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Hormonal regulation of LH receptor mRNA and expression in the rat ovary

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Agonist-induced changes in expression and mRNA levels of luteinizing hormone (LH) receptors were compared during stimulation of ovarian follicular maturation and luteinization by gonadotropic hormones. Three major species of LH receptor mRNA, 5.8, 2.6 and 2.3 kb, were present throughout differentiation and changed similarly, the 5.8 kb species being consistently more abundant than the smaller forms. The increased expression of plasma-membrane LH receptors in preovulatory follicles and luteinized ovaries and their homologous down-regulation during follicular and luteal desensitization were closely correlated with the steady-state receptor mRNA levels. The reappearance of LH receptors following desensitization during the luteal stage was preceded by an increase in mRNA levels. These studies have demonstrated that the expression of LH receptors during follicular maturation, ovulation and desensitization is related to the prevailing levels of receptor mRNA in the ovary.

Ovary; LH receptor; mRNA; Gonadotropin

1. INTRODUCTION

Gonadotropic hormones exert their multiple tissue-specific effects on ovarian function in a temporal and concentration-dependent manner through specific receptors on ovarian cells. Follicle-stimulating hormone (FSH) stimulates follicular growth and induces the expression of LH receptors in granulosa cells [1-4]. Such receptors have high binding affinity and specificity for LH and hCG (human chorionic gonadotropin) and are sometimes referred to as LH/hCG receptors. Moderate and sustained increases of serum LH facilitate the induction of LH receptors by FSH. However, the endogenous LH surge and administration of an ovulatory dose of hCG cause a temporary loss of LH receptors in ovulated follicles. A secondary increase in LH receptors after induction of ovulation by hCG occurs during follicular luteinization and formation of corpora lutea [4].

The PMSG (pregnant mare serum gonadotropin)/hCG primed pseudopregnant rat is a well-established model for the study of regulation of ovarian functions by gonadotropins [5]. The intrinsic FSH and LH activities of PMSG stimulate the granulosa cells of small preantral follicles to grow and differentiate, with acquisition of LH and prolactin receptors and aromatase activity. The subsequent administration of an ovulatory dose of hCG induces the antral follicles to ovulate and luteinize. About 8-10 days after PMSG treatment, the ovulated follicles become heavily luteinized and display maximal increases in LH and prolactin receptors [6-8]. At this stage, further administration of hCG causes a marked loss of LH receptor sites, and desensitization of LH-stimulated adenylate cyclase, cyclic AMP production, and steroidogenesis [6,9]. The developmental stage-specific regulation of LH receptors is a relatively complex process, and could be associated with multiple mechanisms including receptor occupancy, hormone-receptor internalization, receptor unmasking, recycling, degradation, and synthesis. In the present study, we utilized the pseudopregnant rat model to investigate the correlation between steady-state LH receptor mRNA levels and the expression of receptor sites during various stages of ovarian differentiation and homologous receptor down-regulation. Our results have demonstrated coordinate regulation of LH receptors and mRNA levels during gonadotropin-induced up-regulation and homologous down-regulation of the ovarian receptor sites.

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2. MATERIALS AND METHODS

Ovaries were removed at various stages of hormone treatment as shown in Fig. 1, for analysis of LH receptors and mRNA levels. Total ovarian RNA was prepared as described by Chirgwin et al. [10] and poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography. Ovarian LH receptor cDNA was isolated from a size-selected ovarian cDNA library [11]. The library was screened with three end-labelled synthetic oligonucleotides (10^9 dpm/μg) based on regions of the recently cloned cDNA for the rat LH receptor [12] at positions 108-81, 462-435 and 1227-1200. A full-length cDNA clone was characterized and used as the probe in this study. For Northern blot analyses, 7 μg of poly(A)$^+$ RNA were denatured with glyoxal, resolved in 1% agarose gels, transferred to Zetabind nylon filters and subsequently hybridized with random primed labelled full-length (2.4 kb)

