Relationship between pituitary nuclear oestrogen receptors and the release of LH, FSH and prolactin in the ewe

I. J. Clarke, J.W. Funder and J. K. Findlay

Medical Research Centre, Prince Henry's Hospital, St Kilda Road, Melbourne, Victoria 3004, Australia

Summary. Ovariectomized ewes were given a single injection (i.v.) of 100 µg oestradiol-17β. Nuclear oestrogen receptor values in the pituitary, as a function of total receptor concentrations, were 3.0 ± 1.0% in controls, 56.0 ± 5.4% at 1 h (P < 0.001) and 6.5 ± 2.1% at 6 h (P > 0.05) after oestradiol injection. There was a fall in plasma LH values from 5.7 ± 1.0 (preinjection) to 2.1 ± 0.3 ng/ml (P < 0.01) 4–6 h after oestradiol. At 13–21 h after injection plasma levels increased to 37 ± 8 ng/ml (P < 0.001). Plasma FSH levels declined from 840 ± 18 to 506 ± 48 ng/ml after 20–22 h (P < 0.001). Plasma prolactin concentrations fell from 90 ± 16 ng/ml before injection to 29 ± 9 ng/ml at 1 h (P > 0.05), and then rose to a maximum of 1537 ± 24 ng/ml (P < 0.001) 11–14 h later. These results show that transient nuclear compartmentalization of oestrogen receptors after a bolus injection of oestradiol was associated with the feedback effects of oestradiol on LH, FSH and prolactin release.

Introduction

Oestradiol-17β can exert feedback effects on gonadotrophins by actions at the hypothalamic and pituitary levels (Knobil, 1973; Brown-Grant, 1976; Fink, 1979). Although specific oestrogen receptors have been identified in the neuroendocrine tissues of a number of species (Morrell, Kelley & Pfaff, 1975; Kato, 1978), including the sheep (Wise, Payne, Karsch & Jaffe, 1975; Tang & Adams, 1978; Clarke, Burman, Funder & Findlay, 1981), the involvement of these receptors in the feedback actions of oestrogen on gonadotrophin secretion is unclear.

Single i.m. and i.v. injections of oestradiol to intact and ovariectomized monkeys respectively, resulting in transitory elevations in plasma oestradiol, fail to elicit a surge in LH secretion (Yamaji et al., 1971; Yamaji, Dierschke, Bhattacharya & Knobil, 1972) although there was a decrease in plasma LH levels in ovariectomized monkeys (Yamaji et al., 1972). When prolonged elevations of plasma oestradiol values in monkeys are achieved, by the injection of oestradiol benzoate or the use of s.c. implants, plasma LH and FSH concentrations show an initial fall and then a marked elevation (Yamaji et al., 1971; Karsch et al., 1973; Attardi, Hotchkiss & Knobil, 1980). In contrast to the effects of oestradiol in monkeys a single i.v. injection of oestradiol will cause a biphasic alteration in plasma LH levels in ovariectomized ewes (Findlay et al., 1973).

Measurement of oestrogen receptor concentrations in pituitary tissue of ovariectomized monkeys given s.c. oestrogen implants showed prolonged nuclear uptake of receptors and a biphasic pattern of LH and FSH release. If sheep, which exhibit biphasic LH responses after a
single i.v. injection of oestradiol, also showed prolonged retention of nuclear oestrogen receptor complexes, the difference between sheep and monkeys could involve retention time of the receptors in pituitary nuclear acceptor sites. However, if transient nuclear occupancy in the sheep is followed by delayed effects on gonadotrophin secretion, the difference between species would appear more likely to involve post-nuclear protein synthetic events. To distinguish between these possibilities, pituitary cytosol and nuclear oestrogen receptor levels were measured in ovariectomized ewes after a single injection of oestradiol and receptor occupancy was related to changes in plasma LH, FSH and prolactin concentrations.

### Materials and Methods

**Hormones and reagents**

Tritiated oestradiol (sp. act. 4.07 TBq/mmoll) was purchased from New England Nuclear, Boston, Massachusetts, U.S.A., and purified by chromatography on a 10 × 0.5 cm LH-20 Sephadex column developed in chloroform:benzene:methanol (30:60:10 by vol.). Non-radioactive oestradiol and diethylstilboestrol were obtained from Steraloids, Wilton, New Hampshire, U.S.A. All laboratory chemicals were Analar grade.

