Role of GnRH in the regulation of pituitary GnRH receptors in female mice*

S. I. Naik, L. S. Young, G. Saade, A. Kujore, H. M. Charlton† and R. N. Clayton

Department of Medicine, University of Birmingham, Birmingham B15 2TH, and †Department of Human Anatomy, University of Oxford, South Parks Road, Oxford OX1 3QX, U.K.

Summary. The fall in pituitary GnRH receptors in female mice after ovariectomy (Ovx) was further decreased ($> 50\%$), rather than prevented, by treatment with a GnRH antiserum, despite suppression of the post-gonadectomy increase in serum gonadotrophins, suggesting that increased endogenous GnRH secretion is not the mediator of GnRH receptor fall after ovariectomy in mice. Furthermore, GnRH antiserum reduced GnRH receptors by 30–50% in intact normal females, without altering receptor affinity, and rendered serum LH and FSH undetectable but did not reduce receptors in GnRH-deficient, *hpg* mice.

When GnRH was administered to ovariectomized mice this failed to restore receptor values (fmol/pituitary) (intact = 55.3 ± 2.4 ; Ovx = 30.1 ± 2 ; Ovx + GnRH = 31.6 ± 2.8), but serum LH was reduced from high post-ovariectomy values (231 ± 42 ng/ml) to values normal for intact females (24 ± 2 ng/ml). In contrast, multiple GnRH injections to intact female mice increased GnRH receptor by 35%, while serum LH was reduced to just detectable levels. A marked dissociation between GnRH receptor and serum gonadotrophin concentrations was observed.

Administration of oestrogen (E_2) plus progesterone (P) to ovariectomized mice in which endogenous GnRH had been immunoneutralized reversed the inhibitory effect of GnRH antiserum on GnRH receptors and increased values above those of ovariectomized controls, although no increase in serum or pituitary gonadotrophin levels was seen in ovariectomized mice treated with $E_2 + P +$ GnRH antiserum.

Treatment with E_2 and P of intact females receiving GnRH antiserum did not prevent the inhibitory effect of antiserum on receptors, while $E_2 + P$ treatment alone of intact female mice reduced GnRH receptors by 30%. These data suggest that the gonadal steroids reduce GnRH receptors in intact female mice by inhibiting hypothalamic GnRH secretion, and that a certain degree of pituitary exposure to GnRH is required for maintenance of a normal receptor complement.

These results suggest that (1) the fall in GnRH receptors after ovariectomy is primarily attributable to removal of gonadal factors. The fall is not a reflection of alteration in endogenous GnRH interaction with the gonadotroph; (2) homologous ligand 'up-regulation' of GnRH receptors in female mice depends upon the presence of the ovaries; (3) endogenous GnRH is also required for GnRH receptor maintenance in intact female mice; and (4) GnRH receptor and serum gonadotrophin responses to hormonal changes can be dissociated and their relationship is complex.

* Reprint requests to Dr R. N. Clayton.

Introduction

Gonadectomy in rats is invariably associated with a 2–3-fold rise in gonadotrophin-releasing hormone receptors coincident with the rise in serum gonadotrophins (Frager, Pieper, Tonetta, Duncan & Marshall, 1981; Clayton & Catt, 1981a; Conne, Scaglioni, Lang, Sizonenko & Aubert, 1982). These responses can be prevented by immunoneutralization with a specific GnRH antiserum (Frager *et al.*, 1981; Clayton, Popkin & Fraser, 1982) suggesting that increased endogenous GnRH is essential for the post-gonadectomy rise in pituitary GnRH receptors and serum gonadotrophins. Studies of ewes (Fraser & McNeilly, 1982) and castrated rats (Kawakami & Higuchi, 1979) have shown that passive immunoneutralization of endogenous GnRH results in a rapid and sustained decrease of the high serum LH and FSH concentrations. Moreover, immunoneutralization of GnRH prevents the preovulatory surge of LH in rats (Arimura, Debeljuk & Schally, 1974) and sheep (Fraser & McNeilly, 1982). We have demonstrated a GnRH-receptor response to gonadectomy in mice that is opposite that found in rats, although the serum gonadotrophin (Naik, Young, Charlton & Clayton, 1984a) responses are the same. There is therefore no positive correlation between pituitary content of GnRH receptors and serum gonadotrophin secretion after gonadectomy of mice.

