REGULATION OF OVARIAN GONADOTROPIN RECEPTORS AND LH BIOACTIVITY DURING THE ESTROUS CYCLE

A. R. SOLANO, A. Garcia VELA, K. J. CATT and M. L. DUFAU

Molecular Endocrinology Section, Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20205, USA

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1. Introduction

Regulation of LH receptors in the testis [1,2] and ovary [3] by elevation of circulating gonadotropins is a well-recognized phenomenon, but the relation of such changes to the physiological control of gonadal function has not been established. Also, the biological potency of circulating LH has been found to change during the primate estrous cycle [4], suggesting that a more potent form of the hormone is released at mid-cycle. To analyze the contributions of receptor regulation and LH bioactivity to the cyclical control of ovarian function, we measured gonadotropin receptors and bio:immuno (B:I) ratios of pituitary and serum LH throughout the rat estrous cycle. These studies showed that the preovulatory surge of LH is released from a pituitary pool of high biological activity. Also, negative regulation of gonadotropin receptors and positive regulation of prolactin were clearly identified as physiological components of the ovarian cycle.

2. Materials and methods

Ovarian receptors and LH bioactivity were analyzed in animals with 4-day estrous cycles, sacrified at 18 h of estrous, metestrus and diestrus, and at several times before and after the luteinizing hormone (LH) surge at proestrus. Serum and pituitary LH content were measured by radioimmunoassay (RIA) and by rat interstitial cell testosterone bioassay (RICT assay) and expressed in terms of pure rat LH provided by Dr M. Jutisz (1 ng rLH = 22.7 ng rLH RP-1 by RIA; or 45.5 ng rLH RP-1 by RICT assay [5]). Rat prolactin (rPrl), rat FSH (rFSH), estradiol-17β (E_2) and progesterone were measured by RIA.

Ovarian receptors were determined by binding studies with ^{125}I-labeled human chorionic gonadotropin (hCG) and human folicle stimulating hormone (hFSH) for gonadotropin receptors, and human growth hormone (hGH) for prolactin sites [6]. The receptor affinity (K_a) was derived by Scatchard analysis of equilibrium binding data and receptor concentration was measured by Scatchard analysis and/or equilibration of ovarian sites with saturating hormone concentrations. Receptor occupancy was determined by elution of bound endogenous LH at 65°C and subsequent measurement of the released hormone by RIA.

Measurements of LH bio- and immuno-activity in serum and pituitary were performed as in [5].

3. Results and discussion

Initial studies performed to determine the RIA and RICT profiles of serum and pituitary levels throughout the estrous cycle, showed a marked increase in bio- and immunoactivity of serum LH at 20 h of proestrus. The serum LH B:I ratio was relatively constant (0.92 ± 0.08) throughout the cycle, except at the time of the LH surge, when it rose to 1.5 ± 0.2 ($p < 0.01$) (fig.1, above). Pituitary LH content increased to a maximum 6 h before the LH surge, then rapidly decreased to a nadir at the time of the LH surge. A decrease in the B:I ratio of pituitary LH, from 1.36 ± 0.07 at all other times of the cycle to 1.16 ± 0.16 ($p < 0.01$) at the time of the surge, accompanied the increase in serum B:I ratio observed

Address correspondence and reprint requests to M. L. D.: National Institutes of Health, Bldg. 10, 12N-216, Bethesda, MD 20205, USA

