Effect of Adrenocorticotropin and Cortisol on Luteinizing Hormone Surge and Estrous Behavior of Cows

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ABSTRACT
In six cows, twice daily administration of 100 IU corticotropin for 3.5 days during the follicular phase delayed the preovulatoy luteinizing hormone surge and onset of behavioral estrus. Corticotropin increased progesterone and decreased estradiol and basal luteinizing hormone concentrations of blood. Following corticotropin withdrawal, a shortened period of behavioral estrus (50% of control) was accompanied by an apparent luteinizing hormone surge and ovulation. To ascertain if these effects were caused by the elevated corticosteroid concentrations induced by corticotropin, we infused intravenously four heifers with cortisol sodium succinate. During this infusion, luteinizing hormone surge and estrous behavior were inhibited; however, estradiol and basal luteinizing hormone concentrations were not affected. Furthermore, there was no luteinizing hormone surge, ovulation, or behavioral estrus after cessation of cortisol treatment. These results are consistent with a role for corticosteroids in mediating inhibitory effects on reproduction produced by corticotropin administration.

INTRODUCTION
There is interest in the role that stress plays in livestock infertility. One approach to studying this problem has been to mimic the pituitary-adrenal response to stress by administration of adrenocorticotropin (ACTH). Administered ACTH during the follicular phase of the estrous cycle interfered with ovulation in both cattle and swine (6, 17, 18, 19, 27). This interference was not seen, however, if exogenous gonadotropins were given along with ACTH (17, 19), suggesting that ACTH does not prevent ovulation by acting at the ovary but rather by inhibiting the preovulatory surge of luteinizing hormone (LH). However, LH concentrations in blood plasma were not measured in these experiments; thus, the hypothesis that ACTH administration inhibits gonadotropin release has not been confirmed directly.

The mechanism by which ACTH disrupts ovulation is unknown. Effects produced by ACTH suggest a role for adrenal glucocorticoids, but experiments using synthetic and natural corticosteroids have yielded conflicting results. In rats, baboons, and cattle, triamcinolone or dexamethasone will either prevent or delay ovulation (1, 2, 6, 22). In contrast, the intramuscular administration of hydrocortisone acetate had no effect on ovulation in cattle and swine (17, 19). Investigators using hydrocortisone acetate may not have mimicked ACTH's action adequately. In a preliminary study, we found that plasma corticosteroid stayed elevated for less than 3 h following intramuscular administration of hydrocortisone, which agrees with results by Short et al. (26) using hydrocortisone acetate. However, as demonstrated in the present study, the dose of ACTH in previous experiments causes an elevation in plasma corticosteroids for more than 9 h.

Whereas workers have demonstrated that ACTH will delay or prevent ovulation, direct confirmation of ACTH's effects on LH release is still lacking. Furthermore, there is conflicting evidence as to whether corticosteroids mediate effects of ACTH. To elucidate these two problems, we studied effects of ACTH and cortisol on the LH surge in cows. In these experiments, cortisol was infused continuously at a rate which allowed concentrations in the
plasma to mimic those with ACTH administration. In so doing, we found inhibitory effects of both ACTH and cortisol on the preovulatory LH surge in cows.

METHODS

Ten virgin Holstein heifers from the University of California Davis dairy herd aged 12 to 20 mo were in these experiments. Animals were housed prior to experiments in a 19 m × 33 m cement corral along with approximately 20 other heifers of similar age. All animals were fed alfalfa cubes and water ad libitum prior to and during the experiments.

General Experimental Procedures

All animals had estrous cycles synchronized prior to each experiment. Synchronization was accomplished by daily intramuscular injection of 50 mg progesterone (50 mg/ml dissolved in corn oil — Sigma Chemical Co.) for 13 days with 6 mg estradiol valerate (Delestrogen, E. R. Squibb, Inc.) given on day 5 of treatment. In pilot experiments this procedure reliably had produced behavioral estrus accompanied by an LH surge within 150 h of the last progesterone injection. The time of this last progesterone injection was designated time zero, and all times refer to that injection.