**Animals and treatment**

Merino ewes were selected from a single flock and ovariectomized at least 6 months before the study. All experiments were performed during the mating season. The ewes were housed in individual pens. Blood samples were withdrawn from a jugular vein cannulated with polyethylene tubing (PE 205; Intramedic) on the day before experimentation. Cannulae were kept patent with 0.154 M-NaCl containing 125 i.u. heparin/ml. Blood samples were collected into heparinized syringes, centrifuged immediately at 4°C and plasma was stored at −20°C until assay. The ewes were randomly allocated to three groups.

**Group 1.** Three ewes were killed at 1, 3, 6 or 24 h after an i.v. injection of 100 μg oestradiol in 1 ml ethanol. Three other ewes received 1 ml ethanol and served as controls. Within 5 min of exsanguination, pituitaries were removed into ice-cold buffer for receptor analysis (see below).

**Group 2.** Three ewes received 100 μg oestradiol (as above) and 10 ml blood samples were taken from the contralateral jugular at 0, 2.5, 5, 10, 15, 30, 60 and 180 min after injection to measure plasma oestradiol concentrations.

**Group 3.** Four ewes received 100 μg oestradiol via the indwelling jugular cannula and blood samples were taken at hourly intervals from 2 h before the injections until 22 h afterwards. Plasma LH, FSH and prolactin concentrations were measured.

**Oestrogen receptor assays**

Pituitaries were collected into 0.01 M-phosphate buffer containing 0.25 M-sucrose and 1 mM-mercaptoethanol (pH 7.3) at 4°C and homogenized in 5 volumes of the same buffer. The homogenate was centrifuged at 800 g at 4°C for 10 min, and the supernatant re-centrifuged at 105 000 g at 4°C to yield cytosol that was assayed for receptor sites by saturation analysis (Clarke et al., 1981). Nuclear oestrogen receptors were measured in the 800 g pellet according to the method of Roy & McEwen (1977), modified for sheep tissues (Clarke et al., 1981). Aliquots of nuclear pellets and crude homogenates were assayed for DNA (Burton, 1956). After correction for preparative losses in the nuclear assay, the cytosolic and nuclear oestrogen receptor values were expressed per g wet weight.
Hormone assays

**LH and FSH.** Plasma LH values were measured in a radioimmunoassay previously described by Lee et al. (1976) with NIH-LH-S18 as the assay standard. Plasma FSH concentrations were measured as described by Bremner, Findlay, Lee, de Kretser & Cumming (1980), with NIH-FSH-S6 as the assay standard. Samples were read on the portion of the standard curve where intra-assay coefficients of variation (C.V.) were <20% using the computer programme of Burger, Lee & Rennie (1972). For each study all samples were measured in the same assay. Assay sensitivity was 0.3 ng/ml for LH and 15 ng/ml for FSH.

**Prolactin.** The assay was similar to that of McNeilly & Andrews (1974). The antiserum (No. 2532) was developed in a rabbit immunized against NIH-P-S8 and was used at a final dilution of 1/850 000. Highly purified prolactin (AFP-2060-C; NIH, Bethesda, U.S.A.) was used for iodination by the lactoperoxidase method. The second antibody was raised in a goat immunized against rabbit gamma globulin and used at a final dilution of 1/960. The assay standard was NIH-P-S8. No immunological cross-reactivity was observed with ovine LH, FSH or GH or with human TSH or GH at 100 ng/tube. In 20 assays the sensitivity (mean ± s.e.m.) was 11 ± 1 pg/tube; the intra-assay C.V. was <10% over the range 38–88 pg. The between assay C.V. measured by repeated measurement (n = 20) of 4 plasma pools was 18-7% at 65 ng/ml, 18-7% at 133 ng/ml, 17-7% at 209 ng/ml and 8-3% at 420 ng/ml.

**Oestradiol.** The radioimmunoassay described by Carson, Findlay, Clarke & Burger (1981) was used. Plasma aliquots (1 ml) were extracted in 5 ml redistilled hexane : ether (4:1 v/v) and the samples were chromatographed on LH-20 Sephadex mini-columns (England, Niswender & Midgley, 1974). Oestradiol was undetectable in extracts of wether plasma, indicating the absence of a plasma or solvent blank in the assay. All samples were measured in a single assay in which the intra-assay C.V. was <20% and extraction efficiency of oestradiol from plasma was 86%. Assay sensitivity was 3 pg/ml.