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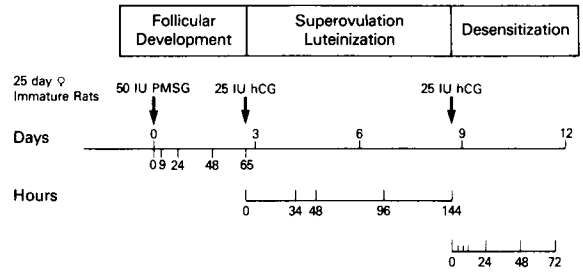

Fig. 1. Diagram of the experimental design. 25-day old immature female rats (Sprague-Dawley) were injected subcutaneously (s.c.) at the time points indicated by arrows with 50 IU PMSG, followed by two injections of 25 IU hCG (1st and 2nd doses). Ovaries were removed at specified times (bars). Overlapping points were allowed for the different groups.

ovarian LH receptor cDNA (spec. act. 1-2 × 10^9 dpm/μg) for 18 h followed by autoradiography [11,13]. Subsequently, blots were stripped and rehybridized to an 0.6 kb β-actin cDNA probe to normalize the quantization of specific LH receptor mRNA species. Radioactivities were quantitated from the blots with a Radioanalytic Imaging System (AMBIS Systems Inc., San Diego, CA). LH receptor binding capacity was derived from ^{125}I-hCG binding studies in ovarian membranes, as previously described [14].

3. RESULTS AND DISCUSSION

Northern blot analyses of ovarian poly(A)$^+$ RNA using the LH receptor cDNA probe revealed that three major bands of 5.8, 2.6 and 2.3 kb, and a minor 4.4. kb band, were present at most stages of ovarian maturation, while three other minor species (8.0, 1.9 and 1.4 kb) were only observed at certain developmental stages (Figs 1 and 2A-D). The immature ovary possesses low levels of both LH receptor mRNA and LH receptors (15 fmol/ovary) (Fig. 3). After administration of PMSG, the levels of the major mRNA species (5.8, 2.6 and 2.3 kb) were significantly elevated at 24 h and increased progressively to 14-fold above the untreated levels at 65 h (Fig. 2A above; Fig. 3 below). One of the minor mRNA species, the 4.4. kb band, was present in untreated immature ovaries and increased with the major mRNAs, while the 1.4 and 1.9 kb bands were barely detectable at 48 and 65 h after PMSG administration. The LH receptors showed an initial significant decrease at 5 h, probably due to occupancy by the exogenous gonadotropin, and then increased in parallel with the mRNA changes and reached maximal levels of 5-fold above pretreatment values at 65 h (Fig. 3, above).

After administration of an ovulatory (first) dose of hCG, receptor mRNA levels declined by 50% within the first 34 h (Fig. 2A and Fig. 3, below). This was followed by a marked and progressive increase to reach maximal levels of the more abundant mRNA species, as well as the minor 4.4, 1.9 and 1.4 kb bands, 6 days after the 1st hCG injection. At days 4 and 6, an additional band of 8.8 kb was observed (Fig. 2B). This species was barely detectable 65 h after PMSG injection and two days after the initial ovulatory dose of hCG. These changes in mRNA levels were accompanied by an initial decrease in ^{125}I-hCG binding within 34 h, followed by progressive increases at 4 and 6 days to levels of 4000 fmol per ovary at the time of maximal luteinization (Fig. 3, above).

Administration of a second (desensitizing) dose of hCG caused a sharp decrease in LH receptor mRNA from 6 h to almost undetectable levels at 24 h (Fig. 2C,D), with partial recovery during the following two days. The LH receptors also declined markedly, to one-tenth of the binding capacity observed at the peak of luteinization. Minor recovery occurred during the following 4 days (Fig. 3), when receptors levels began to increase ($P<0.01$) and returned to the levels (not shown, [6]) observed in heavily luteinized ovaries 8-10 days after the first dose of hCG (Fig. 3, below).

The up-regulation of LH receptors in preovulatory follicles and luteinized ovaries, and their down-regulation during follicular and luteal desensitization, were closely associated with the steady-state receptor mRNA levels. However, the rate of the increase in LH receptors after the 1st hCG dose was 2-fold greater than that of mRNA (0.0288 vs 0.0142, 0.0135 cpm·h^{-1}), indicating that hormone-stimulated variations in cell-surface receptor number are not solely dependent on de novo synthesis.