At +48 h all animals were fitted with indwelling jugular cannulas through which blood samples were drawn. In experiments involving constant infusions, a second cannula was inserted in the contralateral jugular vein. Cannulas were made from silastic tubing (1.0 mm × 2.1 mm, Dow Corning Corp.) and were kept filled with .9% NaCl containing 100 U/ml heparin (Lipo-Hepin, Riker Laboratories). Following cannulation (+51 h) animals were placed in adjacent individual 2.4 m × 6.1 m pens, and one of the treatments described below was initiated. At +72 h blood sampling was begun. Samples were drawn through the jugular cannula into heparinized syringes at +72 h, +81 h, and at 6-h intervals thereafter until signs of estrus were noted. When estrus was observed, the sampling interval was decreased to 3 h, and sampling was continued until estrous behavior ceased or until +150 h. Animals that did not show behavioral estrus were sampled at 6-h intervals until sampling ended at +150 h. All blood samples were centrifuged immediately, and plasma aliquots were stored at −20°C until assayed for LH, progesterone, corticosteroids, and estradiol.

Estrous checks were to estimate timing of the LH surge. These checks were performed by opening gates between two or more of the adjacent pens and allowing animals to interact. During these times, an observer tallied the frequency of the following reproductive behaviors for each animal: mounting, showing the intention to mount (i.e., shifting weight to back feet as if to mount but not doing so), and standing still when mounted. Whereas considerable research has shown that standing when mounted is the only true indication of estrus (14), the first two behaviors also were noted so that if an animal were not mounted during her estrous period and, hence, could not stand to be mounted, we still could have some indication of the time of behavioral estrus. Behavioral observations lasted 10 min and were at least every 12 h. The interval between checks was shortened from 12 to 6 h or even 3 h if an animal appeared to be approaching estrus.

In addition to estrus checks, all animals also were palpated per rectum at 24 to 48-h intervals to determine the approximate time of ovulation.

Experiment 1 — ACTH Treatment

Six animals were in this experiment. All six received both ACTH treatment and control treatment. One hundred international units of ACTH (Adrenomone, Burns Biotec) were injected intramuscularly at time of cannulation (+51 h) and at 12-h intervals until +135 h. Three weeks following the ACTH experiments, each animal was synchronized and used in a control experiment in which no injections were given. During the period of blood sampling, none of the ACTH-treated heifers displayed estrous behavior. For this reason, four of the six animals were observed for estrus twice daily for several days following the normal sampling period (+72 h to +150 h). Upon our observation of estrous behavior, sampling was resumed in three of the animals and continued until estrous behavior was no longer seen.

Experiment 2 — Cortisol Infusion

Six heifers were in this experiment. Four animals were infused with cortisol sodium succinate (Fs) as described above, and two
were infused with the vehicle. All pretreatment procedures were identical to those in Experiment 1 except that both jugular veins were cannulated, one for obtaining blood samples and the other for infusing cortisol or saline. Following cannulation, the animals were moved to individual pens, placed in stanchions, and the infusion was begun. Infusion was continuous for 90 h except for 10-min periods every 12 h when each animal was checked for signs of estrous behavior. At the end of the infusion period (+141 h), heifers were released from their stanchions and blood samples drawn until the sampling period ended 9 h later. Following sampling they were observed regularly for behavioral estrus.

Because cortisol is not soluble in water, its more soluble conjugate cortisol sodium succinate (Solucortef, Upjohn Inc.) was administered by intravenous infusion at an of 1.1 g/min per kilogram body weight by a Harvard Infusion Pump flowing at .1 ml/min. The infusion vehicle was .9% NaCl containing 50 U/ml heparin. In a pilot study, this infusion rate produced corticosteroid in plasma comparable to amounts obtained with ACTH.

Hormone Assays

Luteinizing hormone was assayed according to the radioimmunoassay (RIA) procedure of Geschwind and Dewey (10) with slight modifications in volumes of sample, antisera, and [125I] LH. Anti-LH antibody for the assay was supplied by the late I. Geschwind, and the second antibody was goat antirabbit precipitating antibody purchased from Antibodies Inc., Davis, CA. Luteinizing hormone for iodination was preparation LER--1324--A supplied by L. Reichert, and LH for standards was NIH--LH--B--10 from the National Institutes of Health. Iodination of LH was according to the method of Greenwood et al. (11) with 125I purchased from New England Nuclear. Coefficients of variation (CV) between and within assay for this and all other assays were calculated as described by Rodbard (14). For the LH assay a pool of bovine plasma containing 9.42 ng/ml LH was used. Coefficients of variation between and within assay were 25.7% and 3.2%.