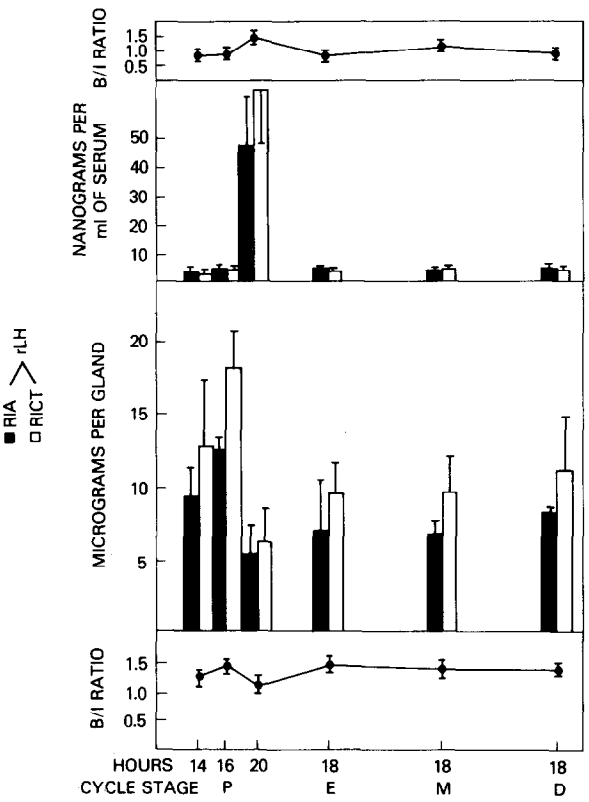

Fig.1. Serum LH levels and pituitary LH content measured by RIA and RICT assay during the rat estrous cycle. The stages of the cycle are indicated as proestrus (P), estrus (E), metestrus (M) and diestrus (D).

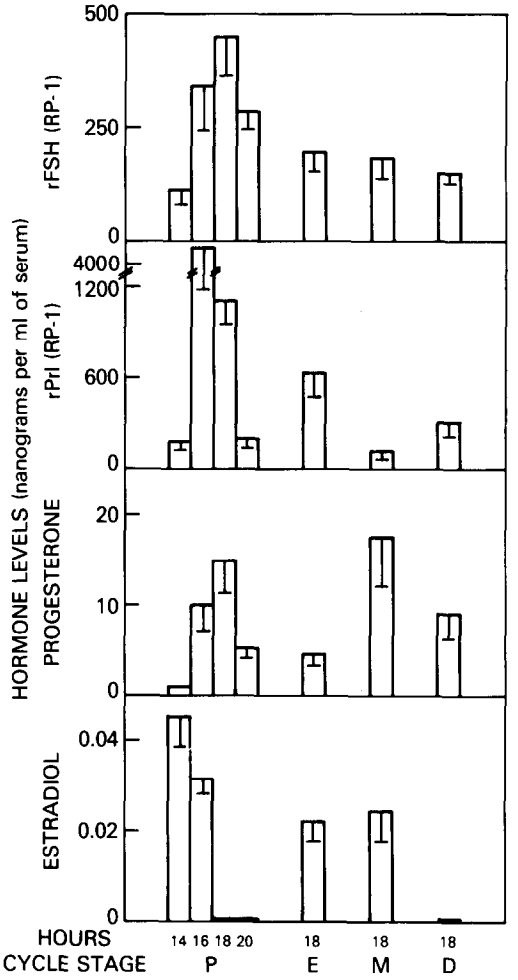

Fig.2. Measurements of serum FSH, prolactin, progesterone and estradiol (E_2) during the rat estrous cycle (mean ± SD, n = 6 animals).

during the LH surge (fig.1, below). We have observed an increase in plasma B:I ratio at the time of the mid-cycle LH peak in the rhesus monkey [4], and in the human during GnRH stimulation in the late follicular phase, near the time of the mid-cycle LH peak [7].

As noted in [8,9], serum FSH levels were highest at the time of the LH surge. Prolactin levels followed a similar pattern as LH and FSH during proestrus, and also showed a second peak at estrus. Serum progesterone increased at proestrus and at metestrus, in both cases following the peak in prolactin levels. Serum estradiol levels were maximal 6 h before the LH surge, and decreased to almost undetectable values at the time of the LH surge (fig.2).

Receptor binding concentration and affinity were determined at each of the stages of the estrous cycle. Analysis of the data derived from equilibrium binding studies by Scatchard analysis or saturation plots showed a single order of binding sites with similar affinities at all stages of the cycle (fig.3, right). The number of ovarian LH receptors was lowest at metestrus, increasing significantly at diestrus and reaching the highest value 2 h before the time of the LH surge. Following the preovulatory LH peak, the LH receptors decreased after 2 h to a nadir at 4 h, and remained low throughout estrus (fig.3, left). This decrease in receptors could not be accounted for by occupancy of receptors by endogenous LH. Also, solubilization of particulate ovarian preparations with Triton X-100 showed a similar degree of receptor loss, ruling out the presence of hindered or unavailable LH receptor sites. The significant decrease in total LH receptors 4 h after the LH surge indicates that down-regulation induced by endogenous hormone occurs as a physiol-

