ACTH responses to CRF and AVP in pregnant and nonpregnant ewes

MAUREEN KELLER-WOOD
Department of Pharmacodynamics, University of Florida
College of Pharmacy, Gainesville, Florida 32610

Keller-Wood, Maureen. ACTH responses to CRF and AVP in pregnant and nonpregnant ewes. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1762–R1768, 1998.—During both ovine and human pregnancy plasma cortisol is increased. In human pregnancy the placenta secretes corticotropin-releasing factor (CRF), but pituitary responses to CRF are decreased. However, in ovine pregnancy there is no measurable placental secretion of CRF. This study tests for changes in pituitary responsiveness to CRF or AVP. Pregnant and nonpregnant ewes were infused with saline or CRF at three doses (3, 9, 45 µg/h), with or without infusion of AVP (9 µg/h). ACTH responses to CRF and AVP were not different between pregnant and nonpregnant ewes. The results suggest that there is no change in pituitary responsiveness to CRF or AVP during ovine pregnancy.

MATERIALS AND METHODS

Experimental design. A total of nine ewes of mixed breeds were studied. Ewes were obtained from a commercial breeder (Tom Morris, Reistertown, MD) or from the herd at the Institute for Food and Agricultural Sciences at the University of Florida. Six time-dated pregnant ewes were studied between days 110 and 144 of gestation (term in these ewes was 148 ± 2 days). A total of nine ewes of mixed breeds were studied between days 110 and 144 of gestation (term in these ewes was 148 ± 2 days). One of these ewes was also studied postpartum (15–35 days after delivery), and three additional nonpregnant ewes were also studied. Eight experiments were performed in each ewe. In three of these experiments, ovine CRF (oCRF) was infused at rates of 3, 9, and 45 µg/h. In three additional experiments, AVP was infused with CRF; the rate of AVP infusion was 9 µg/h. An additional experiment in which CARF was infused at 9 µg/h was performed, and a control experiment in which vehicle alone (saline) was infused was also performed. The order of experiments in both groups was varied among the ewes.

Animal use and care. Bilateral femoral arterial and venous catheters were placed in all ewes. Ewes were anesthetized with halothane (1–2%) in O2. Catheters were filled with sodium heparin (1,000 U/ml; Elkins-Sinn, Cherry Hill, NJ) between uses. All animals were treated with amoxicillin (Polyflex, 500 mg im twice daily; Fort Dodge Animal Health, Fort Dodge, IA) for 5 days postoperatively and on each day in which catheters were used for sampling or were flushed. All ewes were housed in individual pens and provided food and water ad libitum. For each experiment, the catheters were brought out of the pen so that the sampling and infusion could be performed with minimal disturbance of the ewes; the system used for this purpose has been described previously.

ACTIVITY IN THE hypothalamic-pituitary-adrenal axis is altered by pregnancy. In sheep, as well as in women, pregnancy results in an increase in secretion of cortisol (3, 31). There is also a corresponding increase in ACTH when repeated samples are collected under conditions of minimal environmental stimulation (3, 9). In pregnancy, ACTH responses to stimuli are also altered, but are not uniformly increased; the ACTH response to hypoglycemia in ewes is increased (20), whereas the ACTH and AVP responses to hypotension or hypovolemia in ewes and dogs are decreased (5, 20, 21) during pregnancy.

Secretion of ACTH from the pituitary is stimulated by multiple hypothalamic releasing factors. Both the 41-amino acid corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) release ACTH in vivo and in vitro (1, 7, 11, 12, 18, 19, 25, 29, 30, 38). During human pregnancy, CRF levels in plasma are increased due to placental secretion of CRF. The increase in plasma CRF is accompanied by an increase in CRF binding protein in plasma, which reduces the bioactivity of the secreted CRF (28, 29). In pregnant women, the ACTH response to CRF is reduced (41), suggesting that either corticotrope responsiveness is reduced, perhaps due to receptor downregulation, or that the increase in binding protein may reduce the free concentrations available for receptor binding (27, 28). In primates, the ACTH response to CRF is also reduced, although the ACTH response to AVP is increased (15, 16, 41), suggesting that the corticotrope response via the pathway due to AVP binding and protein kinase C activation (29) is not decreased in primate pregnancy.