Progesterone was assayed according to the RIA procedure of Orczyk et al. (21). For this assay, the antiprogesterone antiserum was preparation FO developed by L. Edqvist. The progesterone tracer was [1, 2, 3H] progesterone from New England Nuclear, and the progesterone standard was purchased from Sigma Chemical Company. Standards were dissolved in absolute ethanol which had been distilled twice in our laboratory. Plasma samples (100 liters) were placed in a 12 x 75 mm disposable test tube and extracted for 30 s in 2 ml of nanograde petroleum ether (Mallinkrodt) by vortexing. The tubes then were snap frozen, and the organic phase was poured into another tube and evaporated under air at 37°C. Extraction efficiency for this procedure was 80%. Between and within assay CV’s were 8.8% and 7.8% based on a plasma pool containing 5.8 ng/ml progesterone.

Total corticosteroids of plasma were assayed according to the competitive protein binding procedure of Basset and Hinks (3). For this assay, a buffer solution containing .05M borate, .025M sodium hydroxide, and .015M sodium azide (pH 8.8) was substituted for that in the original protocol. Canine corticosteroid binding globulin was used for the binding protein, [1, 2, 3H] cortisol was purchased from New England Nuclear, and cortisol for standards was obtained from Sigma Chemical Co. Between and within assay CV’s were 4.0 and 11.2% as calculated from a plasma pool containing 14.8 ng/ml cortisol.

For the assay of estradiol, .5 ml aliquots of plasma were extracted twice with 1 ml of benzene (Spectral analyzed, 4–111, Fisher Chemical Co.) by shaking on a metabolic shaker for 10 min. After being shaken, samples were centrifuged at room temperature for 2 to 5 min, and the organic phase was pipetted into a 12 x 75 mm disposable test tube and evaporated under nitrogen at 45° to 55° C. Following evaporation all samples were assayed according to the procedure reported by Turgeon (30) except that standards were dissolved in .01 M phosphate buffer rather than methanol. The estradiol antiserum used was preparation TG-K developed by D. Collins. Tritiated estradiol ([2,4,6,7,16,17-3H]–estradiol) was purchased from New England Nuclear, and estradiol for standards was purchased from Sigma Chemical Company. Extraction efficiency was measured for each assay by extracting plasma to which had been added approximately 3000 cpm of
[\textsuperscript{3}H]-estradiol. By this method extraction efficiency was 82%. In addition, tubes containing plasma with 10.0 pg estradiol were included in approximately one-third of the assays. Estradiol recovery from these tubes was 9.8 ± 1.0 pg corrected for extraction efficiency. A plasma blank consisting of steer plasma was included in all assays. This blank had an average estradiol concentration of 3.75 pg/ml which was subtracted from each unknown sample. Samples from a plasma pool having 14.5 pg/ml estradiol were included in each assay. Between and within assay CV’s calculated from this pool were 16.6% and 12.0%.

Statistics

A t-test for related measures and a t-test for independent means were used in Experiments 1 and 2, respectively, to test for significant differences between means of: time to onset of behavioral estrus, length of behavioral estrus, basal LH concentration, corticosteroid concentration, progesterone concentration, and estradiol concentration. The presence or absence of an LH surge was ascertained according to the method of Christian et al. (5).

Mean corticosteroid and progesterone concentrations were calculated for each trial (Experiment 1)\(^2\) or animal (Experiment 2)\(^2\) by first averaging all measures for the trial or

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\caption{Plasma LH and estradiol concentrations of six heifers during ACTH or control trials.}
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animal and then using these averages for statistical analysis. Basal LH concentrations were calculated in a similar manner except that all means that were part of the LH surge were excluded. Plasma estradiol concentration also was calculated in this way except that in experiments where an LH surge occurred, only those estradiol means which preceded the surge were used.

