Effects of adrenalectomy on photoperiod-induced changes in release of luteinizing hormone and prolactin in ovariectomized ewes

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Summary. Finnish Landrace × Southdown ewes were ovariectomized (OVX) and subjected to daily photoperiods of 16L:8D (Group I) or 8L:16D (Group II) for 84 days. Ewes were then either adrenalectomized (ADX) ($N = 5$ for Group I; $N = 4$ for Group II) or sham ADX ($N = 6$ for Groups I + II). After surgery, ewes in Group I were subjected to 8L:16D for 91 days and 16L:8D for 91 days whereas ewes in Group II were exposed to 16L:8D for 91 days and 8L:16D for 91 days. Oestradiol implants were inserted into all ewes on Day 148. Sequential blood samples were taken at 28, 56, 91, 119, 147 and 168 days after surgery to determine secretory profiles of LH and prolactin. Photoperiod did not influence LH release in Group I in the absence of oestradiol. Although photoperiod influenced frequency and amplitude of LH pulses in Group II before oestradiol treatment, adrenalectomy did not prevent these changes in patterns of LH release. However, in Group II the increase in LH pulse amplitude during exposure to long days was greater ($P < 0.01$) in adrenalectomized ewes than in sham-operated ewes. Mean concentrations of LH increased in ADX ewes on Days 91 ($P = 0.07$) and 119 ($P < 0.05$). Adrenalectomy failed to influence photoperiod-induced changes in mean concentrations of LH, amplitude of LH pulses and frequency of LH pulses in the presence of oestradiol. Concentrations of prolactin were influenced by photoperiod. In Groups I and II concentrations of prolactin increased ($P < 0.01$) after adrenalectomy, but the magnitude of this effect decreased over time. These results suggest that the effects of photoperiod on patterns of LH release are mediated in part by a mechanism that is not dependent on sex steroids and that the adrenal may influence release of prolactin.

Keywords: photoperiod; LH; prolactin; adrenalectomy; sheep

Introduction

The effects of photoperiod on reproduction in the ewe may be mediated by two neuroendocrine mechanisms. A sex steroid-dependent mechanism involves photoperiod-induced changes in responsiveness of the hypothalamic–pituitary axis to the negative feedback action of oestradiol (Legan *et al.*, 1977; Legan & Karsch, 1980). During the breeding season oestradiol is a weak inhibitor of luteinizing hormone (LH) pulse amplitude but increases frequency of LH pulses (Goodman & Karsch, 1980; Karsch *et al.*, 1983). In contrast, oestradiol becomes a potent inhibitor of LH pulse frequency during anoestrus (Goodman *et al.*, 1982; Martin *et al.*, 1983). Recent evidence suggests that oestradiol exerts its negative feedback action on secretion of gonadotrophin-releasing hormone in the sheep (Karsch *et al.*, 1987).

Photoperiod also influences patterns of LH release in ovariectomized ewes not treated with oestradiol (sex steroid-independent effects). Ovariectomized ewes subjected to natural short day-lengths had higher LH pulse frequencies and lower LH pulse amplitudes than did ovariectomized

ewes exposed to natural long daylengths (Goodman *et al.*, 1982). Similar results have been reported for ovariectomized ewes exposed to artificial photoperiods (Pau & Jackson, 1985; Schillo *et al.*, 1985).

The existence of a sex steroid-independent effect of photoperiod has not been proven because seasonal fluctuations in patterns of LH release in ovariectomized ewes may be attributed to changes in responsiveness to negative feedback actions of adrenal oestrogens (MacDonald & Siiteri, 1965; Warren & Cheatum, 1967) or androgens (Abraham, 1974) which can be aromatized to form oestrogens in the brain and other tissues (Naftolin & Ryan, 1975). We tested the hypothesis that sex steroid-independent effects are due to feedback actions of adrenal sex steroids by studying the effects of adrenalectomy on photoperiod-induced changes in patterns of LH release in ovariectomized ewes. We also studied the effects of adrenalectomy on photoperiod-induced changes in prolactin release because previous work suggests that the adrenal gland influences prolactin release in rats (Robyn & Tukumbane, 1983; Krieg *et al.*, 1984).

Materials and Methods

Animals. Adult, Finnish Landrace × Southdown ewes were used. All 24 ewes were maintained on pasture before being placed in photochambers where they were fed alfalfa hay, and had access to water and trace mineralized salt.