Fluctuations in the concentrations of the major 5.8, 2.6 and 2.3 kb mRNA species during follicular development (after PMSG injection) and subsequent luteinization (after 1st hCG dose) were also significantly correlated ($r=0.240 \pm 0.0391$ and 0.263 ± 0.032, respectively). The levels of the 5.8 kb species were significantly higher (4.6 ± 1.3 ($\pm$SD)-fold) than those of the 2.3 and 2.6 species (Fig. 3, below). However, the ratio between the 5.8 and 2.6 plus 2.3 kb species did not vary significantly, indicating that hormonal treatment did not differentially affect the larger and smaller forms. The increase in 5.8 kb mRNA following the marked down-regulation caused by the desensitizing (2nd) dose of hCG was more pronounced than that observed for the smaller forms. The increase in both mRNAs was followed by a modest rise in receptors that was significant on the 4th day after the 2nd hCG dose (Fig. 3, above) and continued to rise thereafter as previously demonstrated [6]. The delay in appearance of receptors in this case could be attributed to induction of luteinization in a further group of mature follicles.

Although the function of these individual mRNA species and their translatable potential to form mature receptor protein is not yet known, it is possible that the larger forms (8.8, 5.8 and 4.4. kb) are precursor mRNA forms while the smaller species (2.6, 2.3, 1.9 and 1.4 kb) represent mature messages. Also, some or all species could arise from alternative splicing since the LH receptor gene, in contrast to the β-adrenergic and most other G-protein receptors, has at least several introns located