RESULTS

Corticotropin Treatment

Plasma LH and estradiol concentrations in both control and ACTH-treated conditions for the six heifers in this experiment are in Figure 1. From these data and from results of rectal palpation, we concluded that in all six control trials, there was an LH surge and ovulation before +150 h. In contrast, during ACTH treatment only one animal had an LH surge and ovulated (Animal #1732). The surge was extremely small in this case. In the remaining five heifers, LH surges and ovulation were prevented during ACTH treatment.

Basal LH concentration and estradiol concentration in ACTH-treated animals also were depressed during treatment. The average basal LH concentration for ACTH-treated animals was .5 ng/ml as opposed to 1.0 ng/ml in control trials ($P<.05$), and the average estradiol concentrations were 8.2 pg/ml for experimentals and 13.2 pg/ml for controls ($P<.05$).

Reproductive behavior also seemed to be inhibited by ACTH treatment. In control trials, behavioral estrus was observed in all six heifers. The mean time to estrus onset was $+99.5 \pm 4.1$ h. In contrast, none of the six heifers showed estrous behavior during treatment with ACTH. Following ACTH treatment, behavioral observations were continued in four of the animals, and a delayed period of estrus was observed in all four animals at $+192 \pm 6.9$ h. This delayed estrus was shorter than the period of estrus in control trials (25.9 h for controls vs 13.0 h: for ACTH, $P<.05$).

In three of four ACTH-treated animals followed past $+150$ h, blood samples were drawn. The LH assay of these samples indicated that in at least two of the cases an LH surge had occurred between the end of ACTH treatment and resumption of sampling. Rectal palpations indicated that animals #1738 and #1739 ovulated.

In Figure 2, the mean corticosteroid concentrations for all six control trials and the five anovulatory ACTH trials are graphed with time. In addition, the corticosteroid concentration for the sixth ACTH trial in which ovulation did occur is presented individually. The corticosteroid concentration in ACTH treated trials was greater than that of controls at all times except $+87$ h. The overall average corticosteroid concentration was also significantly greater than controls in the ACTH-treated condition (35.6 ng/ml vs. 13.0 ng/ml, $P<.05$). The average cortisol concentration in the ACTH treated animal that ovulated was intermediate to the control and treatment means (24.8 ng/ml) and was appreciably lower than that of ACTH-treated animals between $+72$ h and $+86$ h, which was when this animal's LH surge occurred. The marked fluctuations in plasma corticosteroid concentrations in ACTH-treated heifers reflect the effects of ACTH injections. In each case, the peak plasma corticosteroid concentration was 3 h after ACTH injection.

Progesterone concentration also was affected by ACTH treatment (Figure 3). Average progesterone concentration in ACTH-treated trials was greater than during the control trials (1.4 ng/ml vs .6 ng/ml, $P<.05$). Progesterone in the ACTH-treated heifer which ovulated did not
Figure 3. Mean plasma progesterone concentrations of six heifers during ACTH or control treatment. It appears to differ from that of the other five animals (1.1 ng/ml); thus, data from all six were grouped together in Figure 3.

Cortisol Infusion

Plasma LH and estradiol concentrations for the six animals are in Figure 4. During Fs infusion, the LH surge was prevented in three of the four treated animals, but the average basal plasma LH concentration was elevated (2.2 ng/ml vs. 4.8 ng/ml, P<.05). In contrast, estradiol concentration was unaffected by treatment. The mean presurge concentration of
estradiol in control animals was 13.0 pg/ml, whereas the average estradiol concentration in Fs-infused animals was 12.6 pg/ml. This difference was not statistically significant ($P > .05$). Despite the lack of an effect on estradiol concentration, Fs infusion did produce an inhibition of estrous behavior. In both of the saline infused animals, estrous behavior was observed beginning at $+99.0 \pm 3.0$ h and had a duration of $19.5 \pm 4.5$ h. Among the Fs-infused animals, however, only one (#1738, Figure 4) showed any sign of estrous behavior, and this was only for a few seconds when she stood when mounted at $+114$ h. The remaining three Fs-infused heifers did not show estrus either during Fs treatment or for up to 7 days after treatment. Rectal palpations indicated that none of these three animals ovulated either during or following treatment.