**Statistics**

The number of receptors in the nuclear compartment following an injection of oestradiol was compared to the number of receptors in the nuclear compartment of non-treated ovariectomized ewes by Student’s t test. To analyse longitudinal changes in plasma hormone concentrations, the values obtained after injections of oestradiol were compared to preinjection values using Student’s paired t test.

**Results**

**Oestrogen receptors**

The results for Group 1 ewes are shown in Table 1. Total pituitary receptor concentrations did not change over the time course studied, and the overall mean was 6-0 ± 0-6 pmol/g wet wt (mean ± s.e.m.; range 3-8–11.5). In view of the large range of receptor values between animals the intracellular distribution of receptors was analysed in terms of percentage of total receptors that was present in the nuclear compartment (Table 1). Maximal nuclear uptake of oestrogen receptors was observed 1 h after injection; by 6 h nuclear values were not significantly different from those in control ovariectomized ewes.

**Plasma oestradiol concentrations**

Oestradiol values rose rapidly after the i.v. injection of 100 µg oestradiol (Group 2) but clearance was complete within 3 h (Text-fig. 1).
Table 1. Pituitary oestrogen receptor levels (mean ± s.e.m.) in cytosol and nuclei after i.v. injection of 100 μg oestradiol in ovariectomized ewes

<table>
<thead>
<tr>
<th>Time after injection (h)</th>
<th>No. of ewes</th>
<th>Receptor concentration (pmol/g wet wt)</th>
<th>Cytosol</th>
<th>Nucleus</th>
<th>Total</th>
<th>% in nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td></td>
<td>5.35 ± 1.48</td>
<td>0.17 ± 0.05</td>
<td>5.61 ± 1.51</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td></td>
<td>1.93 ± 0.25</td>
<td>2.45 ± 0.31</td>
<td>4.37 ± 0.26</td>
<td>56.0 ± 5.4***</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td></td>
<td>3.89 ± 0.59</td>
<td>1.59 ± 0.48</td>
<td>5.48 ± 0.62</td>
<td>29.0 ± 7.3*</td>
</tr>
<tr>
<td>24</td>
<td>3</td>
<td></td>
<td>7.48 ± 1.50</td>
<td>0.52 ± 0.16</td>
<td>8.00 ± 1.45</td>
<td>6.5 ± 2.1</td>
</tr>
</tbody>
</table>

* P < 0.02; *** P < 0.001 compared to control by Student’s t test.

**Text-fig. 1.** Plasma concentrations of oestradiol in 3 ovariectomized ewes after a single i.v. injection of 100 μg oestradiol. Note the log scale on the vertical axis. The shaded area indicates the detection limit of the assay.

**Effect of oestradiol on plasma LH, FSH and prolactin concentrations**

The results for Group 3 ewes are given in Text-fig. 2. Plasma LH levels were significantly (P < 0.01) depressed from pre-injection values of 5.7 ± 1.0 ng/ml (mean ± s.e.m.) to a minimum of 2.1 ± 0.3 ng/ml at 4-6 h after injection and increased (P < 0.01) to 37.0 ± 8.0 ng/ml 13-21 h later. Plasma FSH concentrations showed a gradual but progressive decline throughout the 22-h period after injection, falling from 809 ± 19 ng/ml before injection to 506 ± 47 ng/ml at 20-22 h (P < 0.001). There was a noticeable but not significant (P > 0.05) decline in plasma prolactin concentrations 1-2 h after injection (90 ± 16 to 29 ± 9 ng/ml). Thereafter concentrations rose to a maximum of 1537 ± 241 ng/ml, 11-14 h after injection (P < 0.001).

