ACTH responses to hypotension and feedback inhibition of ACTH increased by chronic progesterone treatment

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Keller-Wood, Maureen. ACTH responses to hypotension and feedback inhibition of ACTH increased by chronic progesterone treatment. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R81–R87, 1998.—During pregnancy, arterial pressure, baroreceptor sensitivity, and adrenocorticotrophic hormone (ACTH) responses to hypotension are decreased. Basal ACTH and cortisol are increased in pregnancy, suggesting a reduction in cortisol feedback inhibition of ACTH. Acute treatment with progesterone decreases arterial pressure, baroreflex-mediated responses, and corticosterone feedback effects on ACTH. These experiments test the hypotheses that chronic increases in progesterone produce changes in arterial pressure, ACTH responses to stress, and feedback inhibition of ACTH similar to pregnancy. Ewes were treated with progesterone for 60–80 days. This increase in plasma progesterone (to 7.6 ± 0.4 ng/ml) did not alter basal ACTH, cortisol, arterial pressure, or heart rate. However, ACTH and AVP responses to hypotension were augmented in progesterone-treated ewes compared with untreated ewes. Chronic progesterone treatment did not decrease the ACTH response to hypotension or attenuate the feedback control of ACTH secretion, these results suggest that the changes in pituitary-adrenal control during pregnancy do not reflect a simple effect of progesterone alone.

PREGNANCY APPEARS TO ALTER the regulation of both adrenocorticotrophic hormone (ACTH) secretion and blood pressure. During pregnancy, mean arterial pressure (MAP) and baroreflex responsiveness are decreased, and ACTH and AVP responses to hypotension are also attenuated (9, 12, 19). ACTH responses to hypoglycemia, on the other hand, are augmented, suggesting that the alteration of hormonal responses to hypotension is specific to this stimulus pathway. It has been suggested (9, 12, 24) that progesterone plays a role in the altered baroreflex responses during pregnancy. Acute treatment of sheep with progesterone results in a rapid decrease in MAP within minutes (24); a decrease in arterial pressure has also been demonstrated after administration of neurosteroid metabolites of progesterone which bind at γ-amino butyric acidA receptors (9). Treatment of ewes with progesterone for 10–14 days decreased MAP and shifted the midportion of the baroreflex response curve to the left, consistent with reset of the regulated resting pressure to a lower value (18). However, this progesterone treatment does not decrease heart rate, ACTH, or AVP responses to hypotension. Other experiments in rabbits have also suggested that progesterone may not cause all of the changes in cardiovascular control that occur in pregnancy. In that species, the change in pressure and baroreflex responses occurs relatively late in pregnancy, whereas progesterone and estrogen are increased early in gestation (19).

Pregnancy results in an increase in plasma cortisol and plasma ACTH concentrations (2, 5, 16, 23). In women, the elevated plasma cortisol is not completely suppressed by standard doses of glucocorticoids, suggesting that negative feedback efficacy is reduced (16). It has been suggested that estrogens or progesterone may alter glucocorticoid feedback inhibition of ACTH or alter stimulus-induced ACTH secretion (16). Consistent with this hypothesis, acute infusion of progesterone with cortisol decreases the feedback effectiveness of cortisol on ACTH secretion (14). However, we have also found that suppression of ACTH by increases in cortisol is normal or increased in ovine pregnancy (13), suggesting that chronic increases in progesterone might not exert an inhibitory effect on glucocorticoid-mediated actions. On the other hand, we also observed an apparent increase in the set-point for basal plasma cortisol in the pregnant state, suggesting that the altered steroid environment of pregnancy may alter feedback control at low basal levels of cortisol.

Because acute treatment with progesterone produced changes in blood pressure, baroreceptor responsiveness, and control of ACTH similar to those observed in studies of pregnant subjects, we have proposed that progesterone may be responsible for these changes. The purpose of this study is to directly test the hypothesis that a sustained increase in plasma progesterone would produce changes similar to pregnancy: reduced MAP, a reduced ACTH response to hypotension, and an increase in basal ACTH and the ACTH response to hypoglycemia, but normal suppression of stimulated ACTH by cortisol.

