The failure of prolactin to enhance the inhibitory effect of oestradiol-17 β on LH synthesis and release in ewes

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Summary. Groups of 5–6 ovariectomized ewes were treated with oestradiol-17 β (100 mg implant) and/or bromocriptine (1 mg; 2 $\times$ daily) or with vehicle for 30 days during the anoestrous season. Oestradiol increased ($P < 0.05$) the priming effect of 2 $\times$ 1 μ g LH-RH on LH release and decreased ($P < 0.001$) pituitary LH content, plasma LH levels, the frequency and amplitude of pulsatile LH release and the LH response to LH-RH. Bromocriptine decreased prolactin levels to <2.5 ng/ml ($P < 0.001$) and increased the LH response to LH-RH ($P < 0.05$). There were no significant treatment interactions. It is concluded that prolactin does not enhance the inhibitory effect of oestradiol on LH synthesis and release in the ewe.

Introduction

Ovarian acyclicity in ewes is associated with elevated plasma prolactin levels and an increase in the inhibitory effect of oestradiol-17 β on LH release. During both seasonal and post-partum anoestrus, when prolactin levels are raised (Walton, McNeilly, McNeilly & Cunningham, 1977; Thimonier, Ravault & Ortavant, 1978; Kann & Martinet, 1975; Kann, Habert, Meusnier & Ryniewicz, 1977), there is evidence of an enhanced inhibitory effect of oestradiol-17 β on LH release (Legan, Karsch & Foster, 1977; Wright, Geytenbeek, Clarke & Findlay, 1981). Treatments that reduce plasma prolactin levels *post partum*, such as udder denervation (Kann & Martinet, 1975), administration of bromocriptine (Kann, Martinet & Schirar, 1978), or early lamb removal (Mauleon & Dautier, 1965; Shevah, Black, Carr & Land, 1974), can hasten the onset of ovarian cyclicity *post partum*. This suggests that high prolactin levels may be causally related to ovarian acyclicity by increasing the inhibitory effect of oestradiol on LH release. We have investigated the relationship between prolactin levels and the inhibition of LH release by oestradiol in ovariectomized ewes during the anoestrous season.

Materials and Methods

Animals and experimental design

The experiment was conducted at the Animal Research Institute, Werribee, Victoria, Australia, during the anoestrous season (November, 1977).

Twenty-three mature Corriedale ewes were ovariectomized; treatment commenced immediately (Day 1) and continued until autopsy (Day 30). Five ewes received s.c. Silastic (Dow Corning, U.S.A.) implants containing oestradiol-17 β (Ikapharm, Israel, 95–105 mg) (Karsch *et*

al., 1973). Six ewes received bromocriptine (Sandoz, Switzerland; 1 mg in 2 ml 60% ethanol—0.154 M-NaCl i.m.) twice daily to reduce plasma prolactin levels (Niswender, 1974). Six ewes received both oestradiol and bromocriptine treatments and 6 control ewes received 2 ml vehicle (60% ethanol in 0.154 M-NaCl i.m.) twice daily. Blood samples were taken every 20 min via jugular vein cannulae on the day before ovariectomy (Day -1) for 6 h and on Day 21 for 12 h. On Day 23, pituitary responsiveness to LH-RH was assessed; the ewes received two i.v. injections of 1 µg LH-RH (Lutal: Hoechst, West Germany) in 2 ml 0.154 M-NaCl, 1 h apart (Stelmasiak & Galloway, 1977). Blood samples (8 ml) were taken via jugular vein cannulae into heparinized syringes at 10-min intervals from 20 min before to 1 h after the second LH-RH injection. All blood samples were centrifuged promptly and the plasma stored at -20°C until assayed for LH and prolactin.

Seven days after assessment of LH-RH responsiveness the ewes were autopsied (Day 30), the pituitaries removed and stored at -20°C. The procedure for extraction of LH from the pituitary glands was similar to that described by Foster *et al.* (1972). Each pituitary was thawed, diced and homogenized in 10 ml 0.1 M-(NH₄)₂SO₄ (pH 5.5) at 4°C. The homogenate was then centrifuged at 17 000 g at 4°C for 30 min. The supernatant was decanted and stored at -20°C until assay. LH determinations on pituitary extracts were made in quadruplicate at dilutions of 1:20 000 and 1:40 000 in assay buffer (0.05 M-phosphate buffer, pH 7.0, containing NaCl (0.15 M), human albumin (0.1% w/v), iodoacetamide (0.05% w/v) and merthiolate (0.01% w/v)).