Pituitary GnRH receptors can be induced in rats by intermittent administration or continuous infusion of low doses of GnRH or an agonist analogue (Clayton, 1982), and GnRH rapidly increases GnRH receptors in the pituitaries of mice with an inherited absence of hypothalamic GnRH (*hpg*) (Young, Speight, Charlton & Clayton, 1983). Homologous up-regulation of GnRH receptors also occurs in cultured rat pituitary cells (Loumaye & Catt, 1982; Conn, Rogers & Seay, 1984; Young, Naik & Clayton, 1984). Thus, GnRH positively regulates its own pituitary receptors, and pituitary exposure to GnRH seems to be essential for the maintenance of a normal receptor number. The present study was therefore designed to analyse the importance of endogenous GnRH in the fall of GnRH receptors after ovariectomy of mice. We reasoned that, if excessive endogenous GnRH secretion after ovariectomy was responsible for the receptor fall, then this should be prevented by specific immunoneutralization of endogenous GnRH. Alternatively, if the receptor fall after ovariectomy was due to a relative deficiency of endogenous GnRH secretion then it should be reversed by exogenous GnRH treatment. Consequently, the role of GnRH in its own receptor regulation was determined in ovariectomized and intact female mice, and compared with serum and pituitary gonadotrophin changes.

Materials and Methods

Hormones. The GnRH agonist analogue (D-Ser(t But)⁶des Gly¹⁰)GnRH *N*-ethylamide (GnRH-A), used for iodination and as standard for the radioreceptor assay (RRA), was kindly donated by Dr J. Sandow (Hoechst A.G. Frankfurt, West Germany). GnRH antiserum (sheep anti-GnRH serum No. 94) was kindly supplied by Dr H. M. Fraser, Edinburgh.

Animals and sample collection. Normal and *hpg* (GnRH-deficient) male and female mice (C3H/HeH/101H F1 hybrid strain intercrosses) aged 80–180 days (25–35 g) were obtained from the Department of Anatomy, University of Oxford. Female mice were housed, from the time of weaning as 6–8 animals per cage (size = 21 × 28 × 10 cm), in the same room as male mice from birth until experiment, with 14 h light/24 h and free access to Purina chow and tap water. All females were used at random stages of the oestrous cycle. Ovariectomy was performed through a single dorsal incision and orchidectomy was carried out by way of a scrotal incision under light ether anaesthesia. At the completion of experiments, all animals were killed by decapitation, trunk blood was collected and serum separated and stored at –20°C until RIA. Pituitary glands were rapidly removed, snap-frozen in liquid nitrogen and stored at –70°C until GnRH RRA.

Radioreceptor and radioimmunoassays. GnRH receptor numbers were measured in an aliquant of an individual normal mouse pituitary homogenate by equilibration at 4°C for 90 min in a total volume of 135 µl with a near-saturating concentration (1 nM) of GnRH agonist as the ligand, including 100 000 c.p.m. of the radioiodinated analogue. Non-specific binding was measured in the presence of a 200-fold excess of unlabelled GnRH-A. Pituitary receptors of *hpg* female mice were measured under non-saturating ligand conditions. This method allows duplicate determination of total and non-specific binding for each individual pituitary homogenate; the difference between these values, the specific binding, was converted to receptor concentration as femtomoles GnRH-A bound per pituitary. Values were corrected for difference in protein concentration between glands. The details of the methodology were as described previously (Naik *et al.*, 1984a).

Serum and pituitary LH, FSH and prolactin concentrations were measured by RIA as described by Naik *et al.* (1984a, b), and results are expressed in terms of the rat RP-2 standard. The inter- and intra-assay coefficients of variation for LH, FSH and prolactin RIAs were 8–10% and 3–5%, respectively. The lower limit of detectability for serum LH (bound counts/bound counts in the absence of added unlabelled hormone = 0.9) varied between 19 and 25 ng/ml and the value for each experiment is presented in the corresponding Table or Text-figure legend. The limit of detection for serum FSH was 50 ng/ml.

Statistical analysis. When appropriate, differences between groups were examined by the Mann-Whitney U-test or analysis of variance and Student's *t* test.

Results

Dose-response effect of GnRH-antiserum in intact and ovariectomized normal female mice (Table 1)

Groups of ovariectomized normal mice were injected s.c. with 50, 10, 5 or 2 µl GnRH antiserum or non-immune sheep serum once daily for 5 days, commencing at the time of ovariectomy. Two groups of intact normal female mice were given 50 or 10 µl GnRH antiserum once daily for 5 days and a third group of intact normal females was injected with 10 µl non-immune sheep serum (control). Animals were killed 24 h after the last injection of antiserum.