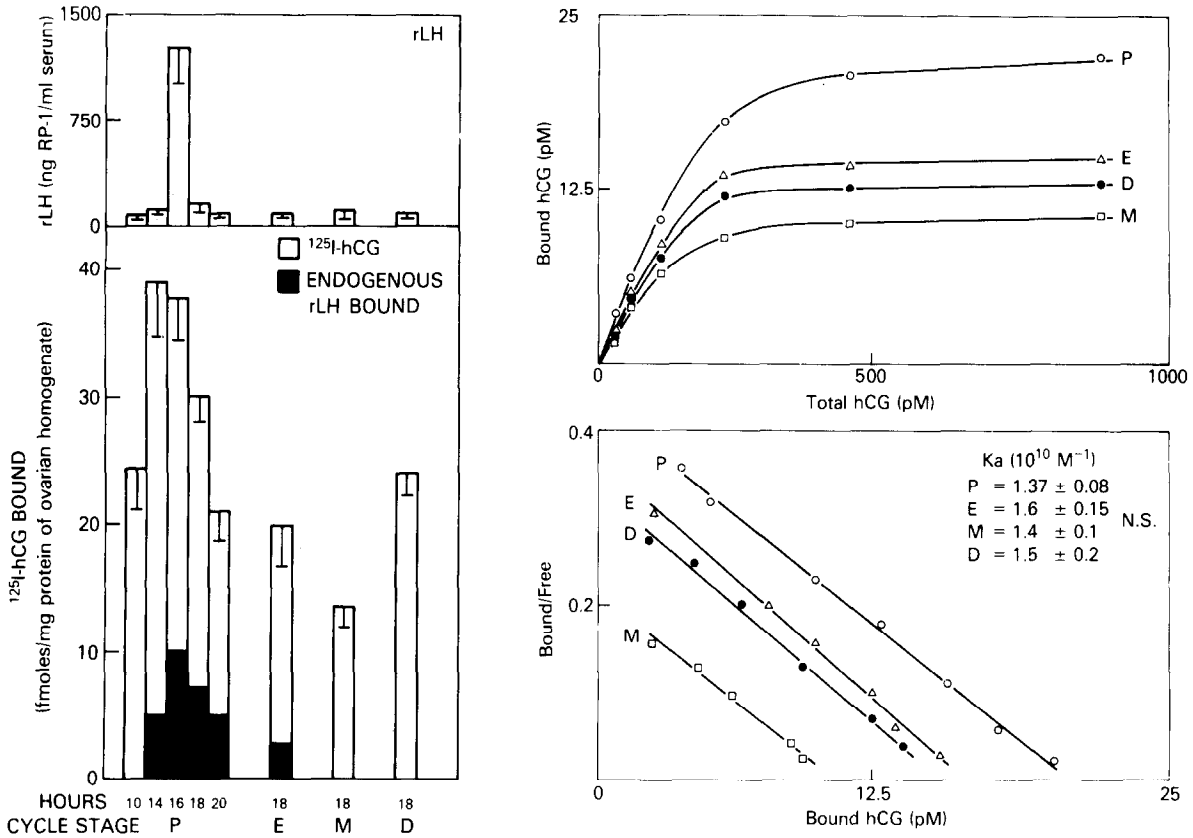

Fig.3. Left: Quantitation of LH receptors, and endogenous occupancy (above) and parallel measurements of serum LH levels by RIA (below). Right: Saturation curves (above) and Scatchard plots (below) of ovarian hCG receptor binding studies. K_a = affinity constant at equilibrium (mean ± SD, n = 5 animals).

ogical response in the ovary during the estrous cycle.

The pattern of FSH receptors during the cycle was similar to that observed for LH, with less prominent changes. The absolute number of FSH receptors was ~20% of the value for LH receptors (fig.4, left). A significant increase in FSH receptors was observed from metestrus to diestrus, with a further small increase at proestrus. The highest values occurred prior to and during the LH and FSH surge, followed by a significant decrease 4 h after the LH peak. No endogenous FSH bound to receptors was detectable at any time during the estrous cycle. The significant decrease in FSH receptors following the LH/FSH surge suggested the occurrence of down-regulation of these receptors by the trophic hormones. The K_a of the FSH receptors was 2×10^{10} M^{-1}, and no significant changes in binding affinity were observed during the estrous cycle. The increase in LH and FSH receptors at proestrus was accompanied by a significant increase in prolactin receptors at this time of the cycle (fig.4, right). Despite the very high serum prolactin levels observed at this stage, the prolactin receptors remained elevated with only a minor decrease 4 h after the surge, and a further decrease was observed at estrus. A second and more prominent increase in prolactin receptors was observed at metestrus, following the increase of serum prolactin at estrus. These results suggest a positive regulatory effect of serum prolactin on the ovarian lactogenic receptors. The K_a of the ovarian lactogenic receptor was 0.3×10^{10} M^{-1}, and did not change at the different stages of the estrous cycle.