Hormone assays

LH. Plasma and pituitary LH concentrations were measured by a double-antibody radioimmunoassay (Stelmasiak & Galloway, 1977), using horse anti-bovine LH serum (Snook, 1968), goat anti-horse gamma globulin, NIH-LH-S18 (biopotency 1.02 × NIH-LH-S1) as standard and Papkoff LH (G3222B; biopotency 2.69 × NIH-LH-S1) for iodination by the chloramine T method (Greenwood, Hunter & Glover, 1963). LH concentrations and assay quality control data were calculated using the methods and computer programme of Burger, Lee & Rennie (1972). The study included 3 assays. The mean (± s.e.m.) sensitivity was 0.15 ± 0.02 ng/ml. The within-assay coefficient of variation was <20% over the range 0.53 ± 0.09 to 26.3 ± 2.2 ng/ml. The interassay coefficients of variation were 13% (0.8 ± 0.06 ng/ml), 20% (12.7 ± 1.4 ng/ml) and 16% (22.0 ± 2.1 ng/ml).

Prolactin. Plasma prolactin levels were measured by a double-antibody assay developed in this laboratory. Rabbit anti-ovine prolactin (NIH-P-S8) serum was used at a final dilution of 1:16 000. Iodination of prolactin (LER-860-2; biopotency 30 i.u./mg) was performed using a lactoperoxidase method similar to that of Rogol & Rosen (1974). The assay standard was NIH-P-S8 (biopotency 28 i.u./mg). Horse anti-rabbit serum was used as second antibody at a final dilution of 1:75. Cross reactivities with other ovine protein hormones were <1% for LH (NIH-LH-S18), FSH (NIH-FSH-S6) and TSH (NIH-TSH-S5) and 5% for GH (NIH-GH-S11). Serial dilutions of a pool of ovine plasma gave values (mean ± s.e.m., *n* = 10) of 1.4 ± 0.10, 0.68 ± 0.02, 0.32 ± 0.06 and 0.16 ± 0.03 ng prolactin/tube (*r* > 0.99; *P* < 0.001). Serial dilutions of ewe plasma and of the prolactin standards in assay buffer gave parallel inhibition curves ($y = 41.7 - 25.7x$, $y = 43.7 - 24.7x$, where $y = B/B_0\%$, $x = \log_e$ ng prolactin/tube).

Prolactin levels were determined in the plasma samples taken on Day 21 over the second 6-h period. Samples were diluted 1:10 in assay buffer (0.04 M-phosphate buffer, pH 7.4, containing 0.15 M-NaCl, 0.01 M-EDTA, 0.5% (w/v) BSA and 0.1% (w/v) sodium azide) and assayed in duplicate using 100 µl diluted plasma. All values were determined in the one assay. Prolactin concentrations and assay quality control data were calculated using the methods and computer programme of Burger *et al.* (1972). The assay sensitivity was 2.5 ng/ml and the intra-assay coefficient of variation was <20% over the range 5.3–161 ng/ml and was 8% at 35 ng/ml.

Analysis of results

Treatment effects were assessed by comparison of hormone levels on a within-assay basis only. For each ewe the plasma LH levels for the series of blood samples taken to determine LH release patterns were analysed in terms of mean LH levels, and of the frequency and amplitude of short-term elevations of plasma LH concentrations that reflected pulsatile discharges (pulses) of the hormone from the pituitary gland. An LH pulse was identified when the plasma concentrations in one or a series of samples was at least 1 ng/ml and greater than the level in preceding and/or following samples by a factor of at least 4 times the coefficient of variation of the assay. Pulse amplitude was the maximum plasma LH concentration associated with the pulse minus the concentration preceding the pulse. These definitions are the same as those used by Wright *et al.* (1981) and similar to those of Baird (1978) and Lincoln (1978). The response to the first injection of LH-RH (1 µg i.v.) was determined as the integrated area under the plasma LH concentration against time curve (ng.ml⁻¹.h) derived from blood samples taken each 10 min for 1 h from injection. The response to the second injection of LH-RH was similarly calculated and corrected for the estimated residual response to the first injection. This was done by assuming a half-life of 24 min for the plasma LH present at the time of the second injection (Geschwind & Dewey, 1968; Kaltenbach, Schroff, Klindt & Dunn, 1972) and subtracting its contribution to the concentration–time curve following the second injection. Peak values were noted 10 or 20 min after each injection in all groups. The LH-RH priming effect ratio, a measure of increasing pituitary responsiveness to successive small doses of LH-RH, was the response to the second injection expressed as a ratio of the response to the first injection. Total LH-RH responsiveness was the integrated area under the plasma LH concentration–time curve from the first injection to 1 h after the second. These methods are similar to those of Stelmasiak & Galloway (1977).