In sheep, CRF is not secreted from the placenta and there is no measurable circulating binding protein (2, 28). The changes in ACTH and cortisol secretion are, therefore, not related to alterations in peripheral levels of CRF, but are more likely to be related to changes in corticotrope sensitivity to CRF and/or AVP or to changes in hypothalamic secretion of CRF or AVP. It is possible that changes in relative corticotrope responses to these releasing factors could explain both the increase in resting ACTH and the divergent changes in responses to hypoglycemia and hypotension, because AVP and CRF may have different relative importance in the response to these stressors (11, 34, 35). These studies were designed to test whether the effects of CRF and AVP on the corticotrope were altered by pregnancy in ewes.
Before each infusion, aliquots of CRF or AVP (oCRF: Peninsula Laboratories, Belmont, CA; AVP: Sigma, St. Louis, MO) were reconstituted with sterile saline and diluted to a final volume of 50 ml. All infusions were delivered intravenously at a rate of 0.67 ml/min. Arterial samples (8 ml each) were collected from each ewe immediately before the start of the infusion and at 10-min intervals during the infusion. Body temperature was measured in each ewe at the end of each experiment.

Analyses. Blood samples were spun at 3,000 rpm, and aliquots of plasma were frozen at −20°C until assay. Plasma CRF, AVP, ACTH, cortisol, and progesterone were measured by RIA. The progesterone RIA was a commercially available kit (Diagnostic Products, Los Angeles, CA). The cortisol and peptide assays were developed in this laboratory in conjunction with Dr. Charles Wood; these assays have all been previously described (3, 36, 43). Each of the peptide assays uses antibodies with high specificity for the peptide for which the antisera was raised: oCRF, AVP, and human ACTH-(1–39), with no significant (<0.1%) crossreactivity with other neuropeptides. The ACTH antibody does crossreact with the higher molecular weight species containing the 11–24 sequences of human ACTH. All peptides were extracted from plasma; CRF was extracted with acetone, ACTH was extracted with a slurry of borosilicate glass (Corning Glass, Corning, NY) in phosphate buffer, and AVP was extracted with a slurry of bentonite (Sigma). Recovery was corrected by extraction of an aliquot of standard.

Plasma ACTH and cortisol values were analyzed by four-way analysis of variance to test for effects of time, CRF, AVP, and pregnancy. Mean CRF, AVP, and ACTH values from 10 to 60 min were calculated, and the data were analyzed by three-way analyses of variance to test for effects of CRF, AVP, and pregnancy. The ACTH responses were compared both as a nonrepeated analysis (n = 5 or 6 pregnant, n = 4 nonpregnant) and as a repeated analysis (n = 5 pregnant, n = 4 nonpregnant). ACTH, AVP, and CRF values were log-transformed before analysis because of nonhomogeneity of variance. Mean plasma progesterone values were compared between groups of animals by t-test. The criterion for significance in all tests was P < 0.05.

**RESULTS**

Plasma progesterone concentrations were significantly greater in pregnant ewes (pregnant: 15 ± 6, nonpregnant: 1.9 ± 0.7 ng/ml) and did not change over the course of the experiments in the pregnant ewes. However, initial concentrations of AVP, CRF, and ACTH were not significantly different between the groups.

The infusions produced dose-related changes in CRF and AVP (Fig. 1) that were not different between pregnant and nonpregnant ewes. Plasma AVP was increased by the infusion to ~200 pg/ml; plasma CRF was increased over the range of ~50–1,000 pg/ml, spanning the range measured in portal blood (7, 11). In response to infusion of AVP, plasma AVP concentrations were significantly increased by 10 min and were not different between 10 and 60 min. The time course of the change in AVP was also not different between pregnant and nonpregnant ewes.

![Fig. 1. Mean plasma corticotropin-releasing factor (CRF; A) and arginine vasopressin (AVP; B) responses during infusions of saline (0) or CRF (3, 9, 45 µg/h) with or without AVP (9 µg/h) in pregnant and nonpregnant ewes. Data are expressed as means ± SE of values between 10 and 60 min. *Significantly different from saline control; †significantly different from AVP alone; ‡significantly different from the same CRF dose without infusion of AVP; ††significantly different from the same AVP dose and lower CRF doses (n = 4 nonpregnant ewes with all doses; n = 6 pregnant ewes with 0 and 6 µg/h CRF, and n = 5 pregnant ewes with 3 and 45 µg/h CRF).](http://ajpregu.physiology.org/doi/fig/1)
The increase in plasma CRF concentration was logarithmically related to the CRF dose (Fig. 1) and was not different between CRF infusion alone and CRF infusion with AVP. Plasma CRF concentrations were also significantly increased by 10 min after the start of infusion of CRF; although mean plasma CRF levels tended to increase over time throughout the 60 min of infusion, the concentrations reached by 20 min were not significantly different from those at 60 min. The CRF response over time was also not significantly different between pregnant and nonpregnant ewes.