MATERIALS AND METHODS

Experimental protocols. Twelve adult ewes were studied. Six ewes were studied 60–80 days after subcutaneous placement of implants containing progesterone. The other six ewes were studied without implants. All ewes were studied between February and April, a time when the ewes would normally be entering the anestrus period. However, five of the six untreated ewes showed patterns of progesterone consistent with normal estrous cyclicity. Six experiments were performed in each ewe: infusion of saline, infusion of nitroprusside at 5 and 10 µg·kg⁻¹·min⁻¹, injection of insulin at a dose of 0.10 and 0.25 U/kg, and infusion of cortisol for 2 h followed by infusion of nitroprusside at 10 µg·kg⁻¹·min⁻¹. The ewes were studied in groups of four ewes: two treated and two untreated. The order of the saline, insulin, and nitroprusside back experiment was usually performed as the final experiment.
Progesterone was administered to the six treated ewes via subcutaneous implants. These implants were prepared as previously described (18) using sterilized sheets of silicone polymer (PlasElast; SF Medical, Hudson, MA) folded into packets (50 × 75 mm), which were filled with crystalline progesterone (Steraloids, Wilton, NH). These packets were incubated at 37°C and then were aseptically placed between the scapulae of the ewes. Four implants were inserted into each ewe. During implantation, the ewes were sedated with ketamine (Ketaset, ~5 mg/kg iv; Fort Dodge Laboratories, Fort Dodge, ID) and locally anesthetized with lidocaine (200–400 mg sc; Abbott Laboratories, North Chicago, IL). Ewes were treated with 500 mg of ampicillin intramuscularly at the time of placement of the implants and one time per day for 5 days after placement of the implants. Experiments were performed at least 60 days after placement of the implants.

All ewes were prepared with indwelling femoral arterial and venous catheters using aseptic techniques, as previously described (2). In the treated ewes, catheters were placed ~2 mo after placement of the progesterone implants. Ewes were studied over a 2- to 3-wk period beginning 5–7 days after surgery.

Ewes were housed in pens with controlled lighting and temperature in the Health Center Animal Resources Department. The ewes were allowed access to food, water, and a salt block ad libitum. At least 16 h before the experiments, catheters were threaded through a vinyl duct and were swivel suspended above the pen. This method allows access to the catheters without disturbing the sheep (2).

Body temperatures were monitored in all ewes after catheter placement and throughout this study; all ewes had normal body temperatures (102–104°F) before study. Experiments in which body temperature was elevated at the end of the experiment, presumably due to catheter clotting or contamination, were repeated. Ewes were treated with antibiotics (Polylex, 500–750 mg) two times daily for 5 days after surgery and then after the end of each experiment.

In each experiment, samples were collected for measurement of hormones, electrolytes, and/or glucose. The volume of blood sampled in each experiment was <0.1 ml (3 ml/kg), which does not stimulate hormonal or blood pressure responses (12, 13). During the experiments in which nitroprusside was infused, samples for hormone measurements were collected before the start of the infusion and at 5, 10, 20, 30, 40, 50, and 60 min. Samples for plasma electrolyte concentrations were collected at 10, 30, and 60 min. Nitroprusside (Elkins-Sinn, Cherry Hill, NJ) was delivered at 5 µg·kg·min⁻¹ and 10 µg·kg⁻¹·min⁻¹ in 5% dextrose infused for 10 min at 1.67 ml/min. During experiments in which saline was infused or insulin was injected, samples for hormones and glucose were collected at 10, 20, 30, 40, 50, 60, 70, 80, and 90 min; samples for plasma electrolyte concentrations were collected at 30, 60, and 90 min. Saline infusions were delivered at a rate of 1.67 ml/min. All blood samples for hormone analysis were collected into tubes containing EDTA (0.15 M tetrasodium EDTA; Sigma, St. Louis, MO). Samples collected for electrolyte analysis were placed in heparinized plastic microcentrifuge tubes. All sample tubes were kept on ice until the end of the experiment; they then were centrifuged at 3,000 g for 20 min in a refrigerated centrifuge (Sorval RT6000B, DuPont, Newtown, CT). The plasma for hormone analysis was aliquoted and frozen at ~20°C.