Table 1. Dose-response effect of GnRH antiserum (AS) in intact and ovariectomized (Ovx) normal female mice

Groups	GnRH receptor (fmol/pituitary)	Serum LH (ng/ml)	Serum FSH (ng/ml)	Pituitary LH (µg/pituitary)	Pituitary FSH (µg/pituitary)
(1) Intact female + vehicle	53.1 ± 2.5	50.5 ± 6.6	82 ± 20	28.3 ± 6.4	2.67 ± 0.21
(2) Intact female + AS (50 µl)	29.0 ± 1.2	ND	ND	29.5 ± 1.7	4.85 ± 0.47
(3) Intact female + AS (10 µl)	53.9 ± 2.9	52.7 ± 8.7	64 ± 15	32.0 ± 5.5	2.9 ± 0.06
(4) Ovx normal female + vehicle	28.18 ± 2.9	263 ± 17	756 ± 48	18.4 ± 2.7	14.5 ± 1.5
(5) Ovx normal female + AS (50 µl)	14.28 ± 1.6	48.7 ± 8.0	154 ± 25	39.2 ± 5.4	23.2 ± 1.5
(6) Ovx normal female + AS (10 µl)	18.18 ± 1.6	32.8 ± 9.2	216 ± 25.7	37.5 ± 4.5	21.23 ± 2.2
(7) Ovx normal female + AS (5 µl)	16.7 ± 1.2	60.4 ± 10.7	307.4 ± 41.9	42.8 ± 6.9	14.4 ± 0.94
(8) Ovx normal female + AS (2 µl)	26.1 ± 2.7	121 ± 24	576 ± 71	35.8 ± 2.9	15.79 ± 1.8

Values are mean ± s.e.m. (5 mice/group).

See text for significance of differences between groups.

ND = not detectable (<25 ng LH/ml serum; <50 ng FSH/ml serum).

Treatment of intact normal female mice with 50 μ l GnRH antiserum decreased GnRH receptors by 45% (Table 1, Group 1 *vs* Group 2, $P < 0.001$) and rendered serum LH and FSH undetectable. However, 10 μ l GnRH antiserum in intact females did not change any of the characteristics measured (Group 1 *vs* Group 3). As previously observed (Naik *et al.*, 1984b), ovariectomy resulted in a 40% decrease in GnRH receptors (Group 4 *vs* Group 1) ($P < 0.001$). Treatment of ovariectomized normal female mice with 50 μ l GnRH antiserum further decreased GnRH receptors by 50% (Group 4 *vs* Group 5, $P < 0.001$), and the post-ovariectomy rise in serum gonadotrophins was prevented ($P < 0.001$).

Treatment of ovariectomized female mice with decreasing amounts of GnRH antiserum caused a dose-dependent increase of the receptor levels, from 14.28 ± 1.6 (50 μ l) to 26.1 ± 2.7 (2 μ l) fmol/pituitary. The lowest dose (2 μ l) did not influence the receptors (Group 4 *vs* Group 8). The fall in receptor concentration with increasing amount of GnRH antiserum was associated with decreasing serum LH concentration, from 121 ± 24 (2 μ l) to 48.7 ± 8.0 (50 μ l) ng/ml. Similarly, a dose-dependent decrease in serum FSH occurred, reaching a minimum value of 154 ± 25 ng/ml with the 50 μ l dose of antiserum. As shown in Table 1, pituitary FSH values in ovariectomized females (Group 4) were 5-fold higher than in untreated intact controls (Group 1), and 50 and 10 μ l GnRH antiserum significantly augmented the pituitary FSH rise (Groups 5 and 6 *vs* Group 4, $P < 0.05$). Treatment of intact females with 50 μ l antiserum increased pituitary FSH 2-fold (Group 2 *vs* Group 1, $P < 0.001$), while 10 μ l antiserum were ineffective in this respect (Group 3 *vs* Group 1).

In contrast to the pituitary FSH rise in ovariectomized females, pituitary LH remained unchanged (Group 1 *vs* Group 4 NS). However, treatment with antiserum (all doses) increased pituitary LH content in ovariectomized females whereas similar treatment of intact females had no effect on pituitary LH content (Groups 2 and 3 *vs* Group 1).

In 5 separate experiments in which mice were injected *s.c.* with 50 μ l antiserum/day for 5–7 days to intact and ovariectomized normal females, a 30–50% fall in pituitary GnRH receptors, accompanied by suppression of serum gonadotrophins, was consistently observed. The same dose of antiserum also decreased GnRH receptors (fmol/pituitary) by 50 and 65% in intact and castrated normal male mice (intact control 40.0 ± 1.9 ; intact + antiserum 20.4 ± 1.5 ; castrate 32.9 ± 2.3 ; castrate + antiserum 11.5 ± 1.3). This treatment suppressed serum gonadotrophin levels in intact male mice to undetectable values and prevented the post-castration rise of serum gonadotrophin values (not shown). GnRH receptors were also decreased by 60% when determined by Scatchard analysis of GnRH-A binding to pooled pituitary homogenate from male mice treated with 50 μ l antiserum once daily for 5 days (25.27 fmol/pituitary) compared with untreated intact controls (62.05 fmol/pituitary), but there was no change in receptor affinity ($K_a = 1.9 \times 10^9$ and 2.7×10^9 M^{-1} respectively). However, similar antiserum treatment (50 μ l/day for 7 days) did not change GnRH receptors (fmol/pituitary) in *hpg* male mice (15.0 ± 2.5 in *hpg* control, 15.5 ± 2.0 in *hpg* + antiserum), and there was no effect on serum LH and FSH values, which remained undetectable in treated and control groups.

