renal steroid action (sodium, potassium) and whole body volume status (plasma protein) on plasma ACTH. In the animals chronically replaced to 0.35 mg·kg\(^{-1}\)·day\(^{-1}\), the logarithm of the plasma ACTH was found to be related only to plasma proteins; this relationship was positive (\(r = 0.454\); \(P < 0.034\), suggesting that increased ACTH secretion may be secondary to volume constriction, which would increase plasma protein, or conversely that ACTH concentrations are lower in ewes with lower plasma protein concentration, reflecting relatively greater blood volume.

**Table 2. Plasma glucose concentrations**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cortisol Dose, mg·kg(^{-1})·day(^{-1})</th>
<th>Nonpregnant (intact)</th>
<th>Pregnant (intact)</th>
<th>Adrenalectomized Nonpregnant</th>
<th>Adrenalectomized Pregnant</th>
<th>Adrenalectomized Nonpregnant + Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenalectomized ewes from study I</td>
<td>0.35</td>
<td>60 ± 1</td>
<td>55 ± 2</td>
<td>58 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.35 + 0.22</td>
<td>67 ± 7</td>
<td>66 ± 10</td>
<td>66 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.35 + 0.72</td>
<td>70 ± 6</td>
<td>64 ± 6</td>
<td>57 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.85 + 1.0</td>
<td>63 ± 6</td>
<td>67 ± 7</td>
<td>59 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal-intact ewes</td>
<td>0</td>
<td>58 ± 2</td>
<td>66 ± 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ 0.35</td>
<td>66 ± 6</td>
<td>54 ± 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenalectomized ewes from study II</td>
<td>+ 0.7</td>
<td>71 ± 7</td>
<td>76 ± 10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as means over 4–24 h of sampling ± SE. Units are mg/100 ml.
are not the major factor in the reset of ACTH in the pregnant state. In vitro studies indicate that progesterone acts as a mineralocorticoid antagonist (26), binding at MR with affinity similar to that of cortisol or aldosterone, but resulting in inhibition of MR activation. In vivo studies in rats indicated that were more MRs available in hippocampal cytosols from rats treated with progesterone (3); the progesterone-treated rats also have increased ACTH concentrations in response to novelty stress. These changes are both consistent with reduced feedback action of corticosterone in the presence of progesterone. In our laboratory, we found that MR availability in cytosol was increased in pregnancy (25). We have also observed increased MR availability in the hippocampal cytosols in progesterone-treated ewes (unpublished). Despite these suggestions that progesterone could reduce action of corticosteroids at MR in regions such as hippocampus, in this study this effect did not result in increased ACTH concentrations, suggesting that altered hippocampal feedback effects of cortisol are not the primary factor leading to increased ACTH in pregnancy.

In humans and other primates, progesterone also can alter cortisol action by displacing cortisol binding to corticosteroid-binding globulin (CBG); in many primate species, progesterone binds to CBG with similar affinity as cortisol. However, in sheep the inhibitory constant for progesterone displacement of cortisol from CBG is 13-fold less than that of cortisol (17). The binding capacity of ovine plasma for cortisol also does not change in the sheep during pregnancy (32). In the present study, there was no effect of pregnancy or of progesterone treatment on the percentage of free cortisol measured using the ultrafiltration method (7). In intact ewes in study I, free plasma cortisol concentration increased from 0.74 ± 0.61 to 1.21 ± 0.69 ng/ml in pregnant ewes as expected. In the adrenalectomized ewes replaced with 0.35 mg·kg⁻¹·day⁻¹ cortisol, mean free plasma cortisol was 0.49 ± 0.22, 0.49 ± 0.18, and 0.69 ± 0.23 ng/ml in the adrenalectomized nonpregnant, adrenalectomized pregnant, and adrenalectomized nonpregnant progesterone-treated groups, respectively; these differences were not significant. Thus it is not likely that increases in free cortisol in the pregnant or nonpregnant progesterone-treated ewes increase the effective cortisol feedback dose, thereby causing the relatively lower ACTH levels observed in the groups with higher progesterone after adrenalectomy and replacement to 0.35 mg·kg⁻¹·day⁻¹.