In the experiment to test the feedback effectiveness of increased plasma cortisol, infusion of nitroprusside was preceded by the infusion of cortisol (cortisol hemisuccinate, Solucortef; Upjohn, Kalamazoo, MI) at 1 µg·kg⁻¹·min⁻¹ for 1 h, beginning 2 h before the start of the nitroprusside infusion (~120 to ~60 min). This infusion was delivered at a rate of 0.5 ml/min.

In all experiments, direct arterial pressure was measured using a Statham P23Db transducer (Gould, Oxnard, CA) and a Gould recorder (Gould, Cleveland, OH). Arterial pressure was sampled from the analog output of the recorder at 10 Hz using a Keithley system 570 analog-to-digital converter (Keithley Instruments, Cleveland, OH), Assistant+ software (Ayyst Software Technologies, Rochester, NY), and an AST 286 microcomputer. MAP was averaged for each minute of study. Heart rate was calculated over 30-s intervals at ~2, 3, 5, and 10 min after the start of the infusion of nitroprusside.

Analyses. Plasma sodium and potassium concentrations were measured using a Nova 1 analyzer (Nova, Waltham, MA). Plasma ACTH and arginine vasopressin (AVP) concentrations were measured using antibodies raised in collaboration with Dr. Charles Wood. The antibody against ACTH has 100% cross-reactivity with ACTH-(11–24), ACTH-(1–24), or hACTH-(1–39) but does not cross-react with ACTH-(4–10), Met-enkephalin, vasopressin, oxytocin, or CRF (2). The antibody against AVP is specific for AVP and does not cross-react against oxytocin, vasotocin, or lysine-vasopressin (20). For both assays, the samples were extracted before assay as described (2, 20). Human ACTH-(1–39) was used as the standard in the ACTH assay, and the lower limit of detection was 20 pg/ml. The lower limit of detection in the vasopressin assay was 1.56 pg/ml. Plasma cortisol was measured using antibody raised in our laboratory, as previously described (32); this antibody does not significantly cross-react with progesterone and has a lower limit of detection of 1 ng/ml using 20 µl of plasma. Plasma progesterone was measured using kits (Diagnostic Products, Los Angeles, CA); the limit of detection of this assay is 0.3 ng/ml.

The data were analyzed by two-way analysis of variance corrected for repeated measures to test for interactions between treatment and time on hormone concentrations during hypoglycemia or during hypotension (30). The data were analyzed by three-way analysis of variance to test for interaction among cortisol, treatment, and time on the response to hypotension. Differences among means were compared by Duncan’s multiple-range test. The analysis of hormone data was performed after logarithmic transformation because the raw data were not normally distributed.

The total ACTH response to hypotension was also compared between the groups by Mann-Whitney’s rank sum test. The total ACTH response was calculated by triangulation (calculation of the area under the curve) of the ACTH values from 0 to 60 min. The degree of suppression of ACTH after the cortisol feedback signal was compared between the groups by calculating the percent suppression of the total response; this comparison was also performed using Mann-Whitney’s rank sum test. The relationship between stimulus intensity and ACTH response was also analyzed for each stimulus. The relationship between the logarithm of the nadir in plasma glucose concentration and the logarithm of the peak ACTH concentration after insulin injection and saline infusion were analyzed in each group by linear regression analysis. The relationship between the integrated change in MAP, calculated by triangulation of the 1-min mean values from 0 to 10 min and the ACTH concentration after 10 min of infusion of nitroprusside were also analyzed in each group by linear regression analysis. The slopes of the stimulus-response relationships were compared between the two groups of ewes by t-test. The criterion for significance in all tests was P < 0.05.
RESULTS

Basal values. Plasma progesterone was increased to 7.6 ± 0.4 ng/ml in the progesterone-treated ewes. This value was significantly greater than the average plasma progesterone concentration of 2.3 ± 1.0 ng/ml in the untreated ewes. The values were also greater than those measured in our lab in previous studies of cycling nonpregnant ewes (mean values of 2.5 ng/ml) or anestrous or ovariectomized ewes (0.3 ng/ml) (2). The values were less than those measured in previous studies in pregnant ewes [mean values of 14.5 ng/ml overall, including both singleton and twin pregnancies (2)]. There were no statistically significant differences in the progesterone values among the six experiments in the untreated ewes.