The effect of multiple GnRH injections in normal and previously ovariectomized normal female mice

Five injections of GnRH daily (60 ng/injection) were given to groups of intact normal mice and mice that had been ovariectomized 7 days earlier. The dose chosen had previously been shown to stimulate gonadotroph function in *hpg* mice (Young *et al.*, 1983), and the frequency of injections was on the basis that 3–4 injections of GnRH/day increase GnRH receptors in rats (Marshall *et al.*, 1983). Groups of intact normal and 7-day ovariectomized females were injected with vehicle (PBS + 0.1% BSA) and served as controls. The injections were given every 4 h during the day, the last injection being at 23:00 h, and the next one at 07:00 h. The treatment was continued for 4 days, and the animals were killed 8 h after the last injection.

Multiple injections of GnRH to intact normal female mice increased GnRH receptors by 35% (55.3 ± 2.4 and 74.1 ± 2.8 fmol/pituitary; $P = 0.005$) (Text-fig. 1). This was not accompanied by

any change in serum FSH concentration or in pituitary LH and FSH content. However, serum LH values were reduced from 67.7 ± 2 to 19.5 ± 1 ng/ml. Identical GnRH treatment of 7-day ovariectomized female mice was without effect on pituitary GnRH receptors, serum FSH or pituitary FSH. As in intact GnRH-treated female mice, serum LH was markedly suppressed from high post-ovariectomy values (231 ± 42 ng/ml) to values (24 ± 2.2 ng/ml) that were significantly ($P < 0.005$) below those of untreated intact female mice (67.7 ± 2 ng/ml). Pituitary LH content was also reduced ($P < 0.001$) in ovariectomized females treated with GnRH.

Text-fig. 1. Effect of multiple injections of GnRH (5×60 ng for 4 days) on (a) GnRH receptors, (b) serum LH and FSH concentrations and (c) pituitary LH and FSH content in normal mice and those ovariectomized 7 days earlier. Lower limit of detection for serum LH = 19 ng/ml. Values represent the mean $\pm$ s.e.m. of 6 animals/group.

The effect of GnRH treatment immediately after ovariectomy

GnRH (60 ng) or vehicle was injected at 8-h intervals for 24 h or 4 days to ovariectomized mice, starting at the time of ovariectomy. Control animals were injected for the same time with vehicle alone. The animals were killed 8 h after the last injection. When measured 32 h after ovariectomy (50.2 ± 4 compared with 66.5 ± 3.9 fmol/pituitary in intact females; $P = 0.05$; $n = 5$), the fall in GnRH receptors was not as marked as at 5 or 7 days after ovariectomy (Naik *et al.*,

1984b). Three injections of GnRH accentuated rather than prevented this early receptor fall (ovariectomy + GnRH = 37.0 ± 4.3 fmol/pituitary, $P = <0.05$ vs ovariectomy alone at 32 h). If the GnRH treatment was continued for 4 days from the time of ovariectomy it neither accentuated nor prevented the receptor fall (intact female control = 63.3 ± 3.4 fmol/pituitary, $n = 5$; ovariectomy alone (4 days) = 46.2 ± 3.0 ; ovariectomy + GnRH = 49.5 ± 4.0 ; $P = <0.01$ vs intact control for ovariectomy with or without GnRH).

Effect of oestradiol and progesterone in intact and ovariectomized normal female mice treated with GnRH antiserum

The dose of antiserum (50 μ l) was chosen on the basis of the dose-response study data. Groups of normal female mice were ovariectomized and injected with (i) 50 μ l GnRH antiserum; (ii) 300 ng oestradiol/day plus 375 μ g progesterone/day; (iii) the combination of (i) and (ii); or (iv) vehicle alone (sesame oil), once daily for 7 days starting at the time of ovariectomy. Groups of intact normal female mice were similarly treated. All the injections were in 0.1 ml. Animals were killed 24 h after the last injection of steroids and antiserum.

