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 $3.0 \pm 1.0\%$ in controls, $56.0 \pm 5.4\%$ at 1 h ($P < 0.001$) and $6.5 \pm 2.1\%$ 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/mmol) 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_{av} 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 $\pm$ 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 $\pm$ 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 μ g oestradiol (Group 2) but clearance was complete within 3 h (Text-fig. 1).

Table 1. Pituitary oestrogen receptor levels (mean $\pm$ s.e.m.) in cytosol and nuclei after i.v. injection of 100 μ g oestradiol in ovariectomized ewes

Time after injection (h)	No. of ewes	Receptor concentration (pmol/g wet wt)			% in nucleus
		Cytosol	Nucleus	Total	
Control	4	5.35 $\pm$ 1.48	0.17 $\pm$ 0.05	5.51 $\pm$ 1.51	3.0 $\pm$ 1.0
1	3	1.93 $\pm$ 0.25	2.45 $\pm$ 0.31	4.37 $\pm$ 0.26	56.0 $\pm$ 5.4***
3	3	3.89 $\pm$ 0.59	1.59 $\pm$ 0.48	5.48 $\pm$ 0.62	29.0 $\pm$ 7.3*
6	3	7.48 $\pm$ 1.50	0.52 $\pm$ 0.16	8.00 $\pm$ 1.45	6.5 $\pm$ 2.1
24	3	6.77 $\pm$ 2.28	0.21 $\pm$ 0.05	6.98 $\pm$ 2.29	3.0 $\pm$ 1.4

* $P < 0.02$; *** $P < 0.001$ 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 μ g 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 $\pm$ 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

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.

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 Dielen, 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).

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.

We thank Mr B. Doughton, Mr T. Gill, Ms Kathy Burman and Mrs L. Salamonsen for technical assistance; Dr L. Reichert and Dr A. F. Parlow for immunoassay reagents; Dr A. S. McNeilly for prolactin antiserum; and the Director of the Animal Research Institute at Werribee for co-operation and facilities. This work was supported by a grant from the National Health and Medical Research Council of Australia.

References

- Attardi, B., Hotchkiss, J. & Knobil, E. (1980) Monkey pituitary oestrogen receptors and the biphasic action of oestradiol on gonadotrophin secretion. *Nature, Lond.* **285**, 252–254.
- Baird, D.T. (1978) Pulsatile secretion of LH and ovarian estradiol during the follicular phase of the sheep estrous cycle. *Biol. Reprod.* **18**, 359–364.
- Bremner, W.J., Findlay, J.K., Lee, V.W.K., de Kretser, D.M. & Cumming, I.A. (1980) Feedback effects of the testis on pituitary responsiveness to luteinizing hormone-releasing hormone infusions in the ram. *Endocrinology* **106**, 329–336.
- Brown-Grant, K. (1976) Control of gonadotrophin secretion. In *Subcellular Mechanisms in Reproductive Neuroendocrinology*, pp. 485–501. Eds F. Naftolin, K. J. Ryan & I. J. Davies. Elsevier, Amsterdam.
- Burger, H.G., Lee, V.W.K. & Rennie, G.C. (1972) A generalised computer program for the treatment of data from competitive protein-binding assays including radioimmunoassay. *J. Lab. Clin. Med.* **80**, 302–312.
- Burton, K. (1956) A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**, 315–321.
- Carson, R.S., Findlay, J.K., Clarke, I.J. & Burger, H.G. (1981) Estradiol, testosterone and androstenedione in ovine follicular fluid during growth and atresia of ovarian follicles. *Biol. Reprod.* **24**, 105–113.
- Clarke, I.J. & Findlay, J.K. (1980) Catecholestrogens and gonadotrophin secretion in the ewe: affinity for pituitary oestrogen receptors *in vitro* and action on gonadotrophin secretion *in vivo*. *J. Endocr.* **85**, 503–509.
- Clarke, I.J., Burman, K., Funder, J.W. & Findlay, J.K.