Treatments. Ewes were assigned randomly to one of two treatment groups (Fig. 1). All ewes were ovariectomized on 12 and 13 September 1985. After surgery, ewes were placed into photochambers. Ewes in Group I were exposed to daily photoperiods of 16 h light:8 h dark (16L:8D; long days) for 84 days, 8L:16D (short days) for 91 days and 16L:8D for 91 days. Ewes in Group II were exposed to 8L:16D for 84 days, 16L:8D for 91 days and 8L:16D for 91 days. Using automatic timers, lights were turned on at 04:00 h and off at 20:00 h during long-day treatments and on at 08:00 h and off at 16:00 h for short-day treatments. Ambient temperature was maintained at 20°C throughout the experiment.

The experiment began after the initial 84-day period. Animals were subjected to bilateral adrenalectomy or sham adrenalectomy during the final 17 days of this period (3–20 December 1985). There were 6 sham-operated ewes in Groups I and II. Numbers of adrenalectomized ewes in Groups I and II were 5 and 4, respectively, because of losses due to surgery.

Fig. 1. Experimental design used to determine the effects of adrenalectomy (ADX) on photoperiod-induced changes in release of luteinizing hormone and prolactin in ovariectomized (OVX) ewes (see text for details).

Blood samples were taken at 10-min intervals for 4 h between 11:30 and 15:30 h (sequential bleeding) at 28, 56, 91, 119 and 147 days to assess patterns of LH release. Serum concentrations of prolactin were measured in samples taken at 60-min intervals during these first 5 sequential bleedings. On Day 148, a Silastic (Dow Corning, Midland, MI, U.S.A.) capsule containing 10 mm packed oestradiol-17 β (1.47 mm i.d. × 1.96 mm o.d.) was implanted subcutaneously in the axillary region under the right front leg of each sheep. This type of implant produced serum concentrations of ~1 pg/ml (Goodman *et al.*, 1982). On Day 168, animals were subjected to an additional sequential bleeding to evaluate the effects of adrenalectomy on LH release in the presence of oestradiol.

The following procedure was used to determine whether adrenalectomies were complete. At the conclusion of the experiment all sheep were injected intramuscularly with 40 U adrenocorticotrophic hormone (ACTH; Butler, Columbus, OH, U.S.A.). Blood samples were taken 15 min before injection, immediately before injection, and at 15, 45, 75, 105, 165 and 195 min after injection to determine serum concentrations of cortisol.

Surgery. Ovariectomies were performed via midventral laparotomy using sodium thiamylal to induce anaesthesia and halothane to maintain anaesthesia. Each ewe was given intramuscular injections containing 60 000 U penicillin and 600 000 U streptomycin (Combiotic, Butler, Columbus, OH, U.S.A.) on the day of surgery, and on each of 5 consecutive days after surgery.

Bilateral adrenalectomies were performed according to the procedure described by Thompson & Wagner (1974). Adrenals were removed by a two-step procedure which required two horizontal incisions, one on each side of the ewe along the transverse processes of the lumbar vertebrae. In sham-operated ewes, adrenals were isolated, then manipulated, but were not removed. All ewes received antibiotic therapy as described for ovariectomies. Adrenalectomized ewes received replacement therapy of 0.2 mg dexamethasone and 2.5 mg 11-deoxycorticosterone acetate intramuscularly each day at ~09:00 h throughout the experiment. Dexamethasone replacement therapy was withdrawn 24 h before each sequential bleeding. Ewes were haltered and tethered 1 h before and during bleeding periods. Blood samples (5 ml) were taken via jugular venepuncture.

Radioimmunoassays. Blood samples were allowed to clot overnight at 4°C and serum was recovered and stored at -20°C until assayed. Serum concentrations of LH were determined by the method of Niswender *et al.* (1969) using ovine LH antiserum (GDN-15) at an initial dilution of 1:50 000 (200 µl). NIADDK-oLH-25 was used for radioiodination and as a standard. Precipitation of bound hormone was accomplished by using goat antirabbit gamma globulin (Biotek Research, Inc., Lenexa, KS, U.S.A.) at an initial dilution of 1:50 (100 µl). Parallelism was demonstrated by showing that estimates of LH concentration were not influenced by volume of serum assayed (25–200 µl). Recoveries of 0.5, 1, 2, 4, 8 and 16 ng LH added to serum were 110%, 101%, 90%, 96.5%, 91% and 97%, respectively. Assay sensitivity was defined as the amount of standard at which binding of labelled hormone was 95% of that in assay tubes not containing standard and averaged 0.025 ng/tube. Intra- and interassay coefficients of variation were 3.4% and 17.7%, respectively.