The plasma corticosteroid concentrations for Fs infused and control animals are illustrated in Figure 5. The Fs infusion produced an elevation in plasma corticosteroids similar to that with ACTH. In this experiment, the mean plasma corticosteroid concentration of Fs-infused heifers was $46.7 \pm 1.7$ ng/ml vs. $17.8 \pm 0.8$ ng/ml for controls ($P < .05$). Unlike ACTH treatment, however, Fs infusion produced no elevation in plasma progesterone. As in Figure 6, progesterone concentration remained well below 1 ng/ml with the averages of progesterone for the two groups $0.4 \pm 0.1$ ng/ml for controls and $0.5 \pm 0.1$ ng/ml for Fs-infused heifers ($P > .05$).

**DISCUSSION**

Two major conclusions may be drawn from this study. The first is that ACTH administration to the heifer during the follicular phase blocks preovulatory LH surge. In all control animals, as well as in all seven animals in a related study (28), the synchronization procedure produced an LH surge by $150$ h. During ACTH treatment, however, an LH surge was seen in only one of six cases. This finding confirms the hypothesis suggested by Liptrap and McNally (19) that the disruption of ovulation with ACTH administration (13, 17–19) is from an effect on pituitary gonadotrophin release.

The second conclusion is that adrenal corticosteroids could have mediated ACTH's effects. All ACTH-treated animals had elevated corticosteroid. However, the one animal that ovulated in spite of ACTH treatment had substantially lower corticosteroid than the five that failed to ovulate, and this difference was most pronounced during the first portion of the experiment when the LH surge occurred. An alternative explanation is that ACTH's effects on ovulation may be mediated by progesterone as ACTH causes progesterone secretion by the adrenal cortex (23, 31). In our study, ACTH elevated both corticosteroid and progesterone concentrations; during Fs infusion progesterone concentrations were not elevated above controls. Thus, the inhibitory effects of Fs infusion on ovulation and LH surge could not have been caused by elevated progesterone concentration.

In addition to blocking the LH surge, the two treatments in this study also may have disrupted reproductive behavior. Usually a cow...
displays estrous behavior for approximately 24 h (14), beginning at the same time as the LH surge (15). All control trials reflected this pattern. During both ACTH and Fs treatment, however, estrous behavior was either prevented or attenuated greatly in all cases including the two trials in which ovulation occurred prior to the end of treatment (Experiment 1, #1732, Experiment 2, #1738).

The mechanism or mechanisms by which all of these effects were produced are not certain; however, for the LH surge, at least, available evidence suggests that cortisol feedback at the pituitary could have been involved. Cortisol decreased LHRH-induced LH release in cultured bovine pituitary cells (16). In addition, corticosteroid treatment in both humans (25) and rats (1, 2) or hypercortisolemia due to Cushing’s syndrome in humans (4, 29) also will prevent or reduce the LHRH-induced release of LH. Thus, apparently corticosteroids can inhibit the LH surge by lowering the pituitary’s sensitivity to LHRH.

Effects on estrous behavior are more difficult to interpret. Observations of behavior were intended only to predict the approximate timing of the LH surge. For this reason, the observation periods were shorter than those required for a careful behavioral study, and it may be that the experimental animals showed some estrous behavior which was not seen. However, it was the observer’s subjective impression that ACTH or Fs treated animals showed no indication of behavioral estrus. Thus, the possible blocking effect of corticosteroids on estrous behavior in this study should not be ignored. Furthermore, there is evidence that corticosteroids can disrupt reproductive behavior in the rat (7), gilt (8), and monkey (9). Thus, whereas the behavioral data should be viewed with caution, they also may reflect an important influence of corticosteroids on reproduction. Further research in this area is needed.

All of these results represent ways in which hormones of the pituitary-adrenal axis can disrupt the process of reproduction. The question arises, however, as to what relevance this work has to the study of normal function. One problem with any experiment in which exogenous hormones are administered is that the effects may not reflect physiological conditions. We feel that our findings are of considerable relevance to the study of stress and its effects on reproduction, because corticosteroid concentrations in plasma achieved in our study are similar to those in heifers during acute stress (28). Furthermore, inhibitory effects on ovulation (12), basal LH secretion (20), and estrous behavior (20) also have been reported for animals placed in prolonged stressful situations. Thus, the results that we have reported here do have relevance to physiological situations and apparently represent a model for at least some of the inhibitory effects of stress on reproduction.

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