GnRH antiserum (50 μ l/day) treatment of intact and ovariectomized female mice reduced GnRH receptors by 40% (Group 1 vs Group 2, $P < 0.001$; Group 5 vs Group 6, $P < 0.001$; Table 2). Administration of oestradiol and progesterone to ovariectomized mice treated with GnRH antiserum (Group 6) increased GnRH receptors 3-fold compared to values for Group 7 (ovariectomy + antiserum) ($P < 0.001$). The receptor values in Group 7 mice were identical to those of ovariectomized animals receiving steroids without antiserum (Group 8; NS) but did not reach the values for intact females (Group 1). However, steroid treatment of intact female mice receiving antiserum (Group 3) did not prevent the inhibitory effect of antiserum on receptor numbers (Group 2) (Group 3 vs Group 1, $P < 0.01$). Although intact female mice treated with steroids but not antiserum (Group 4) had a 30% reduction in GnRH receptors compared to Group 1, these values were significantly higher ($P < 0.05$) than those of ovariectomized mice treated with steroids only (Group 8).

Table 2. Effects of oestradiol (E_2) + progesterone (P) in intact and ovariectomized (Ovx) normal female mice treated with GnRH antiserum (AS)

Groups	GnRH receptor (fmol/pituitary)	Serum LH (ng/ml)	Serum FSH (ng/ml)	Pituitary LH (μ g/pituitary)	Pituitary FSH (μ g/pituitary)
(1) Intact female + vehicle	71.56 ± 5.2	52.6 ± 8.7	120 ± 43	45.5 ± 5.1	2.42 ± 0.39
(2) Intact female + AS	41.5 ± 3.1	26.93 ± 3.8	57 ± 5.0	46.9 ± 2.7	4.85 ± 0.47
(3) Intact female + AS + E_2 + P	45.57 ± 2.2	ND	<50	34.2 ± 3.6	3.37 ± 0.18
(4) Intact female + E_2 + P	52.4 ± 4.2	24.6 ± 5.1	<50	39.4 ± 4.2	3.1 ± 0.16
(5) Ovx + vehicle	25.7 ± 2.4	274 ± 34	652 ± 35	55.6 ± 3.1	8.0 ± 0.37
(6) Ovx + AS	15.0 ± 2.5	48.7 ± 8.1	254 ± 22	57.8 ± 2.2	9.2 ± 0.44
(7) Ovx + AS + E_2 + P	45.0 ± 4.7	ND	204 ± 17	41.9 ± 0.42	7.85 ± 0.34
(8) Ovx + E_2 + P	40.0 ± 0.24	26.4 ± 7.3	240 ± 23	40.9 ± 4.2	8.24 ± 0.31

Values are the mean $\pm$ s.e.m. for 5–6 animals/group. See text for significance of difference between groups. ND = undetectable (<20 ng LH/ml serum).

As shown in Table 2, GnRH antiserum or steroid treatment of intact female mice significantly ($P < 0.001$) reduced serum LH and FSH values to just detectable levels, whereas in the previous study (Table 1) both hormones were rendered undetectable by the same dose of antiserum. However, steroid treatment in combination with GnRH antiserum rendered serum LH undetectable in intact and ovariectomized animals. In intact females serum FSH was halved by antiserum treatment and rendered undetectable by steroid treatment either alone or when combined with antiserum. However, steroid treatment of ovariectomized females did not produce further suppression of serum FSH when combined with GnRH antiserum than did the antiserum alone (Groups 6, 7 and 8 *vs* Group 5, $P = <0.001$; Groups 7 and 8 *vs* Group 6, NS).

Treatment of intact normal female mice with antiserum or steroids or their combination had no significant effect on pituitary LH content (NS, for all treated groups), although pituitary FSH was increased 2-fold ($P < 0.01$) by antiserum treatment alone. Pituitary LH in ovariectomized females was not significantly increased compared to intact female values and remained unchanged by antiserum treatment alone, while treatment with steroids with or without antiserum reduced pituitary LH by 25% (Groups 9 and 8 *vs* Group 5, $P < 0.05$). However, pituitary FSH in ovariectomized females was increased 3–4-fold ($P < 0.001$) compared to intact controls, but none of the treatments had any effect compared to the values for the respective vehicle-treated controls.

Discussion

The most striking finding of these experiments was not only the failure of GnRH immunoneutralizatoin to prevent the post-ovariectomy GnRH receptor fall but the fact that this fall was accentuated. The GnRH antiserum treatment was clearly effective at abolishing pituitary stimulation by endogenous GnRH since it prevented the post-ovariectomy rise in serum gonadotrophins. Therefore, the proposal (Naik *et al.*, 1984a) that the post-ovariectomy fall in GnRH receptors resulted from receptor occupancy or down-regulation consequent upon increased endogenous GnRH secretion seems most unlikely. Further evidence against increased receptor occupancy/down-regulation was the observation that additional repetitive exogenous GnRH treatment of ovariectomized mice did not further reduce GnRH receptors.