The most significant findings of this study are summarized in the composite diagram of the relative changes of ovarian gonadotropin receptors through the cycle (fig.5). The receptor concentrations are plotted as percent of the maximal binding, to compare the relative changes observed in the different

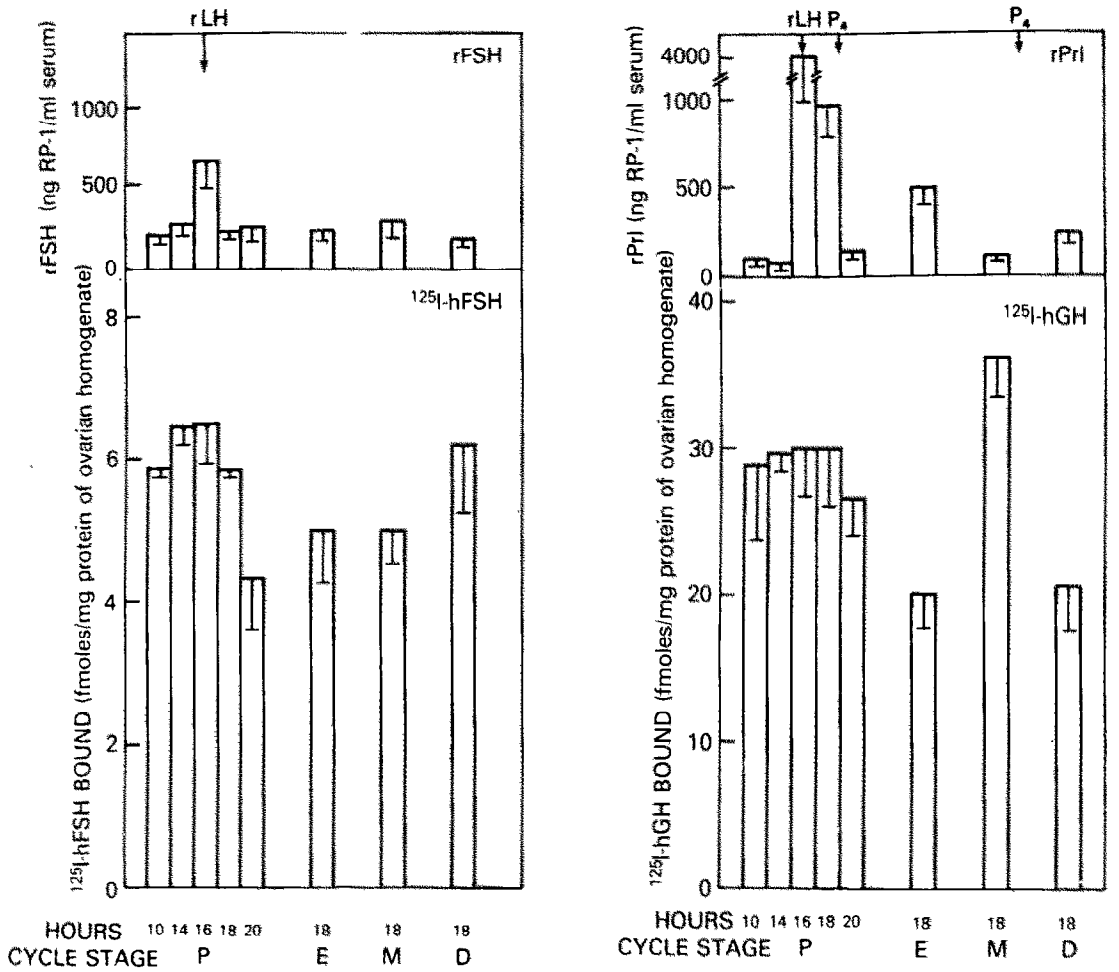

Fig.4. FSH (left) and prolactin (right) ovarian receptor concentration at the different stages of the estrous cycle (below). Serum levels of FSH and prolactin measured by RIA are shown above (mean ± SD, $n = 5$ animals). Progesterone = P_4.