Plasma ACTH concentrations were significantly increased by both CRF and AVP (Figs. 2 and 3). Plasma ACTH concentrations were increased within 10 min...
during all rates of infusion of CRF and/or AVP (Fig. 2). Peak responses to CRF and/or AVP occurred by 20 min; with the highest rate of infusion of CRF these peak levels were not sustained for the 60 min of the infusion (Fig. 2). There was no difference in the time course of the ACTH response between pregnant and nonpregnant ewes.

The mean ACTH response to the infusions (Fig. 3) was not different between the pregnant and nonpregnant ewes. The mean ACTH response was increased by the infusion of AVP at all doses of CRF. The mean plasma ACTH concentrations during the infusions were CRF dose related; although the mean response to 3 and 6 µg/h CRF were not significantly different, the response to 45 µg/h CRF was significantly greater than the response to 6 µg/h (Fig. 3). With all doses of CRF, the ACTH response in the presence of AVP was significantly greater than in the absence of AVP.

Although synergism between CRF and AVP is not obvious when the response is graphed as the logarithm of the ACTH values, there was a statistically significant interaction between CRF and AVP when the data are analyzed using within-animal comparisons. The plasma ACTH response to CRF was augmented by the coinfusion with AVP when within-ewe effects of AVP and CRF on ACTH were analyzed (Fig. 4). Although there was an overall effect of pregnancy in this analysis, the interaction between CRF and AVP was not different between the groups.

Plasma cortisol was increased by both infusion of AVP and CRF; plasma cortisol concentrations were linearly related to the logarithm of the plasma ACTH concentrations produced (Fig. 5). There was no significant interaction between CRF and AVP and no significant differences in the cortisol response to CRF or AVP between the pregnant and nonpregnant ewes.

DISCUSSION

The results demonstrate that pregnancy does not alter pituitary responses to ovine releasing factors, indicating that the changes in ACTH responses to stress are not the result of altered pituitary factors to CRF or CRF during pregnancy.

In these experiments, the dose of AVP was chosen based on previous studies (32) describing ACTH responses to the infusion of AVP and CRF in sheep in vivo. The AVP concentrations that were effective at increasing ACTH secretion were similar to those achieved in these experiments (~250 pM), but are much lower than the EC50 for AVP stimulation of ACTH in vitro (2.3 nM; Ref. 29) or the portal plasma concentrations measured during stresses (1–2 nM; Refs. 7, 11). The plasma CRF concentrations in our study (~12–170 pM), while also
similar to the CRF concentrations reported to be effective in other infusion experiments (30) or measured in portal blood (20–127 pM), are also much lower than the EC_{50} for CRF reported in vitro (9.2 nM; Ref. 29). Our results, therefore, confirm the relatively high in vivo responsiveness to CRF and AVP compared with the in vitro sensitivity of cultured cells, as has been previously noted (39).

Our experiments were not designed to test for a direct effect of AVP on adrenal cortisol production, as has been hypothesized by others (33, 40). In previous studies with human and canine adrenal cells, vasopres- sin stimulated cortisol secretion at levels of AVP similar to those produced by the infusions in this study. If one compares the cortisol response at the highest dose of CRF without AVP to the cortisol response with AVP only (Fig. 5), the cortisol appears to be increased by AVP. However, because the range of plasma ACTH concentrations is greater during infusion of vasopres- sin than with CRF alone, it is not possible using our experimental design to directly test the hypothesis that the response to ACTH is augmented by vasopressin.

One difference between the results of this study and the previous in vivo study of ACTH responses to AVP and CRF is the significant response to AVP alone. A significant ACTH response occurred at a plasma AVP concentration that is in the range measured in sheep in response to hypotension (21). The results suggest that concentrations of AVP in the peripheral circulation, as well as the levels in portal blood, could contribute to ACTH responses during stressors such as hypotension. This is not surprising; in dogs neurohypophysectomy reduced ACTH responses to hypotension, but infusion of AVP to increase plasma AVP concentrations to values in the range that normally occur during hypotension restored the response (37).