The data suggest, in contrast to our original hypothesis, that, under conditions of low circulating cortisol, progesterone is

### Table 3. Concentrations of plasma electrolytes and protein in ewes in study II

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Plasma Sodium, meq/l</th>
<th>Plasma Potassium, meq/l</th>
<th>Plasma Protein, mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>aNP</td>
<td>6</td>
<td>145.7 ± 1.1</td>
<td>3.15 ± 0.33</td>
<td>7.5 ± 0.4</td>
</tr>
<tr>
<td>aP</td>
<td>5</td>
<td>144.4 ± 1.7</td>
<td>4.39 ± 0.23</td>
<td>7.5 ± 0.3</td>
</tr>
<tr>
<td>aNP4</td>
<td>4</td>
<td>146.0 ± 1.8</td>
<td>3.51 ± 0.52</td>
<td>7.5 ± 0.7</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; n, no. of ewes. aNP, adrenalectomized nonpregnant ewes; aP, adrenalectomized pregnant ewes; aNP4 adrenalectomized nonpregnant ewes treated with progesterone. All adrenalectomized ewes in study II were treated with 0.5 mg cortisol·kg⁻¹·day⁻¹ and 3 μg aldosterone·kg⁻¹·day⁻¹.

**DISCUSSION**

The results of this study disproved the hypothesis that increased progesterone treatment would mimic the effects of pregnancy, resulting in greater ACTH concentrations when plasma cortisol concentrations are reduced. The decrease in plasma cortisol with replacement to 0.35 mg·kg⁻¹·day⁻¹ did not result in higher plasma ACTH in the pregnant and progesterone-treated ewes than in the ovariectomized nonpregnant ewes. Thus progesterone of endogenous or exogenous origin is not simply reducing the feedback effects of endogenous cortisol and thereby resetting regulation of cortisol in the pregnant state. ACTH concentrations were greater in pregnant ewes replaced to 0.5 mg·kg⁻¹·day⁻¹ than in nonpregnant ewes, as we had found in previous studies (13, 16), whether the cortisol replacement was chronic over days (as in study I and in the previous studies) or acute over hours (as in study II after the additional infusion of 0.2 mg·kg⁻¹·day⁻¹). As with the replacement to 0.35 mg·kg⁻¹·day⁻¹, plasma ACTH concentrations were lowest in the progesterone-treated ewes with the 0.5 mg·kg⁻¹·day⁻¹ replacement dose. The greater ACTH concentrations in pregnant ewes compared with nonpregnant ewes at these low levels of cortisol are therefore also not attributable to an inhibitory effect of progesterone on cortisol feedback.

The results of these studies suggest therefore that progesterone inhibition of corticosteroid receptor actions at MR
either acting as a corticosteroid receptor agonist, thereby increasing the effective cortisol dose, and/or that progesterone ameliorates some stimulus secondary to low cortisol concentrations. There is evidence supporting both of these possibilities. First, despite evidence that progesterone is an antagonist at MRs (26), progesterone acts as a weak agonist at glucocorticoid receptors (26). In vivo, the weak agonist properties of progesterone result in an inhibition of action of cortisol when cortisol is elevated (15) but an agonist action in terms of delayed feedback inhibition of ACTH when progesterone is high relative to corticosteroid concentrations (10). Second, progesterone can increase or maintain blood volume, thereby reducing stimulation of ACTH in a hypoadrenal state. Glucocorticoids have been implicated in control of both plasma and interstitial volumes (19) and are critical in the restitution of volume after hemorrhage (23). Conversely, hypoadrenocorticism or adrenalectomy is associated with reduced blood volume in humans and reduced ability to restore plasma volume in rodents (5). We have found that infusion of cortisol increases plasma volume in nonpregnant ewes (unpublished observations); progesterone treatment also increases plasma volume in nonpregnant ewes (21), and in nonpregnant women administration of progesterone increases extracellular fluid volume and plasma volume (30). Thus progesterone may attenuate the reduction in blood volume that occurs at low cortisol concentrations, thereby reducing the stimulation of ACTH.

Because changes in the regulation of blood volume have been suggested to drive the chronic expansion of blood volume that occurs in the pregnant state, increased ACTH in pregnancy may in fact be driven by reduced sensitivity to volume underfill (3). The blood volume might be relatively lower in pregnant than in the nonpregnant adrenalectomized ewes. Further studies will be required to test the hypotheses that the increases in ACTH in the adrenalectomized ewes with low plasma cortisol are related to reductions in plasma volume, that progesterone ameliorates the reduction in plasma volume secondary to decreased plasma cortisol, and that relative underfilling of blood volume is a chronic stimulus to ACTH in pregnant ewes.

REFERENCES

26. Rupprecht R, Reul JM, van Steensel B, Spengler D, Soder M, Berning B, Holzboer F, Damk K. Pharmacological and functional characteriza-