The effects of bromocriptine and of oestradiol on pituitary LH content, plasma LH levels, the frequency and amplitude of LH pulse, LH-RH responsiveness and LH-RH priming effect, and plasma prolactin levels were analysed using 2 × 2 analyses of variance. An alteration (increase or decrease) in the inhibitory effects of oestradiol on characters reflecting LH synthesis and release associated with bromocriptine-induced reduction in plasma prolactin levels would manifest itself as a significant interaction effect. When necessary log transformations were performed to render variances compatible.

Results

Before ovariectomy, the overall mean (± s.e.m.) plasma LH level, pulse frequency and amplitude were 2.13 ± 0.23 ng/ml, 1.46 ± 0.23 pulses/6 h and 1.8 ± 0.14 ng/ml. Oestradiol treatment of ovariectomized ewes increased ($P < 0.05$) the LH-RH priming effect ratio and decreased ($P < 0.001$) the pituitary LH content, plasma LH levels, LH-RH responsiveness, LH pulse frequency and amplitude (Tables 1 and 2).

Bromocriptine treatment reduced plasma prolactin levels ($P < 0.001$), and increased LH-RH responsiveness ($P < 0.05$) but had no significant effects ($P > 0.05$) on plasma LH levels, LH pulse frequency or amplitude or on pituitary LH content ($0.05 < P < 0.1$). There were no significant interactions (Table 2).

Discussion

Chronic treatment of ovariectomized ewes with oestradiol during the anoestrous season had a pronounced inhibitory effect on plasma LH levels, a finding similar to that described by Legan *et*

Table 1. The effect of oestradiol-17 β and bromocriptine on pituitary LH and on plasma LH and prolactin in ovariectomized Corriedale ewes during the anoestrous season

	Treatment			
	Control	Oestradiol	Bromocriptine	Oestradiol + bromocriptine
No. of ewes	6	5	6	6
Plasma LH (ng/ml)	5.03 $\pm$ 0.68	0.58 $\pm$ 0.07	6.08 $\pm$ 1.16	0.76 $\pm$ 0.04
No. ewes with LH pulses	6	2	6	3
LH pulse frequency (pulses/12 h)	11.9 $\pm$ 0.7	0.3 $\pm$ 0.2	12.2 $\pm$ 0.9	0.6 $\pm$ 0.3
LH pulse amplitude (ng/ml)	6.2 $\pm$ 0.9	2.8 $\pm$ 0.3	8.7 $\pm$ 2.3	2.7 $\pm$ 0.3
LH-RH responsiveness (ng/ml . h)	34 $\pm$ 5.4	4.7 $\pm$ 0.6	47 $\pm$ 9.2	9.2 $\pm$ 1.9
LH-RH priming effect ratio	1.3 $\pm$ 0.2	1.6 $\pm$ 0.3	1.4 $\pm$ 0.08	1.9 $\pm$ 0.18
Pituitary LH content (mg/gland)	1.51 $\pm$ 0.17	0.26 $\pm$ 0.02	1.71 $\pm$ 0.3	0.39 $\pm$ 0.04
Plasma prolactin (ng/ml)	71 $\pm$ 11	89 $\pm$ 19	<2.5	<2.5

Values are mean $\pm$ s.e.m.**Table 2.** The significance of main effects (oestrogen, bromocriptine) and of the interaction effect derived from 2 $\times$ 2 analyses of variance of plasma prolactin levels and of characters reflecting LH synthesis and release