Progesterone treatment did not significantly alter the basal values of MAP, heart rate, plasma glucose, or cortisol concentrations measured during the infusion of saline (Table 1).

Responses to hypotension. Infusion of nitroprusside at 5 or 10 µg·kg⁻¹·min⁻¹ significantly decreased MAP in both groups of ewes. The progesterone treatment did not alter the change in MAP during the infusion of 5 µg·kg⁻¹·min⁻¹ nitroprusside but resulted in a smaller decrease in MAP after the infusion of 10 µg·kg⁻¹·min⁻¹ nitroprusside (Fig. 1). The progesterone treatment did not significantly alter the heart rate response to the infusion of nitroprusside (Table 2).

Although there was no significant main effect of treatment on plasma ACTH concentrations during nitroprusside, ACTH concentrations were significantly greater at 10–30 min after 5 µg·kg⁻¹·min⁻¹ nitroprusside and at 5 and 20–40 min after 10 µg·kg⁻¹·min⁻¹ nitroprusside in the progesterone-treated ewes (Fig. 1). These differences in the ACTH response resulted in a significantly smaller total ACTH response in the progesterone-treated ewes (Table 3). The ACTH response was significantly related to the degree of hypotension in both groups of ewes. The slope of the relationship between MAP and ACTH was significantly increased by progesterone treatment [for untreated ewes, log ACTH(10min) = −2.396 Σ MAP(0–10min) + 1.675, r = 0.75; for progesterone-treated ewes, logACTH(10min) = −4.455 Σ MAP(0–10min) + 1.729, r = 0.81]. This change in slope reflects the greater ACTH response relative to the degree of hypotension in the progesterone-treated ewes.

Plasma AVP concentrations were also measured to determine if the AVP response to hypotension was similarly increased by progesterone treatment. The AVP response to hypotension produced by the infusion

Table 1. Basal values of plasma glucose concentration, MAP, plasma ACTH concentration, and plasma cortisol concentration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose, mg/100 ml</th>
<th>MAP, mmHg</th>
<th>ACTH, pg/ml</th>
<th>Cortisol, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>62 ± 2</td>
<td>89 ± 3</td>
<td>38 ± 6</td>
<td>6.4 ± 1.8</td>
</tr>
<tr>
<td>Progesterone treated</td>
<td>66 ± 4</td>
<td>90 ± 3</td>
<td>42 ± 8</td>
<td>5.3 ± 1.2</td>
</tr>
</tbody>
</table>

Data are means over the 90-min saline infusion ± SE for these data points; n = 6 animals per group. MAP, mean arterial pressure; ACTH, adrenocorticotropic hormone.

Table 2. Heart rate response to infusion of nitroprusside

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Untreated ewes</th>
<th>Progesterone-treated ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart Rate, beats/min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–2</td>
<td>63 ± 6</td>
<td>68 ± 3</td>
</tr>
<tr>
<td>3</td>
<td>129 ± 14</td>
<td>114 ± 5</td>
</tr>
<tr>
<td>5</td>
<td>107 ± 17</td>
<td>94 ± 6</td>
</tr>
<tr>
<td>10</td>
<td>85 ± 12</td>
<td>82 ± 6</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6 animals per group. Time is expressed as minutes relative to infusion (10 µg·kg⁻¹·min⁻¹) of nitroprusside from 0 to 10 minutes.

Fig. 1. Mean arterial pressure (MAP), plasma adrenocorticotropic hormone (ACTH), and plasma arginine vasopressin (AVP) concentrations in progesterone-treated (●) or untreated (○) ewes during and after infusion of nitroprusside at 5 (A) or 10 µg·kg⁻¹·min⁻¹ (B) from 0 to 10 min. Data are means ± SE. *Values in progesterone-treated ewes significantly different from those of untreated ewes by Duncan’s multiple-range test.
Responses to hypoglycemia. The doses of insulin used in this study produced moderate to severe acute hypoglycemia without causing hypotension (Fig. 2). The progesterone treatment did not alter MAP after injection of insulin; this is consistent with the lack of progesterone effect on MAP values measured during saline infusion. Progesterone treatment significantly altered the glucose and ACTH responses over time after insulin injection. Both the glucose and ACTH responses were delayed in the progesterone-treated ewes (Fig. 2), although there was no difference in the total ACTH response after insulin injection (Table 3). This difference in timing of the response can also be taken into account by analyzing the relationship between the nadir in plasma glucose and the resulting peak in plasma ACTH; there was no difference in this relationship between the treated and untreated ewes (for untreated ewes, log ACTH = −1.537(log glucose) + 4.455, r = 0.64; for progesterone-treated ewes, log ACTH = −1.642(log glucose) + 4.572, r = 0.56).