In rats, it appears that CRF is the predominate secretagogue; CRF is more potent than AVP (38) and is present in higher concentrations in portal blood than is AVP (34, 35). In the sheep, however, the relative importance of AVP and CRF is less clear. In cultured ovine pituitary cells, AVP is generally found to be both more potent and more efficacious as a stimulator of ACTH (12, 25, 29). In vivo, however, AVP may not be effective without the presence of CRF (18). Portal blood levels of AVP in sheep, while very variable, are usually higher than levels of CRF (7, 11). However, immunization of rams with antiserum to AVP does not reduce basal ACTH secretion (19). On the other hand, CRF immunization reduces basal ACTH and reduces both the number and amplitude of the pulses of ACTH secretion (18). Our experiments were not designed to test the relative sensitivity of the pituitary to AVP compared with CRF within each group of ewes. Our results, along with those of an in vitro study by Kempainen and co-workers (25), do suggest a similar sensitivity to AVP and CRF on a molar basis in the range of basal AVP and AVP concentrations that are stimulated by mild stressors. However, a greater maximum ACTH response appears with CRF alone than with AVP alone. The maximum response to AVP alone occurs at a concentration of AVP (100 pM) that is not likely to occur in vivo in portal blood, but the maximum response in the presence of CRF occurs with AVP concentrations expected during stress (25).

We have found that ACTH responses to stresses are not uniformly increased by pregnancy, despite the increase in basal ACTH that is measured under conditions of environmental isolation (3). In this present study we have found that ACTH and cortisol concentrations were not greater in the pregnant ewes than in the nonpregnant ewes in some instances were significantly lower in the pregnant ewes than in the nonpregnant ewes; these differences occurred with AVP alone without CRF and with the lowest doses of CRF without AVP. We have previously hypothesized that this is the result of a greater response of nonpregnant ewes to environmental stimuli, such as presence of the investigators, because the nonpregnant ewes appear more easily agitated. When we have studied ewes using conditions that isolate the investigator from the animals, we find a small increase in ACTH in pregnant ewes relative to nonpregnant ewes (of ~10 pg/ml) and an approximate doubling of cortisol concentrations in pregnant compared with nonpregnant ewes. The higher ACTH concentrations in the nonpregnant ewes at lower exogenous doses of CRF and AVP in the present study would be consistent with greater endogenous releasing-factor release, possibly stimulated by mild environmental stress.

During pregnancy, ACTH responses to hypotension are decreased, which is consistent with the regulation of blood pressure at lower levels during pregnancy (5, 20, 21). On the other hand, ACTH responses to hypoglycemia are increased (20). The relative importance of AVP and CRF as releasing factors has been suggested to be stimulus-type specific; therefore it was important to test for ACTH responses to AVP and CRF in pregnant and nonpregnant ewes. In both rats and sheep there appears to be a difference in the relative release of AVP and CRF from the hypothalamus, depending on the type of stimulus. Hypoglycemia causes a greater increase in AVP than CRF in rats and causes a markedly greater increase in AVP in sheep (12, 34). During hemorrhage, both AVP and CRF increase dramatically, although the increase in AVP is greater (35). Immunization of rams with antiserum to vasopressin reduces ACTH responses to hypoglycemia; on the other hand, immunization with antiserum to CRF results in complete inhibition of the response to hypoglycemia or restraint and reduces the effect of lysine vasopressin injection (18, 19). The results of the present study indicate that the differential increase or decrease in ACTH and AVP responses to stimuli in pregnancy must reflect differential processing of these stimuli at the level of the brain, rather than changes in pituitary responses.