Character	Oestrogen		Bromocriptine		Interaction	
	F	P	F	P	F	P
Before ovariectomy						
Plasma LH	0.14	NS	0.47	NS	0.31	NS
Pulse frequency	0.52	NS	0.08	NS	0.26	NS
Pulse amplitude	2.7	NS	1.2	NS	0.07	NS
After ovariectomy						
Plasma LH	247	<0.001	2.9	NS	0.3	NS
LH pulse frequency	363	<0.001	2.1	NS	1.9	NS
LH pulse amplitude	24.1	<0.001	0.9	NS	0.6	NS
LH-RH priming effect ratio	4.8	<0.05	1.1	NS	0.5	NS
LH-RH responsiveness	116	<0.001	7.1	<0.05	1.0	NS
Pituitary LH concentration	171	<0.001	4.0	NS	1.3	NS
Plasma prolactin	1.5	NS	1030	<0.001	1.5	NS

NS, not significant, $P > 0.05$.

al. (1977). This study extends their findings by demonstrating associated marked falls in pituitary LH content and in LH-RH responsiveness. Studies of sheep (Coppings & Malven, 1976), rats (Cooper, Fawcett & McCann, 1974) and pigs (Pomerantz, Foxcroft & Nalbandov, 1975) suggest that the inhibitory effect of oestradiol on plasma LH involves a decreased release of LH-RH and decreased pituitary responsiveness to LH-RH. The decreased responsiveness to LH-RH in oestrogen-treated ewes probably reflected a direct effect of oestradiol on pituitary responsiveness to LH-RH and also the reduced pituitary LH content. The small increase in priming effect ratio reflected an action of oestrogen described previously for rats (Aiyer, Sood & Brown-Grant, 1976), women (Lasley, Wang & Yen, 1975) and ewes (Wright, Stelmasiak & Chamley, 1980).

The increased LH-RH responsiveness associated with bromocriptine treatment may have reflected the increased ($P < 0.1$) pituitary LH content. Bromocriptine treatment resulting in increased pituitary LH in rats was considered to be due to a failure of LH release associated with reduced synthesis and release of LH-RH (Sheth & Shah, 1978). However, in this study there was indirect evidence that bromocriptine treatment did not alter LH-RH release since there were

no alterations in plasma LH levels, pulse frequencies or amplitudes. The administration of prolactin to lactating monkeys (Maneckjee, Srinath & Moudgal, 1976) and rats (Muralidhar, Maneckjee & Moudgal, 1977) decreased pituitary LH-RH responsiveness. However, it is unlikely that the increased pituitary LH-RH responsiveness in bromocriptine-treated ewes was due to a removal of an inhibitory effect of prolactin since alterations in prolactin levels in lactating ewes by treatment with bromocriptine (Kann *et al.*, 1978) or ergocornine (Louw *et al.*, 1976) and in ovariectomized ewes by prolactin infusions (Chamley, 1978) did not affect LH-RH responsiveness. Short-term treatment of ovariectomized cows with prolactin failed to influence the pattern of release of LH (Forrest, Fleege, Long, Sorenson & Harins, 1980), suggesting that in this species also alterations in plasma prolactin levels were not associated with alterations in LH-RH release or in pituitary LH-RH responsiveness. An understanding of the basis of any changes occurring in studies of bromocriptine-treated ewes is complicated by the confounding of the effects of bromocriptine and of lowered prolactin levels. Interpretation is further complicated because bromocriptine is a dopamine agonist (Fuxe *et al.*, 1978); prolactin, in rats at least, increases dopamine turnover (Hökfelt & Fuxe, 1972) and the effect of dopamine on LH release is unclear (Jackson, 1977). Bromocriptine did not, however, alter plasma LH levels in this study or in one with cyclic ewes (Niswender, 1974).

This study produced no evidence that the inhibitory effects of oestradiol on plasma LH levels, pituitary LH content and LH-RH responsiveness were enhanced by the high plasma prolactin levels associated with the anoestrous season in sheep. This suggests that there is no causal relationship between elevated plasma prolactin levels and the increased inhibitory effect of oestradiol on plasma LH levels described for ewes in both seasonal (Legan *et al.*, 1977) and post-partum (Wright *et al.*, 1981) anoestrus.

We thank Anne Bartlett and Bev Lando for skilled technical assistance; Dr H. Papkoff and NIH for hormone standards; Dr I. A. Cumming and the Victorian Department of Agriculture for generous co-operation; and Lynn Magill and Jill Volfsbergs for typing. These studies were supported by grants from the Australian Meat Research Committee, the Australian Wool Research Trust Funds and the National Health and Medical Research Council of Australia.

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Received 17 November 1980