Feedback effect of cortisol. Infusion of cortisol increased plasma cortisol to similar levels in the progesterone-treated and untreated ewes (Table 4). Infusion of cortisol before the induction of hypotension significantly reduced the ACTH response in both groups of ewes (Fig. 3); ACTH concentrations at all time points were significantly reduced after prior infusion of cortisol in both groups of ewes.

Chronic treatment with progesterone did not decrease the feedback effectiveness of cortisol and in fact appeared to increase the degree of suppression of the ACTH response to hypotension. The ACTH concentrations at 10 min were lower in the progesterone-treated ewes after cortisol than in the untreated ewes after cortisol. The total ACTH response over the 60 min was also lower in the progesterone-treated ewes (Table 3). Therefore, the degree of inhibition was significantly greater in the progesterone-treated ewes than in the untreated ewes; ACTH was suppressed by 90.7 ± 2.8% in the progesterone-treated ewes and 72.8 ± 5.5% in the untreated ewes. The greater suppression of ACTH in the progesterone-treated ewes is also reflected in the reduced cortisol response to hypotension in this group; the cortisol response to hypotension was only reduced in the progesterone-treated ewes (Fig. 4).

### DISCUSSION

On the basis of the effects of acute treatment with progesterone, it was predicted that chronic progesterone treatment would reduce blood pressure and ACTH responses to hypotension but augment ACTH responses to hypoglycemia. It was also predicted that chronic progesterone treatment would decrease feed-

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Table 3. Integrated (total) ACTH response during administration of insulin or nitroprusside

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin 0.1 U/kg</th>
<th>Insulin 0.25 U/kg</th>
<th>Nitroprusside 5 µg·kg⁻¹·min⁻¹</th>
<th>Nitroprusside 10 µg·kg⁻¹·min⁻¹</th>
<th>Nitroprusside 10 µg·kg⁻¹·min⁻¹ After cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>3.01 ± 1.26</td>
<td>8.99 ± 2.31</td>
<td>3.16 ± 2.29</td>
<td>9.22 ± 2.38</td>
<td>1.58 ± 0.51</td>
</tr>
<tr>
<td>Progesterone-treated</td>
<td>3.44 ± 3.53</td>
<td>7.45 ± 4.47</td>
<td>9.61 ± 3.74</td>
<td>22.31 ± 15.49*</td>
<td>0.64 ± 0.67*</td>
</tr>
</tbody>
</table>

Values are means ± SE in ng·min·ml⁻¹. *Significantly different from untreated ewes.
treated (side (10 µg·kg

Fig. 3. MAP (9, 24) or over days to weeks (18), chronic regulation of arterial pressure, which are observed suggest that despite the acute effects of progesterone on curve at resting MAPs (18). The results therefore decrease in MAP and a shift in the baroreflex response to hypotension; however, we had found a response to hypoglycemia; the degree of suppression of ACTH produced by infusion of cortisol was also increased. Chronic treatment with progesterone did not change MAP.

Fig. 4. Plasma cortisol concentrations in progesterone-treated (●) or untreated (○) ewes in response to infusion of nitroprusside (10 µg·kg⁻¹·min⁻¹) after no prior infusion of cortisol (A) or after prior infusion of cortisol (B) from −120 to −60 min. Data are means ± SE. *Values in progesterone-treated ewes significantly different from those of untreated ewes by Duncan’s multiple-range test.

