Prostaglandin F-2α and LH release in immature ewes

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Summary. The effect of PGF-2α on LH release in immature ewes was studied. One intramuscular injection of PGF-2α induced LH release 36–72 h after treatment in 3/4 ewes. The LH variations were not associated with changes in the plasma concentration of progesterone and oestrogens. These results suggest that the LH release in immature ewes is due to PGF-2α acting at central structures.

Introduction

The effect of prostaglandin (PG) F-2α on the release of gonadotrophins has not been studied in immature ewes. Carlson, Barcikowski & McCracken (1973) obtained LH release following PGF-2α treatment in the ewe only during the breeding season. Hafs (1975) showed that PGF-2α releases LH in the cow as a consequence of a fall in progesterone and a central action of PGF-2α in LH release was excluded. Changes in LH would be due to alterations in ovarian hormone secretion induced by PGF-2α rather than to an effect of PGF-2α on the pituitary or hypothalamus. On the other hand, the hypothalamus has been shown to be the principal target of the action of prostaglandins in causing prolactin and growth hormone release (Hafs, 1975).

The aim of the present study was to determine whether treatment with PGF-2α would eventually induce LH release in immature ewes.

Materials and Methods

Animals

Massese ewes, about 8 months old and weighing 25–30 kg (i.e. about 45–55% of mature weight), were used. The experiment was performed in the Bologna area during the months of January and February. The animals were fed ad libitum with a ration of hay, concentrate and sorghum stalks and kept in an open yard with a covered feeding area.

Before treatment blood samples were taken every 48 h for 30 days for measurement of plasma progesterone concentrations and hence to establish which animals were cyclic. Jugular vein blood was collected by venepuncture from each ewe and was centrifuged immediately at 2300 g for 10 min at 4°C. Plasma was removed and stored at −20°C until assay.

Experimental design

The experimental groups each contained 4 ewes. Those in Group A were treated at 08:00 h with a single i.m. injection of a synthetic prostaglandin F-2α (Prostin: Upjohn) at a dose of 170 µg/kg. PGF-2α was in a water solution containing benzyl alcohol. Ewes in Group B received only the vehicle injection.
Blood samples were collected from each animal into heparinized tubes before the injections and then after \( \frac{1}{2}, 1, 3, 6, 12, 24, 36, 48, 60 \) and 72 h. From 72 to 288 h after treatment blood samples were taken at intervals of 24 h.

**Hormone assays**

**Progesterone.** Progesterone was determined by radioimmunoassay as described by Seren, Leopold & Boelli (1974). The antiserum was raised in a rabbit to \( 11\alpha \)-hydroxyprogesterone-hemisuccinate–BSA and was used at a dilution of 1:12 000. Cross-reactions (%) of other steroids were: \( 11\alpha \)-hydroxyprogesterone, 83.3; \( 11\beta \)-hydroxyprogesterone, 15.7; 21-hydroxyprogesterone, 4.0; 17\( \alpha \)-hydroxyprogesterone, 1.7; 20\( \alpha \)-dihydroprogesterone, <0.1. The sensitivity of assay, defined as the mass of hormone required to suppress the binding of the labelled hormone to 90\% of the binding achieved with no hormone added (B/Bo), was 21.9 ± 0.74 (s.e.m.) pg/tube. The recovery of \([1,2,6,7-\text{3H}]\)progesterone was 84.77 ± 1.21\%. The blank value obtained by extracting, under the same plasma conditions, an equal amount of double-distilled water was <0.01 ng/ml.

**Oestrogens.** Oestrogens were determined by RIA as described by Seren et al. (1974). The antiserum was raised in a rabbit to oestradiol-16,17-disuccinate–BSA and used at a dilution of 1:9000. The values reported are regarded as total oestrogen concentrations because this antibody shows the following % cross-reactions: oestradiol-17\( \beta \), 100; oestrone, 92.5; oestradiol, 58.1; oestradiol-17\( \alpha \), 51.5. The sensitivity of the assay was 7.8 ± 0.53 pg/tube for oestradiol-17\( \beta \). The recovery of \([2,4,6,7-\text{3H}]\)oestradiol-17\( \beta \) was 79.29 ± 0.28\%. The average blank value obtained was 7.2 ± 0.53 pg/ml.

**Corticoestrogens.** Cortisol and corticosterone levels were assayed by the protein-binding technique as described by Murphy (1967) and modified by Seren (1973). Blood samples were previously washed with petroleum ether. Cross-reactions were: cortisol, 100\%; corticosterone, 81.6\%; progesterone, 50.0\%. Sensitivity of the assay was 0.35 ± 0.02 ng/tube. The recoveries of \([1,2-\text{3H}]\)cortisol and \([1,2-\text{3H}]\)corticosterone were 92.3 and 93.4\%, respectively. The blank value was <0.01 ng/ml.