Serum concentrations of prolactin were determined by the assay described by Pau & Jackson (1984). Ovine prolactin antiserum (273-11) was used at an initial dilution of 1:100 000. NIADDK-oPRL-I-1 was used as a standard and for radioiodination. Goat antirabbit gamma globulin was used at a working dilution of 1:40 (100 µl) to precipitate bound hormone. Estimates of prolactin concentration were not influenced by sample volume (25–200 µl). Recoveries of 0.5, 0.8, 1.0 and 1.5 ng prolactin added to serum were 90%, 102.5%, 96% and 107%, respectively. Assay sensitivity averaged 0.17 ng/tube. Intra- and interassay coefficients of variation were 6.6% and 13.6%, respectively.

Serum concentrations of cortisol were measured by a solid phase radioimmunoassay kit purchased from Diagnostic Products, Inc. (Los Angeles, CA, U.S.A.). Cross-reactivities for deoxycorticosterone and dexamethasone were 1.5% and 0.53%, respectively. The assay was run according to the directions provided by the manufacturer except that the 0.5 ng standard was diluted 1:1 to provide an additional standard (0.25 ng), and 50 µl aliquants of serum were assayed. Estimates of cortisol concentration were not influenced by dilution of sample. Recoveries of 1 and 10 ng cortisol from serum were 118% and 101.5%, respectively. Intra- and interassay coefficients of variation were 7.7% and 5.2%, respectively.

Statistical analyses. Reduction of radioimmunoassay data was performed by the computer program RIA AID (Robert Maciel Associates, Inc., Arlington, MS, U.S.A.) using a Compaq computer interfaced with a Micromedic Microstat gamma counter (Micromedic Systems Inc., Horsham, PA, U.S.A.). A four-parameter logistic curve (Rodbard & Hutt, 1974) was used to describe the relationship between percentage binding of labelled hormone and log of the amount of standard.

Mean concentrations of prolactin were calculated for the first 5 sequential bleedings. Mean concentrations of LH, LH pulse frequency (pulses/4 h) and LH pulse amplitude were calculated for all sequential bleedings. Pulses of LH were identified by a modification of the procedures described by Martin *et al.* (1983) and Schillo *et al.* (1985). Basal concentration of LH was defined as the mean of the three lowest concentrations encountered during each sequential bleeding. An LH pulse was defined as an increase in LH concentrations that consisted of a minimum of two values that exceeded the basal concentration by at least two within-assay standard deviations for LH concentrations comparable to the basal concentration.

Data for the first 5 sequential bleedings were subjected to analysis of variance for repeated measurements (Gill, 1978) to determine effects of group, surgery, bleeding (time) and appropriate interactions. If the group × time interaction was significant, then data from each group were subjected to separate analyses of variance using orthogonal polynomials to describe effects of time. If the surgery × time interaction was significant, then separate analyses of variance were done to determine effects of surgery at each time. If the group × surgery × time interaction was significant, then analyses of variance were done to determine effects of surgery in Groups I and II. In addition, effects of surgery were determined at each time for Groups I and II using *t* tests (Gill, 1978). Data from the last sequential bleeding were subjected to analysis of variance for a 2 × 2 factorial design to determine effects of group, surgery and the group × surgery interaction.

Results

ACTH challenge

Injections of 40 U ACTH stimulated cortisol release in sham-operated ewes, but not in adrenalectomized ewes (Fig. 2). Concentrations of cortisol were similar ($P > 0.1$) for both groups

Fig. 2. Mean ($\pm$ s.e.m.) concentrations of cortisol in ovariectomized ewes that were adrenalectomized (●) or sham-adrenalectomized (○). Arrows designate times of injections of ACTH (40 U; i.m.).

of sham-operated ewes. Serum concentrations of cortisol were undetectable (<2.5 ng/ml) in adrenalectomized ewes both before and after ACTH, indicating that adrenalectomies were complete.

Prolactin

Serum concentrations of prolactin are shown in Fig. 3(a). Analysis of variance revealed a significant ($P < 0.01$) group $\times$ time interaction since the short days:long days sequence caused prolactin to decrease and then increase in Group I (quadratic effect of time; $P < 0.01$), whereas the long days:short days sequence caused prolactin to decrease in a linear manner ($P < 0.01$) in Group II.