It might be argued that the GnRH antiserum itself cross-reacted with the GnRH receptor *in vivo* leading to reduced ligand–receptor interaction in the in-vitro radioreceptor assay and that this was the explanation of the further reduction in GnRH receptors in the ovariectomized GnRH antiserum treated mice. However, this could not be the case because the same treatment of GnRH-deficient *hpg* mice did not reduce the already low concentration of GnRH receptors. Furthermore, in-vitro experiments have clearly demonstrated that this antiserum does not interact with GnRH receptors (Clayton *et al.*, 1982).

The observation that the GnRH antiserum further reduced GnRH receptors in ovariectomized mice probably indicates that endogenous GnRH, acting directly on the gonadotroph, is required even to maintain the reduced number of GnRH receptors. This conclusion also applies to male mice, based on a similar reduction in receptors after castration and GnRH antiserum treatment. GnRH receptor levels in ovariectomized and antiserum-treated mice were very similar to those of untreated *hpg* female mice (Naik *et al.*, 1985), indicating that the antiserum treatment had induced GnRH deficiency in the normal ovariectomized females. Further evidence for a role of GnRH in receptor maintenance in mice is provided from the studies of intact male and female animals in which GnRH antiserum reduced the receptor complement, as is the case with intact rats so treated (Frager *et al.*, 1981; Clayton *et al.*, 1982). In addition, GnRH treatment of *hpg* male (Young *et al.*, 1983; Detta, Naik, Charlton, Young & Clayton, 1984) and female (Naik *et al.*, 1985) mice indicates that homologous ligand up-regulation of its own receptors clearly occurs in mice, as in rats. The fact that this up-regulation occurs to the same extent in gonadectomized *hpg* animals indicates that it is

the result of GnRH acting directly on the gonadotroph, rather than being secondary to GnRH stimulation of gonadal steroids which can also up-regulate GnRH receptors at least in mice (Naik *et al.*, 1985).

The hypothesis of homologous ligand GnRH-receptor autoregulation was based entirely on studies in rats (Clayton & Catt, 1981b; Marshall *et al.*, 1983, for review) and was that GnRH was the primary hormonal regulator of pituitary GnRH receptor content. The corollary to this is that GnRH receptor levels reflect the prior exposure of the pituitary to endogenous GnRH. On this basis the post-gonadectomy fall in GnRH receptors in mice should represent a relative deficiency of endogenous GnRH secretion, an unlikely explanation in view of the high serum gonadotrophin concentrations observed. If this were the case then exogenous GnRH should prevent or reverse the receptor fall after ovariectomy. However, treatment of 7-day ovariectomized or immediately ovariectomized mice with GnRH, in a dose previously shown to elevate GnRH receptors in *hpg* mice (Young *et al.*, 1983), was completely unable to restore or prevent the receptor fall. It is unlikely that the GnRH 'replacement' regimens were inappropriate since with the 5 times per day regimen for 4 days pituitary LH desensitization occurred as high post-ovariectomy serum LH levels were reduced to intact control values. Moreover the same treatment schedule significantly increased GnRH receptors in intact female mice. Failure of exogenous GnRH to influence the receptor fall in mice after ovariectomy clearly indicates that the fall is unrelated to reduced pituitary exposure to endogenous GnRH. Therefore, the view that pituitary GnRH-receptor content reflects solely the degree of exposure to endogenous GnRH clearly does not apply to the post-gonadectomy state in mice, in which the post-ovariectomy receptor fall is independent of changes in endogenous hypothalamic GnRH secretion.

While this study indicates that exogenous GnRH can up-regulate GnRH receptors in intact female mice, this was dependent upon the gonads and therefore all the changes in GnRH receptors *in vivo* are not necessarily a consequence of direct GnRH action on the gonadotroph. Gonadal steroids, increased secondarily to GnRH-stimulated gonadotrophin release, acting directly on the pituitary (Loumaye & Forni, 1982) may also be involved.