Table 4. Plasma cortisol concentrations in response to infusion of cortisol.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Plasma Cortisol Concentration, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated ewes</td>
</tr>
<tr>
<td>−120</td>
<td>9.2 ± 3.1</td>
</tr>
<tr>
<td>−105</td>
<td>25.0 ± 2.9</td>
</tr>
<tr>
<td>−90</td>
<td>23.2 ± 1.0</td>
</tr>
<tr>
<td>−75</td>
<td>26.4 ± 1.7</td>
</tr>
<tr>
<td>−60</td>
<td>25.8 ± 0.9</td>
</tr>
<tr>
<td>−30</td>
<td>8.2 ± 1.4</td>
</tr>
<tr>
<td>0</td>
<td>5.4 ± 1.1</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6 animals per group. Times are expressed relative to onset of nitroprusside infusion at 0 min. Cortisol was infused from −120 to −60 min.

back effectiveness of cortisol, which might increase basal ACTH and cortisol levels. However, the results show a dramatically different pattern of effects with chronic progesterone treatment than had been found with acute progesterone treatment. Chronic treatment with progesterone increased the ACTH and AVP responses to hypotension without altering the ACTH response to hypoglycemia; the degree of suppression of ACTH produced by infusion of cortisol was also increased. Chronic treatment with progesterone did not change basal concentrations of ACTH or cortisol or change MAP.

Chronic progesterone treatment also did not significantly decrease resting MAP, as occurs during pregnancy, and did not appear to alter the heart rate response to hypotension. Therefore, these results suggest that baroreflex responsiveness is not dramatically altered by chronic exposure to increased levels of progesterone. In previous experiments in our lab, we had also failed to find an effect of 10–14 days of progesterone treatment on the heart rate and ACTH response to hypotension; however, we had found a decrease in MAP and a shift in the baroreflex response curve at resting MAPs (18). The results therefore suggest that despite the acute effects of progesterone on regulation of arterial pressure, which are observed rapidly (9, 24) or over days to weeks (18), chronic exposure to elevated plasma progesterone is not the major or sole factor causing alteration of resting pressure or reflex regulation of pressure in pregnancy. However, the results do not exclude the possibility that progesterone may act in concert with changes in other factors or hormones in pregnancy to effect these changes.

Plasma ACTH and cortisol also were not increased in the progesterone-treated ewes. The increase in plasma ACTH during pregnancy is not easily demonstrated and requires careful control of environmental stressors or within subject comparison (2, 5, 23). However, the increase in plasma cortisol with pregnancy is a consistent finding in pregnant ewes and in women (2, 13). The finding that progesterone treatment did not result in an increase in cortisol concentrations suggests that other factors, such as stimulation of the hypothalamic-pituitary axis and adrenal sensitivity by estrogens (3, 4, 26, 28) are more important in the regulation of basal ACTH in pregnancy.

The increase in the ACTH and AVP responses to hypotension with progesterone treatment in these experiments was not expected based on the results of previous studies. A possible explanation of the increased ACTH response could be a reduced effect of cortisol negative feedback inhibition of ACTH by endogenous circulating cortisol. Progesterone is a weak glucocorticoid receptor (GR) agonist and therefore can act as an antagonist to glucocorticoid action (25). However, in these ewes, the degree of suppression of ACTH was increased in the group chronically exposed to increased circulating progesterone. This result suggests that chronic exposure to progesterone does not reduce glucocorticoid action. However, we cannot exclude the possibility that chronic progesterone exposure reduces the effectiveness of the hypotension-induced increase in cortisol in limiting the ACTH response by a fast-feedback mechanism. The results of studies in the rat (22) suggest that estrogens may be necessary for fast feedback but that progesterone may reduce the effectiveness of endogenous corticosteroids as a fast-feedback inhibitor of ACTH. The effect of chronic progesterone on the ACTH response to hypotension in the ewes is most
marked from 20 to 40 min, suggesting that progesterone primarily acts to increase the duration of the response. This could occur if the rapid effect of cortisol was impaired. A difficulty in this interpretation of the data is that although fast feedback has been demonstrated in humans, rats, and dogs, cortisol does not appear to rapidly inhibit ACTH responses in ewes (31). However, it is possible that endogenous progesterone may have been a confounding variable because it was not measured in that study.