Concentrations of prolactin were significantly ($P < 0.01$) higher in adrenalectomized ewes than in sham-operated ewes. A significant ($P < 0.01$) surgery $\times$ time interaction was detected because the effects of adrenalectomy were significant ($P < 0.05$) during the first three sequential bleedings, but not during the last two sequential bleedings ($P > 0.2$).

Luteinizing hormone

Analysis of variance for mean concentrations of LH revealed significant surgery $\times$ time ($P < 0.05$) and group $\times$ time ($P < 0.01$) interactions. Mean concentrations of LH were higher in adrenalectomized ewes than in sham-operated ewes at 91 ($P = 0.07$), and 119 ($P < 0.01$) days (Fig. 3b). The group $\times$ time interaction was due to the fact that mean concentrations of LH increased in a quadratic manner ($P < 0.01$) in Group II whereas the effect of time was not significant ($P > 0.1$) for Group I.

Adrenalectomy did not influence frequency of LH pulses (Fig. 3c). However, analysis of variance revealed a significant ($P < 0.01$) group $\times$ time interaction due to the fact that photoperiod influenced LH pulse frequency in Group II, but not in Group I. Analysis of variance showed that the effects of time on frequency of LH pulses in Group II consisted of linear ($P < 0.01$), quadratic ($P < 0.05$) and cubic ($P < 0.01$) components because frequency of LH pulses decreased after exposure to long days for 56 days and then increased after exposure to short days for 66 days.

Fig. 3. Mean ($\pm$ s.e.m.) concentrations of (a) prolactin and (b) LH, and (c) frequencies and (d) amplitudes of LH pulses in ovariectomized ewes that were adrenalectomized (●) or sham-adrenalectomized (○) and subjected to different photoperiod sequences.

Amplitudes of LH pulses are shown in Fig. 3(d). Analysis of variance revealed significant effects of surgery ($P < 0.05$) as well as group $\times$ time ($P < 0.01$), surgery $\times$ time ($P < 0.01$) and group $\times$ surgery $\times$ time ($P < 0.05$) interactions. Amplitudes of LH pulses were higher ($P < 0.05$) in adrenalectomized ewes than in sham-operated ewes in Group II, but not in Group I ($P > 0.1$). In Group II the effects of adrenalectomy on LH pulse amplitude were significant ($P < 0.05$) on Day 119.

In Group I, there was a small but significant ($P < 0.01$) decrease in LH pulse amplitude after exposure to short days. This effect of time consisted of both linear ($P < 0.01$) and quadratic ($P = 0.05$) trends. The effect of time was also significant ($P < 0.01$) in Group II and consisted of linear ($P < 0.01$), quadratic ($P < 0.01$) and cubic ($P < 0.05$) components since pulse amplitudes increased after exposure to long days for 56 days and then decreased after exposure to short days for 28 days.

Figure 4 shows characteristics of LH release 2 weeks after insertion of oestradiol implants. Adrenalectomy failed to influence mean concentrations of LH, LH pulse amplitude and LH pulse frequency in the presence of oestradiol. Ewes exposed to short days (Group II) had higher

Fig. 4. Effects of daylength on mean ($\pm$ s.e.m.) concentrations of LH, LH pulse amplitudes, and LH pulse frequencies 2 weeks after insertion of oestradiol implants in ovariectomized ewes adrenalectomized (Adx) or sham-adrenalectomized (sham).

($P < 0.01$) mean concentrations of LH and higher ($P < 0.01$) frequencies of LH pulses than ewes exposed to long days (Group I). Amplitudes of LH pulses were similar ($P > 0.1$) for both groups.

Discussion

Exposure of ovariectomized ewes to either natural or artificial long daylengths decreases frequency of LH pulses and increases amplitude of LH pulses, whereas exposure to short daylengths increases frequency of LH pulses and decreases amplitude of LH pulses (Goodman *et al.*, 1982; Pau & Jackson, 1985; Schillo *et al.*, 1985). In the present study, a photoperiod sequence of short days followed by long days (Group I) did not influence LH release, whereas a sequence of long days followed by short days (Group II) influenced frequency and amplitude of LH pulses in ovariectomized ewes not treated with oestradiol.