We have previously reported (Naik *et al.*, 1984b) that oestrogen, and oestrogen combined with progesterone, can largely, although not completely, reverse the GnRH receptor fall after ovariectomy of mice with intact hypothalamic GnRH secretion. Although unlikely from the evidence discussed above, it was possible that gonadal steroid reversal of the post-ovariectomy receptor fall resulted from a subtle change in endogenous GnRH secretion which in turn stimulated GnRH receptor increase. This possibility is rendered even more unlikely by the finding that oestradiol and progesterone can elevate GnRH receptors in ovariectomized mice in which endogenous GnRH has been immunoneutralized. This provides direct evidence that oestradiol and progesterone act at the level of the gonadotroph to increase GnRH receptors, and that the post-ovariectomy fall in receptors results from removal of the receptor-maintaining influence of gonadal steroids. GnRH receptor regulation in female mice is therefore multifactorial, gonadal steroids being the primary regulator and homologous ligand up-regulation by GnRH having a secondary role. Thus, the receptor fall in intact female mice after GnRH immunoneutralization is a consequence not only of removal of the up-regulatory action of the ligand but also of the secondary hypogonadism that ensues.

In several experiments we have observed that treatment of intact female mice with oestradiol and progesterone reduces GnRH receptors to below untreated control values which are similar to those of ovariectomized females treated with oestradiol and progesterone. This, and the failure of steroid treatment to restore GnRH receptors to intact female values indicates that the steroid treatment suppresses endogenous GnRH secretion with the result that GnRH receptors fall in the intact animals and fail to reach intact values in the ovariectomized oestradiol + progesterone-treated group.

In rats there is often a positive correlation between pituitary GnRH receptors and serum gonadotrophin concentrations, particularly LH (reviewed by Clayton & Catt, 1981a). This

correlation is not as apparent in mice (Naik *et al.*, 1984a, b). The present study with mice also highlights the discrepancy between changes in GnRH receptor and serum LH levels. LH desensitization occurred in intact and ovariectomized mice treated with GnRH when GnRH receptors either increased or remained unchanged, respectively. However, serum FSH values did not change, indicating the relative resistance of FSH to desensitization, at least in these experiments. In addition, oestrogen and progesterone treatment of ovariectomized mice suppressed both high serum LH and FSH levels while increasing GnRH receptors. Therefore, the regulation of gonadotrophin secretion both by GnRH and ovarian steroids in mice is largely independent of GnRH receptor changes and presumably occurs at post-receptor sites. This conclusion was also reached from earlier studies of rats *in vivo* (Clayton, 1982). Pituitary desensitization by GnRH *in vitro* also occurs at post-receptor sites (Smith, Perrin & Vale, 1983; L. S. Young & R. N. Clayton, unpublished data), being largely independent of GnRH receptor down-regulation. Moreover, the LH desensitization by GnRH could not be accounted for by reduced pituitary LH synthesis *in vitro* (Smith *et al.*, 1983) or *in vivo* in the present experiments since LH content was unchanged in intact female mice and only marginally reduced in the ovariectomized group.

In the present experiments there were variable effects of GnRH antiserum treatment on pituitary gonadotrophin content, while both serum gonadotrophins were consistently suppressed. In ovariectomized animals treated for 5 days with antiserum pituitary LH and FSH were increased, although this was not observed with 7 days of antiserum treatment. The reason for this is not clear, although between 4 and 7 days pituitary LH content is beginning to rise after an initial fall after ovariectomy (Naik *et al.*, 1984b). Nevertheless, it is apparent that gonadotrophin biosynthesis, at least in the short term, proceeds despite marked reduction in GnRH receptors and GnRH stimulation of the pituitary. The increased pituitary LH content after ovariectomy was prevented by gonadal steroid treatment both in the presence and absence of concurrent GnRH immunoneutralization. Therefore, at least a component of the negative feedback action of oestradiol and progesterone on gonadotrophin synthesis is exerted at the pituitary level.

In summary, the GnRH receptor fall after ovariectomy of mice is not a consequence of altered endogenous GnRH secretion, but results primarily from removal of the receptor-maintaining action of oestradiol and progesterone. Nevertheless, a minor component of homologous ligand receptor regulation is evident after ovariectomy of normal female mice, although in these animals the gonads are essential for receptor up-regulation by exogenous GnRH. A marked discrepancy between hormonal regulation of GnRH receptors and gonadotrophin secretion indicates the complexity of the inter-relationship between these events.

We thank Dr H. M. Fraser, MRC Unit of Reproductive Biology, Edinburgh, for supplies of the GnRH antiserum. The work was supported by grants from the MRC (R.N.C. and H.M.C.), the Central Birmingham Health District Social Trustees (I.S.Y.) and the Government of Pakistan (S.I.N.).