- (1981) Estrogen receptors in the neuroendocrine tissues of the ewe in relation to breed, season and stage of the estrous cycle. *Biol. Reprod.* **24**, 323–331.
- Coppings, R.J. & Malven, P.V.** (1976) Biphasic effect of estradiol on mechanisms regulating LH release in ovariectomized sheep. *Neuroendocrinology* **21**, 146–156.
- Cumming, I.A., Brown, J.M., Goding, J.R., Bryant, G.D. & Greenwood, F.C.** (1972) Secretion of prolactin and luteinizing hormone at oestrus in the ewe. *J. Endocr.* **54**, 207–213.
- de Koning, J., van Dielen, J.A.M.J., Tijssen, A.M.I. & van Rees, G.P.** (1979) Studies on a protein synthesis dependent step in LH release by LH-RH. *Acta endocr., Copenh.* **92**, 648–657.
- England, B.G., Niswender, G.D. & Midgley, A.R., Jr** (1974) Radioimmunoassay of estradiol-17 β without chromatography. *J. clin. Endocr. Metab.* **38**, 42–50.
- Fell, L.R., Beck, C., Brown, J.M., Catt, K.J., Cumming, I.A. & Goding, J.R.** (1972) Solid phase radioimmunoassay of ovine prolactin in antibody-coated tubes. Prolactin secretion during estradiol treatment, at parturition, and during milking. *Endocrinology* **91**, 1329–1336.
- Findlay, J.K., Buckmaster, J.M., Chamley, W.A., Cumming, I.A., Hearnshaw, H. & Goding, J.R.** (1973) Release of luteinizing hormone by oestradiol-17 β and a gonadotrophin-releasing hormone in ewes affected with clover disease. *Neuroendocrinology* **11**, 57–66.
- Fink, G.** (1979) Neuroendocrine control of gonadotrophin secretion. *Br. med. Bull.* **35**, 155–160.
- Fraser, H.M., Clarke, I.J. & McNeilly, A.S.** (1981) Effect of oestrogen and an LH-RH agonist on the release of gonadotrophins in ovariectomized ewes deprived of LH-RH. *J. Reprod. Fert.* **62**, 131–140.
- Greeley, G.H., Muldoon, T.G. & Mahesh, V.B.** (1975) Correlative aspects of luteinizing hormone-releasing hormone sensitivity and cytoplasmic estrogen receptor concentration in the anterior pituitary and hypothalamus of the cycling rat. *Biol. Reprod.* **13**, 505–512.
- Jackson, G.L.** (1972) Effect of actinomycin D on estrogen-induced release of luteinizing hormone in ovariectomized rats. *Endocrinology* **91**, 1284–1287.
- Jackson, G.L.** (1973) Time interval between injection of estradiol benzoate and LH release in the rat and effect of actinomycin D or cycloheximide. *Endocrinology* **93**, 887–892.
- Jonas, H.A., Salamonsen, L.A., Burger, H.G., Chamley, W.A., Cumming, I.A., Findlay, J.K. & Goding, J.R.** (1973) Release of FSH after administration of gonadotrophin-releasing hormone or estradiol to the anestrus ewe. *Endocrinology* **92**, 862–865.
- Kann, G. & Denamur, R.** (1974) Possible role of prolactin during the oestrous cycle and gestation in the ewe. *J. Reprod. Fert.* **39**, 473–483.
- Karsch, F.J., Weick, R.F., Butler, W.R., Dierschke, D.J., Krey, L.C., Weiss, G., Hotchkiss, J., Yamaji, T. & Knobil, E.** (1973) Induced LH surges in the rhesus monkey: strength-duration characteristics of the estrogen stimulus. *Endocrinology* **92**, 1740–1747.
- Kato, J.** (1978) Brain receptors for sex steroid hormones and their characterization and functional implications. In *Brain-Endocrine Interactions. III. Neural Hormones and Reproduction*, pp. 286–301. Eds D. E. Scott, G. P. Kozlowski & A. Weindl. Karger AG, Basel.
- Kelner, K.L., Miller, A.L. & Peck, E.J.** (1980) Estrogens and the hypothalamus: nuclear receptor and RNA polymerase activation. *J. Receptor Res.* **1**, 215–237.
- Knobil, E.** (1973) On the control of gonadotrophin secretion in the rhesus monkey. *Recent Prog. Horm. Res.* **30**, 1–35.
- Lee, V.W.K., Cumming, I.A., de Kretser, D.M., Findlay, J.K., Hudson, B. & Keogh, E.J.** (1976) Regulation of gonadotrophin secretion in rams from birth to sexual maturity. *J. Reprod. Fert.* **46**, 1–6.
- L'Hermite, M., Niswender, G.D., Reichert, L.E. & Midgley, A.R.** (1972) Serum follicle-stimulating hormone in sheep as measured by radioimmunoassay. *Biol. Reprod.* **6**, 325–332.
- Liu, T.-C. & Jackson, G.L.** (1977) Effect of *in vivo* treatment with estrogen on luteinizing hormone synthesis and release by rat pituitaries *in vivo*. *Endocrinology* **100**, 1294–1302.
- McEwen, B.S., Krey, L.C. & Luine, V.N.** (1978) Steroid hormone action in the neuroendocrine system: when is the genome involved? In *The Hypothalamus*, pp. 255–268. Eds S. Reichlin, R. J. Baldessarini & J. B. Martin. Raven Press, New York.
- McNeilly, A.S. & Andrews, P.** (1974) Purification and characterization of caprine prolactin. *J. Endocr.* **60**, 359–367.
- Morrell, J.I., Kelley, D.B. & Pfaff, D.W.** (1975) Sex steroid binding in the brains of vertebrates. In *Brain-Endocrine Interactions. II. The Ventricular System*, pp. 230–256. Eds K. M. Knigge, D. E. Scott, H. Kobayashi & S. Ishii. Karger AG, Basel.
- Muldoon, T.G. & Watson, G.H.** (1980) Steroid hormone receptor functionality in the brain and anterior pituitary. In *Endocrinology 1980*, pp. 106–109. Eds I. A. Cumming, J.W. Funder & F. A. O. Mendelsohn. Australian Academy of Science, Canberra.
- Pant, H.C.** (1977) Effect of oestradiol infusion on plasma gonadotrophins and ovarian activity in progesterone-primed and unprimed anoestrous ewes. *J. Endocr.* **75**, 227–233.
- Pickering, A.J.M.C. & Fink, G.** (1976) Priming effect of luteinizing hormone releasing factor: in-vitro and in-vivo evidence consistent with its dependence upon protein and RNA synthesis. *J. Endocr.* **69**, 373–379.
- Reeves, J.J., Arimura, A. & Schally, A.V.** (1970) Serum levels of prolactin and luteinizing hormone (LH) in the ewe at various stages of the estrous cycle. *Proc. Soc. exp. Biol. Med.* **134**, 938–942.
- Robinson, J.A. & Leavitt, W.W.** (1971) Estrogen-related changes in anterior pituitary RNA levels. *Proc. Soc. exp. Biol. Med.* **139**, 471–475.
- Roy, E.J. & McEwen, B.S.** (1977) An exchange assay for estrogen receptors in cell nuclei of the adult rat brain. *Steroids* **30**, 657–669.
- Salamonsen, L.A., Jonas, H.A., Burger, H.G., Buckmaster, J.M., Chamley, W.A., Cumming, I.A., Findlay, J.K. & Goding, J.R.** (1973) A heterologous radioimmunoassay for follicle-stimulating hormone: application to measurement of FSH in the ovine estrous cycle and in several other species including man. *Endocrinology* **93**, 610–618.
- Scaramuzzi, R.J., Tillson, S.A., Thorneycroft, I.H. & Caldwell, B.V.** (1971) Action of exogenous pro-