**LH.** LH was estimated by a modification of the double-antibody method of Niswender, Reichert, Midgley & Nalbandov (1969). Purified ovine LH (LER-1374 A) was radiiodinated at room temperature. Then 50 µl 0-05 M-PBS, pH 7.5, containing 3 µg purified hormone were added to 1 mCi \( ^{125}\text{I} \) in 10 µl, and 50 µg chloramine-T in 50 µl 0-05 M-PBS were added for 60 sec. The reaction was stopped by adding 100 µg sodium metabisulphite in 100 µl PBS. KI (100 µl of 10 µg/µl) and bovine serum albumin (100 µl of 1%), both in 0-05 M-PBS, were then added. The reaction mixture was placed onto a chromatography column (15 × 1 cm) packed with Bio-Gel P 60 in 0-5 M-PBS and previously slurried with 50 mg BSA in the same buffer. Fractions of 0-5 ml were collected and diluted to a final activity of about 70 000 d.p.m. in 100 µl 0-05 M-PBS containing 1% BSA. The specific activity of the \( ^{125}\text{I} \)-labelled antigen was 120 µCi/µg.

Freeze-dried anti-ovine LH serum (1st antibody) and normal rabbit serum (NRS) were reconstituted at 1:400 and stored frozen at \(-20^\circ\text{C}\). Before assay the anti-ovine LH serum was diluted 1:400 with NRS in 0-04 M-EDTA–PBS at pH 7.0 to give a working solution of 1:40 000. The amounts of anti-rabbit \( \gamma \)-globulin (2nd antibody) were chosen in order to precipitate 3-02 µg rabbit \( \gamma \)-globulin in each tube.

The specific antiovine LH antibody (200 µl) was added to 100 µl whole plasma or plasma diluted with 0-04 M-EDTA–PBS and incubated at 4\°C for 24 h before addition of 100 µl \( ^{125}\text{I} \)-labelled LH containing \( \sim 10 000 \) d.p.m. to each tube, mixing and incubation at 4\°C. After 24 h 200 µl anti-RGG in 0-04 M-EDTA–PBS were added, and after incubation for a further 24 h at 4\°C, 2 ml cold PBS were added to dilute the unbound hormone radioactivity and the antibody-bound hormone was separated from the free hormone by centrifugation at 3200 g for 15 min at 4\°C. The supernatant was decanted and the precipitate counted.
A standard curve was produced with the NIH-LH-S20 preparation, and the LH values were expressed in ng equivalents of this standard. The antibody used showed high sensitivity to ovine LH: cross-reactions to other hypophysial hormones were negligible. Sensitivity was $32.4 \pm 1.52$ pg.

The inter-assay and intra-assay precisions for all hormones were expressed as the coefficient of variation for replicate determinations of a pool of plasma and the values were always <12%.

**Results**

None of the ewes were cyclic. During the pretreatment period the progesterone concentrations were low and did not show any significant variations (mean value 0.06 ng/ml). Plasma LH concentrations in Group A ewes are shown in Text-fig. 1. Three of the 4 ewes treated with PGF-2α had a significant rise in LH levels between 36 and 72 h after injection (peak values 6.8 to 18.5 ng/ml), to about 10 times higher than basal values. Blood corticosteroid levels rose rapidly 30–60 min after treatment (peak values 29.6 to 49.2 ng/ml), but had returned to basal values by 4 h. Plasma progesterone remained steady at pretreatment levels (mean values 0.02 ng/ml). No significant variations in blood oestrogens were observed, their levels ranging from 2.1 to 14.6 pg/ml. There were no significant variations in the blood concentration of the hormones studied in Group B ewes over the entire experimental period.

**Discussion**

Intramuscularly injected PGF-2α induces LH release in immature ewes 36–72 h after treatment. Although an approximately 10-fold increase in blood LH levels with respect to basal values was seen, these values are still very much lower than those recorded for ovulating ewes by

The LH variations in this study were not associated with changes in plasma oestrogen and progesterone concentrations but marked changes were recorded for corticosteroid. It was clear that intramuscularly injected PGF-2α did not induce any variations in ovarian activity, but did exert some effect by activating the hypothalamo–hypophysial–adrenal axis as well as the release of LH from the hypophysis. Carlson et al. (1973) recorded no variations in LH after PGF-2α treatment of adult ewes during seasonal anoestrus, and Chamley & Christie (1973) were unable to show release of LH in ovariectomized ewes treated with PGF-2α.

Changes in blood concentrations of cortisol, LH, prolactin and GH were observed in cows treated with luteolytic doses of PGF-2α (Hafs, 1975) or their analogues (Seren, Tamanini, Bono & Leopold, 1977), and the rise in plasma LH occurred via a fall in progesterone.

In contrast to earlier reports, the present findings have indicated that PGF-2α does have the ability to release LH in immature ewes by a central action. On the other hand, the central action of prostaglandins in releasing GH and prolactin and indirectly also ACTH has been largely demonstrated. This difference is probably due to the fact that in the immature ewes under examination the hypothalamic areas had a different sensitivity threshold to humoral stimuli.

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References


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