References

- Arimura, A., Debeljuk, L. & Schally, A.V. (1974) Blockade of the preovulatory surge of LH and FSH and of ovulation by anti LH-RH serum in rats. *Endocrinology* **95**, 323–325.
- Clayton, R.N. (1982) Gonadotrophin-releasing hormone modulation of its own pituitary receptors: evidence for biphasic regulation. *Endocrinology* **111**, 152–161.
- Clayton, R.N. & Catt, K.J. (1981a) Regulation of pituitary gonadotrophin releasing hormone receptors by gonadal hormones. *Endocrinology* **108**, 887–895.
- Clayton, R.N. & Catt, K.J. (1981b) Gonadotrophin-releasing hormone receptors: characterisation, physiological regulation, and relationship to reproductive function. *Endocr. Rev.* **2**, 186–209.
- Clayton, R.N., Popkin, R.M. & Fraser, H.M. (1982) Hypothalamic regulation of pituitary gonadotrophin releasing hormone receptors. Effects of gonadotrophin releasing hormone immunoneutralization. *Endocrinology* **110**, 1116–1123.
- Conn, P.M., Rogers, D.C. & Seay, S.G. (1984) Biphasic

- regulation of the GnRH receptor by receptor micro-aggregation and intracellular Ca^{2+} levels. *Molec. Pharmac.* **25**, 51–55.
- Conne, B.S., Scaglioni, S., Lang, U., Sizonenko, P.C. & Aubert, M.L. (1982) Pituitary receptor sites for GnRH; effects of castration and substitutive therapy with sex steroids in the male rat. *Endocrinology* **110**, 70–79.
- Detta, A., Naik, S.I., Charlton, H.M., Young, L.S. & Clayton, R.N. (1984) Homologous ligand induction of pituitary gonadotrophin releasing hormone receptors in vivo is protein synthesis dependent. *Molec. cell. Endocr.* **37**, 139–144.
- Frager, M.G., Pieper, D.R., Tonetta, S., Duncan, J.A. & Marshall, J.C. (1981) Pituitary gonadotrophin releasing hormone (GnRH) receptors: effects of castration, steroid replacement and the role of GnRH in modulating receptors in the rat. *J. clin. Invest.* **67**, 615–623.
- Fraser, H.M. & McNeilly, A.S. (1982) Effects of immunoneutralization of luteinizing hormone-releasing hormone surges in the ewe. *Biol. Reprod.* **27**, 548–555.
- Kawakami, M. & Higuchi, T. (1979) Effects of active and passive immunization with LH-RH on the gonadotrophin secretion in the female rat. *Acta endocr., Copenh.* **91**, 616–628.
- Loumaye, E. & Catt, K.J. (1982) Homologous regulation of gonadotrophin-releasing hormone receptors in cultured pituitary cells. *Science, N.Y.* **215**, 983–985.
- Loumaye, E. & Forni, L. (1982) Regulatory actions of 17β -estradiol and progesterone upon pituitary GnRH receptors in vitro. *Endocrinology* **110**, Suppl., p. 286, Abstr. 825.
- Marshall, J.C., Barkan, A., Bourne, G.A., Duncan, J.A., Garcia-Rodriguez, A., Pieper, D.A. & Regiani, S. (1983) Physiology of pituitary gonadotrophin releasing hormone receptors. In *Recent Advances in Male Reproduction: Molecular Basis and Clinical Implications*, pp. 205–213. Eds R. D. Agata, M. Lipsett, P. Polosa & H. J. van der Molen. Raven Press, New York.
- Naik, S.I., Young, L.S., Charlton, H.M. & Clayton, R.N. (1984a) Pituitary gonadotrophin-releasing hormone receptor regulation in mice. I. Males. *Endocrinology* **115**, 106–113.
- Naik, S.I., Young, L.S., Charlton, H.M. & Clayton, R.N. (1984b) Pituitary gonadotrophin-releasing hormone receptor regulation in mice. II. Females. *Endocrinology* **115**, 114–120.
- Naik, S.I., Young, L.S., Charlton, H.M. & Clayton, R.N. (1985) Evidence for a pituitary site of gonadal steroid stimulation of GnRH receptors in female mice. *J. Reprod. Fert.* **74**, 615–624.
- Smith, M.A., Perrin, M.H. & Vale, W.W. (1983) Desensitization of cultured pituitary cells to gonadotrophin releasing hormone: evidence for a post-receptor mechanism. *Molec. cell. Endocr.* **30**, 85–96.
- Young, L.S., Speight, A., Charlton, H.M. & Clayton, R.N. (1983) Pituitary gonadotrophin releasing hormone receptor regulation in the hypogonadotrophic hypogonadal (hpg) mouse. *Endocrinology* **113**, 55–61.
- Young, L.S., Naik, S.I. & Clayton, R.N. (1984) Adenosine 3',5'-monophosphate derivatives induce GnRH receptors in cultured pituitary cells. *Endocrinology* **114**, 2114–2122.

Received 18 October 1984