The increased ACTH response to hypotension with chronic progesterone treatment is interesting in view of our previous finding that long-term removal of ovarian steroids causes a decrease in the ACTH response to hypotension without altering the ACTH response to hypoglycemia or corticotropin-releasing factor (CRF) infusion, or basal ACTH or blood pressure (17). This effect was observed 4–7 mo postovariectomy but not 2–4 wk after ovariectomy. Similarly, we have found that the ACTH response to hypotension occurs in ewes after 2–3 mo of increased circulating progesterone but not after 10–14 days of treatment (18). This suggests that the decreased response to hypotension in chronically ovariectomized ewes may be the result of a long-term absence of progesterone. The effect of progesterone is therefore likely to be a neurotrophic effect rather than a neurostimulatory effect, which one would expect to be expressed more acutely.

The failure to find decreased effectiveness of delayed glucocorticoid feedback is surprising in view of our previous experiments in which an acute increase in plasma progesterone concentration at the time of the increase in cortisol led to reduced suppression of the ACTH response to hypotension (14). In vivo experiments have also found that progesterone decreases the inhibition of endorphin secretion from the anterior pituitary by cortisol and reduces corticosterone-induced inhibition of stimulated ACTH and CRF secretion by the pituitary and hypothalamus, respectively (1, 8, 10, 11). The fact that a chronic increase in progesterone did not reduce glucocorticoid feedback inhibition of ACTH is not completely surprising; pregnancy also does not result in a decrease in feedback effectiveness of cortisol. In these studies, as in our studies of pregnant ewes (13), we found a slightly increased suppression of ACTH by a delayed cortisol feedback signal. This suggests that despite the long-term exposure of GR to increased progesterone in both cases and the fact that GR has an affinity for progesterone consistent with progesterone binding to GR at physiological concentrations, there is no decrease in GR availability to cortisol. Because other experiments have shown decreased GR availability with more acute progesterone treatment (6), our results suggest that either this change is not sufficient to change glucocorticoid function as a feedback signal or that during chronic treatment GR production might be increased to match the reduction in GR availability.

In summary, chronic treatment with progesterone does not mimic the cardiovascular or endocrine changes of pregnancy. Chronic progesterone treatment does increase the AVP and ACTH responses to hypotension; this effect does not appear to be mediated by a change in baroreflex responsiveness because the heart rate response is not altered. Chronic progesterone treatment also does not alter basal ACTH or cortisol or the response to hypoglycemia, suggesting that the effect of progesterone is not a generalized increase in ACTH secretion. Because both the AVP and ACTH responses are increased and peripheral plasma AVP is known to be a component of the ACTH response to hypotension (21), it is possible that the increased AVP response is a factor in increasing the ACTH response. In our previous studies, less-chronic increases in progesterone increased basal AVP without altering AVP responses to stimuli (18). The site and mechanism of progesterone effects on AVP are not known; however, there are both GR and progesterone receptors in several areas of the hypothalamus responsible for control of AVP (27). Therefore, it is possible that the effect of progesterone on hypotension-stimulated ACTH secretion is the result of progesterone effects on AVP production and/or secretion from the paraventricular nucleus. Progesterone may also alter CRF production and/or secretion.

Perspectives

This study demonstrates the difficulty of explaining the complex changes in ACTH, AVP, and cardiovascular control in pregnancy by extrapolation from known acute effects of treatment with progesterone or other hormones. Progesterone has been shown to have both antimineralocorticoid and antiglucocorticoid effects in various animal models and in vitro systems (1, 7, 10, 11, 25, 29), which could reasonably explain the changes in both blood pressure and ACTH levels in pregnancy. However, chronic treatment with progesterone alone did not produce either of these changes, suggesting that chronic progesterone exposure does not result in production of a new steady state in which these effects of progesterone predominate. This suggests either that progesterone is unimportant as a regulator of these changes in pregnancy or that progesterone requires changes in other factors, stimulated by other mechanisms, for these effects of progesterone to be chronically expressed. There are many other factors that have been implicated in the control of blood pressure in pregnancy, including estrogens (15), prostaglandins, and nitric oxide (7). Estrogens have also been shown to modulate ACTH responses to some stimuli (3, 4, 26, 28).

Understanding the mechanisms of changes in ACTH will require a more thorough understanding of the altered control of blood pressure by these agents as well as a more complete understanding of the interactions between progesterone, cortisol, and estrogens and of regulation of corticosteroid receptors by these hormones and in pregnancy.

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