- gestosterone and estrogen on behavioral estrus and luteinizing hormone levels in the ovariectomized ewe. *Endocrinology* **88**, 1184–1189.
- Shupnick, M.A., Baxter, L.A., French, L.R. & Gorski, J.** (1979) *In vivo* effects of estrogen on ovine pituitaries: prolactin and growth hormone biosynthesis and messenger ribonucleic acid translation. *Endocrinology* **104**, 729–735.
- Tang, B.Y. & Adams, N.R.** (1978) Changes in oestradiol-17 β binding in the hypothalami and pituitary glands of persistently infertile ewes previously exposed to oestrogenic subterranean clover: evidence of an alteration in oestradiol receptors. *J. Endocr.* **78**, 171–177.
- Walton, J.S., Evins, J.D., Fitzgerald, B.P. & Cunningham, F.J.** (1980) Abrupt decrease in daylength and short-term changes in the plasma concentrations of FSH, LH and prolactin in anoestrous ewes. *J. Reprod. Fert.* **59**, 163–171.
- Wise, P.M., Payne, A.H., Karsch, F.J. & Jaffe, R.B.** (1975) Cytoplasmic oestrogen receptor complex of female ovine pituitary: changes associated with the reproductive state and oestradiol treatment. *J. Endocr.* **67**, 447–452.
- Yamaji, T., Dierschke, D.J., Hotchkiss, J., Bhattacharya, A.N., Surve, A.H. & Knobil, E.** (1971) Estrogen induction of LH release in the rhesus monkey. *Endocrinology* **89**, 1034–1041.
- Yamaji, T., Dierschke, D.J., Bhattacharya, A.N. & Knobil, E.** (1972) The negative feedback control by estradiol and progesterone of LH secretion in the ovariectomized rhesus monkey. *Endocrinology* **90**, 771–777.

Received 14 April 1981