In this study we did not measure arterial or atrial blood pressure during the infusions of CRF or AVP, although both substances can increase blood pressure (10, 13). In a previous study in dogs, infusion of AVP that increased AVP to ~70 pg/ml increased atrial pressure (4) and decreased unstimulated plasma ACTH by ~30 pg/ml. However, in a previous study in dogs, a
similar infusion rate of CRF did not increase mean arterial pressure (23). Others have also not found changes in mean arterial pressure in baroreceptor-intact animals with increases in AVP similar to those in this study (10). It is possible that the infusion of AVP in these experiments also increased atrial pressure and decreased basal ACTH. However, there is no evidence to suggest that it could decrease the ACTH response of the pituitary to exogenous AVP or CRF; the effect of atrial pressure and arterial pressure on ACTH both are mediated at the brain stem and hypothalamus (14). Therefore, even if the infusion of AVP or CRF increased atrial or arterial pressure, the effect on ACTH could only be to decrease the unstimulated secretion of ACTH. If the magnitude of suppression was similar to that in the study in dogs, we would expect inhibition of 30–60 pg/ml. If this is occurring in the ewes, then the effect would be to decrease the apparent response in the presence of AVP by this amount regardless of the CRF dose. This would not appreciably change the ACTH responses, except in the case of infusion of AVP alone. However, it is possible that the lack of sustained response to AVP and CRF is due in part to increases in arterial or atrial pressure, which would decrease the secretion of ACTH stimulated by endogenous releasing factors.

It is also possible that the effect of increased pressure could be greater in nonpregnant than pregnant ewes, because baroreflex-mediated responses are reduced in pregnancy (5). However, this would lead to the conclusion that sensitivity of the ACTH response is reduced in pregnant ewes relative to nonpregnant ewes with increases in AVP alone. Although it is possible that endogenous CRF and AVP release could be reduced by increases in blood pressure during the infusions, it is unlikely that the suppression of endogenous releasing factors would cause an appreciable decrease in the response at higher doses of infused AVP and CRF.

Perspectives

Our results in sheep differ from those in pregnant women and primates in that ovine pregnancy does not result in decreased pituitary response to CRF and increased response to AVP (15, 17, 41). The results suggest that the decreased pituitary response to CRF in primates is related to the chronic increase in plasma CRF. The decreased ACTH response to circulating CRF in primates appears to be the result of downregulation of the pituitary response to CRF (44) or the result of the binding of CRF to circulating binding proteins (27, 28). The decreased response to CRF does not appear to be the result of an increased feedback effectiveness of increased cortisol, which would be expected to occur in pregnant ewes as well as pregnant monkeys or humans. In fact, other studies have suggested feedback effects of cortisol on basal ACTH are reduced in pregnancy (22).

The lack of an increase in peripheral plasma CRF levels in pregnant sheep is demonstrated in our measurement of CRF in basal samples and during saline infusion. We have previously found that the ovine placenta does not secrete CRF or contain measurable quantities of this peptide (24); others have found that plasma from pregnant sheep does not contain CRF binding protein, because this binding protein is not produced in ovine liver or placenta (2). Our results suggest that the changes in pituitary responses in humans are related to the secretion of CRF and CRF binding protein from the placenta and not to effects of other factors, such as placental steroids, on the pituitary response to hypothalamic stimuli.

Our results also suggest that the increase in basal ACTH and ACTH responses to hypoglycemia are not related to increased pituitary sensitivity to releasing factors, but rather result from a difference in central processing of afferent inputs, release of CRF and/or AVP, or feedback actions of steroids. We have previously found that chronically ovariectomized ewes have reduced responses to stress, but normal ACTH responses to infused CRF and AVP (29). This result suggested that in vivo secretion of CRF and AVP in response to stimuli is altered, but that pituitary responsiveness is unchanged with chronic absence of the ovaries. These differences are not related to the circulating level of gonadal steroids at the time of the experiment, because acute ovariectomy does not alter responses to stimuli. We have hypothesized that this effect may be related to chronic effects of progesterone on the stress response (32).

It has been suggested that pregnancy may alter ACTH concentrations through actions of either progesterone or estrogens on ACTH responses; effects of both estrogen and progesterone on ACTH have been previously demonstrated in rats (6, 8, 25, 42). The results of this study would suggest that, whatever the site of these effects, it is not likely to involve changes in pituitary response to hypothalamic releasing factors.

I acknowledge the technical assistance of Ellen Manlove and Sara Caldwell in the care of animals, performance of the experiments, and assay of peptide hormone concentrations.

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

Address for reprint requests: M. Keller-Wood, Dept. of Pharmacodynamics, Box 100487, Univ. of Florida, Gainesville, FL 32610.

Received 10 November 1997; accepted in final form 9 March 1998.

REFERENCES


R1768 ACTH RESPONSES TO CRF AND AVP IN OVINE PREGNANCY