Adrenalectomy did not abolish photoperiod-induced changes in LH release in Group II, suggesting that a sex steroid-independent mechanism is involved with mediating the effects of photoperiod on LH release in ewes. Our results agree with those of Montgomery *et al.* (1987) who showed that adrenalectomy did not influence patterns of LH release in ovariectomized ewes during the mid-anoestrous season. The existence of a truly steroid-independent mechanism could not be verified in our study because adrenalectomized ewes were given replacement therapy consisting of a mineralocorticoid and a glucocorticoid, steroids which might influence LH release. The results of Montgomery *et al.* (1987) suggest that deoxycorticosterone acetate acts as a progestagen and inhibits LH release in sheep. Photoperiod influenced LH release in ovariectomized, adrenalectomized hamsters not given steroid replacement therapy (Bittman & Goldman, 1979), suggesting that a steroid-independent mechanism exists in this species.

At 28 and 56 days, ewes in Group II had high LH pulse frequencies and low LH pulse amplitudes due to the effects of prior exposure to short days. In these animals 56–91 days of exposure to long days was required to decrease pulse frequency and increase pulse amplitude, whereas 56 days

of exposure to short days was required to increase pulse frequency and decrease pulse amplitude. These lags in response to artificial photoperiods are similar to those reported for ovariectomized ewes given oestradiol (Legan & Karsch, 1980).

Patterns of LH in Group I did not reflect the photoperiod treatments imposed after adrenalectomy. Since ewes in Group I were exposed to natural long days (> 12 h) before entering the photochamber and were subjected to artificial long days before adrenalectomy, it is likely that these sheep became refractory to long days (Robinson *et al.*, 1985) and therefore exhibited short-day patterns of LH release before exposure to artificial short days. This would explain the high LH pulse frequencies observed in these sheep on Day 28. The effects of long-day treatment on LH release in the absence of oestradiol are difficult to assess in Group I because responses were monitored for only 56 days and previous results suggest that approximately 90 days are required for a maximum response (Legan & Karsch, 1980). However, patterns of LH in Group I in the presence of oestradiol (77 days of long-day treatment) indicate that these ewes did respond to the long photoperiod.

In the presence of oestradiol ewes exposed to short day lengths (Group II) had higher mean concentrations of LH and greater frequencies of LH pulses than did ewes exposed to long days (Group I). This is consistent with the observations of Goodman *et al.* (1982) and Martin *et al.* (1983). We did not detect a difference in amplitudes of LH pulses between oestradiol-treated ewes exposed to different daylengths. This disagrees with Goodman *et al.* (1982) who reported higher LH pulse amplitudes in oestradiol-implanted ewes exposed to long days, but agrees with Martin *et al.* (1983) who failed to detect seasonal changes in LH pulse amplitude in ovariectomized ewes treated with oestradiol.

Mean concentrations of LH increased in both groups of adrenalectomized ewes. This effect was due to an increase in LH pulse amplitude in Group II. We feel that these responses were probably not due to adrenalectomy *per se* because they occurred long after adrenal hormones would have been cleared from the circulation.

It is well established that long days increase and short days decrease circulating concentrations of prolactin in sheep (Pelletier, 1973; Pau & Jackson, 1984). Our results in Groups I and II agree with these previous observations. The decrease in prolactin concentrations in Group II during long days was unexpected, but may have been due to random fluctuations in prolactin release. Subsequent exposure of Group II ewes to short days caused a precipitous decrease in prolactin as expected.

Adrenalectomy caused concentrations of prolactin to increase, suggesting that an adrenal product exerts an inhibitory effect on prolactin release. Glucocorticoids have been shown to inhibit prolactin release in rats (Harms *et al.*, 1975; Euker *et al.*, 1975) suggesting that these steroids could mediate adrenal inhibition of prolactin release in sheep. Although adrenalectomized sheep received daily injections of dexamethasone, it is possible that the resultant glucocorticoid activity in serum was too low to inhibit prolactin release. It is also possible that repeated administrations of this chemical caused a subsequent inhibition of prolactin release. This might explain the gradual decrease in the effect of adrenalectomy on prolactin concentrations.

In summary, adrenalectomy did not interfere with the effects of photoperiod on the LH pulse generator, suggesting that a sex steroid-independent mechanism may play a role in mediating the effects of photoperiod on LH release. Adrenalectomy did cause prolactin to increase indicating that the adrenal gland may exert negative feedback actions on release of this pituitary hormone.

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