hormone receptors. The most pronounced change in receptor number was observed for the LH receptors, which were maximum at proestrus here and in [10,11]. A similar but less prominent pattern was observed for the FSH receptors. In contrast, only a minor decrease of prolactin receptors were observed at proestrus, and a marked increase was found at metestrus following the estrus serum prolactin peak. This could represent a positive regulatory effect of prolactin on its own receptors at this stage of the estrous cycle, and may be important in the control of corpus luteum function. Such positive regulation has been described for hepatic prolactin receptors following pituitary grafts in hypophysectomized rats [12], but has not been reported during physiological receptor modulation by endogenous hormones. The increases of serum progesterone were concomitant with the increased prolactin receptor concentration. These findings are consistent with a permissive action of prolactin on steroidogenic function and secretion of the corpus luteum. This effect is possibly exerted through increases in early precursors of steroidogenesis, since prolactin has been found to increase cholesterol esterase activity and cholesterol ester pools in the ovary [13].

These studies on LH bioactivity and ovarian receptors have provided several insights into the regulation of pituitary–ovarian function during the estrous cycle.

(1) The preovulatory surge of LH is released from a pituitary pool of high biological activity. Recent studies [14] have indicated that the preovulatory rise in estrogen secretion could be responsible for release of LH with high bioactivity from the pitu-

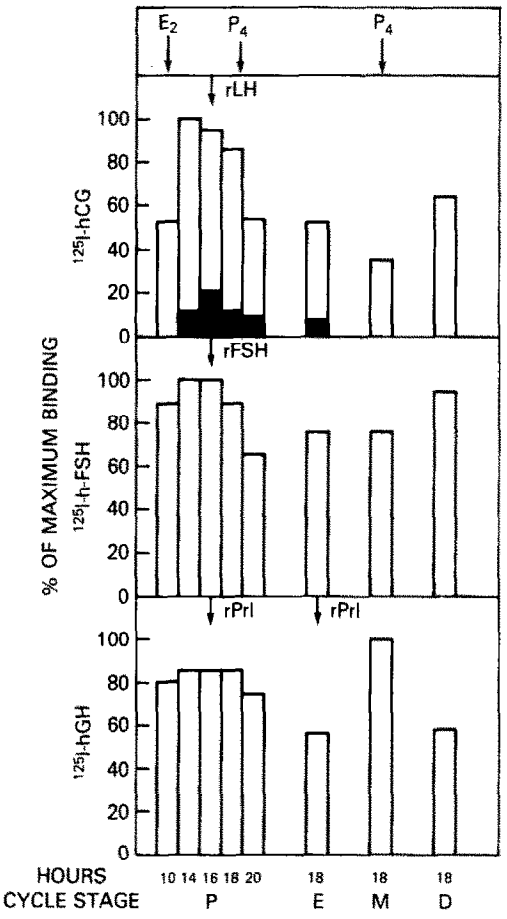

Fig.5. LH, FSH, and prolactin receptor concentrations throughout the estrous cycle, expressed as percent of maximal binding capacity to compare the relative changes observed in the individual hormone receptors.

itary into the circulation at the time of the mid-cycle surge. When castration was performed prior to the maximal increase in estrogen, the preovulatory rise in serum B:I ratio was prevented, despite the presence of an LH pool with high biological activity in the pituitary.

(2) The LH surge is followed by a rapid decrease in ovarian LH and FSH receptors during the day of proestrus, and loss of these receptors was not due to occupancy by endogenous hormone.

(3) Up-regulation of prolactin receptors appears to occur at metestrus after the endogenous increase of serum prolactin at estrus. The concomitant increases in serum progesterone which occurred at the time of the increase of prolactin receptors could be related to prolactin action upon the ovary. These changes in hormone receptors are particularly significant since the marked up-and-down regulation observed in the rat ovary is detectable above the background binding due to the concomitant presence of follicles and corpora lutea from several cycles that occurs in incomplete ovulators such as the rat.

Such findings provide clearcut evidence that endogenous regulation of gonadotropin and prolactin receptors are important physiological events in the normal ovarian cycle.

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