**Discussion**

The fact that brief exposure to oestradiol, resulting in a transient elevation of nuclear oestrogen receptors in the pituitary gland, is followed by alteration in LH, FSH and prolactin secretion in ovariectomized ewes demonstrates that prolonged nuclear occupancy of oestrogen receptors is
not obligatory for delayed feedback effects (see 'Introduction'). The time course of nuclear oestrogen receptor binding in the sheep hypothalamus is not known because we are unable to validate an acceptable exchange assay for this tissue. A study of oestrogen receptors in the rat hypothalamus (Kelner, Miller & Peck, 1980) yielded results similar to these for the sheep pituitary. In addition, the distribution of oestrogen receptors between the cytoplasmic and nuclear compartments is qualitatively similar in the rat hypothalamus and pituitary throughout the oestrous cycle (Greeley, Muldoon & Mahesh, 1975; Muldoon & Watson, 1980).

The biphasic effect of oestradiol on LH secretion in ovariectomized ewes, originally reported by Scaramuzzi, Tillson, Thorneycroft & Caldwell (1971), occurs after transient occupancy of pituitary oestrogen receptors. The negative feedback phase is dose-related and at 1–4 h after an oestradiol injection plasma LH concentrations are significantly reduced compared to preinjection values (Clarke & Findlay, 1980). The time course of this phase could be explained by a steroid effect on the genome or a non-genomic effect as suggested by McEwen, Krey & Luine (1978). The positive feedback phase of LH secretion occurs well after nuclear oestrogen receptor values return to baseline and 9–12 h after administration of oestradiol, and this could suggest that post-nuclear events are involved in this response. These events may include oestrogen sensitization of the pituitary to LH-RH (Coppings & Malven, 1976), the self-priming effect of LH-RH which is dependent upon protein synthesis (Pickering & Fink, 1976; de Koning, van Dieten, Tijssen & van Rees, 1979), and induction of synthesis of RNA (Robinson & Leavitt, 1971) and LH (Liu & Jackson, 1977). The positive feedback effect of oestradiol on LH release can be blocked by inhibitors of RNA and protein synthesis (Jackson, 1972, 1973).

**Text-fig. 2.** Plasma LH and FSH and prolactin concentrations in 4 ovariectomized ewes in relation to a single i.v. injection of 100 µg oestradiol (arrow). Note the log scale on the vertical axis.
The prolonged negative feedback effect of oestradiol on FSH concentrations in ovariectomized ewes has previously been reported (Fraser, Clarke & McNeilly, 1981). As with LH, the initial suppression may or may not involve receptor-mediated responses via the genome. The prolonged effect, however, persists for at least 22 h, long after nuclear oestrogen receptor concentrations have returned to basal values. This prolonged effect presumably involves post-nuclear events yet to be identified. Prolonged oestrogen treatment produces a biphasic pattern of FSH release in the ovariectomized monkey (Attardi et al., 1980) but not in the ovariectomized ewe. A preovulatory surge in FSH secretion, coincident with the LH surge, is apparent in entire cyclic ewes (L'Hermite, Niswender, Reichert & Midgley, 1972; Salamonsen et al., 1973) and in anoestrous ewes given oestradiol infusions (Jonas et al., 1973; Pant, 1977). At present we have no explanation for this discrepancy.

The stimulatory effect of oestradiol on prolactin secretion in ovariectomized ewes is similar to that previously demonstrated in anoestrous ewes (Fell et al., 1972). The magnitude of this response is substantially greater than the early morning diurnal rise reported by Walton, Evins, Fitzgerald & Cunningham (1980). Unlike the LH response, there is no rapid initial suppression of plasma prolactin concentrations by oestrogen. This is consistent with the study of Shupnick, Baxter, French & Gorski (1979) which demonstrated that chronic oestrogen treatment of ewes increased pituitary concentrations mRNA for pre-prolactin. Previous studies have shown that prolactin secretion in ewes is increased following luteal regression (Reeves, Arimura & Schally, 1970; Cumming, Brown, Goding, Bryant & Greenwood, 1972; Kann & Denamur, 1974) when oestrogen concentrations are elevated (Baird, 1978).

We conclude that delayed effects of a single injection of oestradiol on LH, FSH and prolactin secretion in the ewe are associated with only transient increases of nuclear oestrogen receptors in the pituitary gland. If nuclear oestrogen receptor uptake is required for the expression of these pituitary responses, then the period of nuclear occupancy necessary to bring about these responses is less in the sheep than in the rhesus monkey, suggesting that the difference between the species involves post-nuclear protein synthetic events.

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