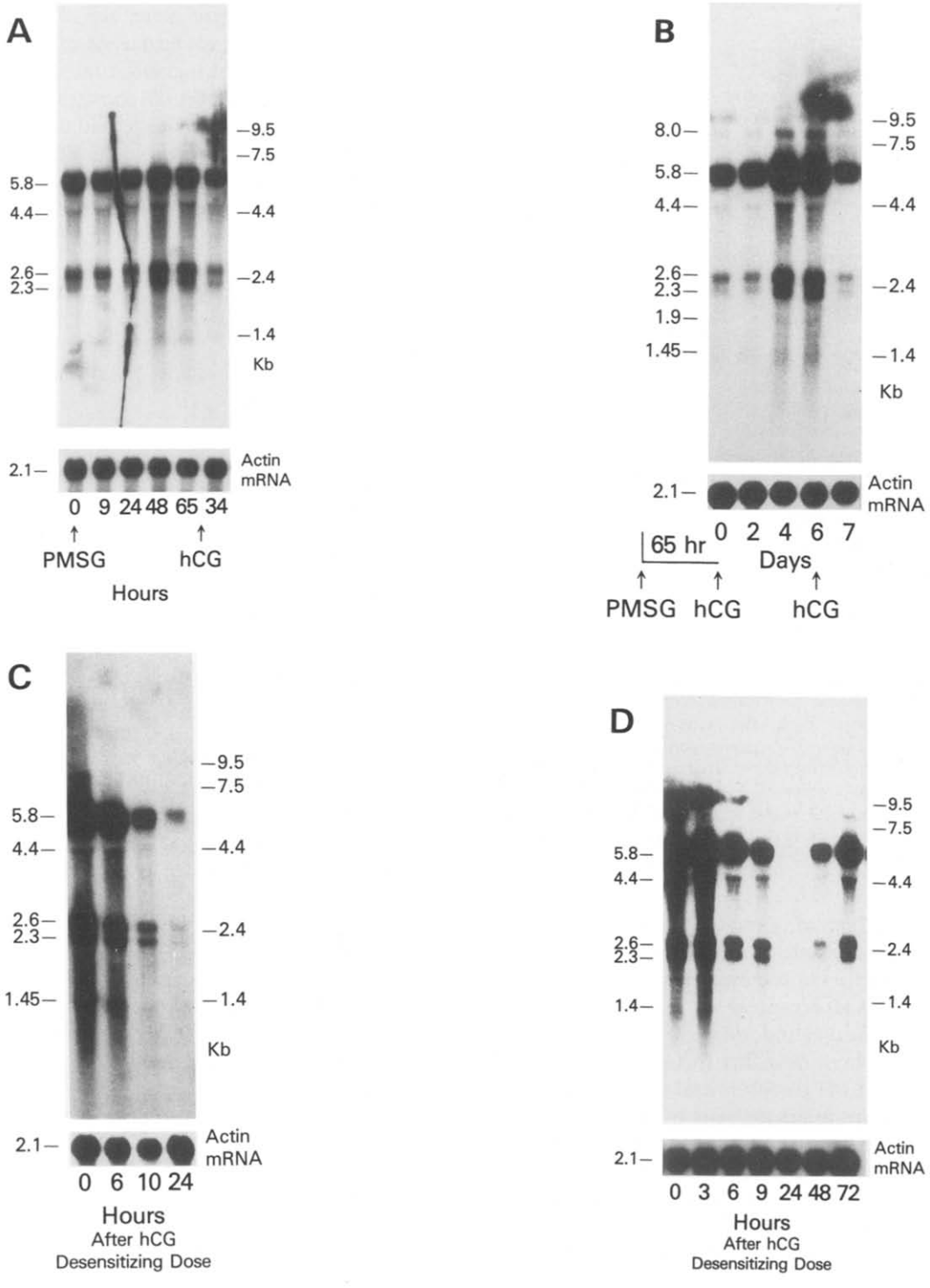

Fig. 2. Northern blot analyses of LH receptor mRNA. (Upper panel) Shows the autoradiographs (A and B) of ovarian LH receptor mRNAs during follicular development, superovulation and luteinization. Lanes are: (A) 0 (control), 9, 24, 48 and 65 h following PMSG treatment; 34 h after hCG (1st dose); (B) 0 (65 h after PMSG), 2, 4, and 6 days after 1st hCG dose and 24 h after 2nd hCG dose. (Lower panel) Shows the autoradiographs (C and D) of LH receptor mRNAs of luteinized ovaries at 0–72 h following the 2nd hCG dose. RNA standards are indicated on the right, and the deduced sizes of mRNA species on the left side of each blot. On the bottom of each blot are shown the corresponding β-actin mRNA levels.

within sequences coding for the extracellular domain of the receptors [15,16]. Several cDNA species have been isolated from pig and rat testicular and ovarian libraries including a 2.1 kb cDNA lacking sequences that code for transmembrane regions [15–17]. Genomic analysis of the LH receptor gene indicates that this species is a product of alternative splicing [15].

The concentration-dependent and developmental

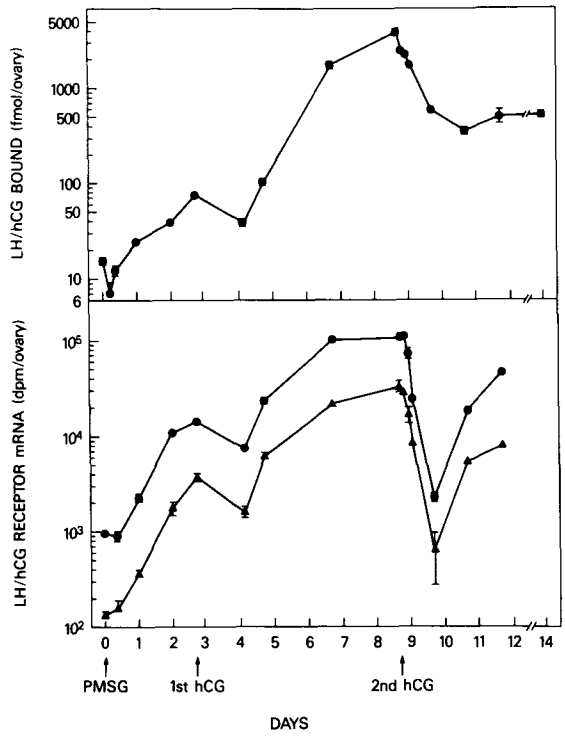

Fig. 3. Changes in LH/hCG receptor binding capacity and steady-state receptor mRNA levels. The upper panel shows the binding of ^{125}I-hCG to the membrane preparation from various stages of pseudopregnant rat ovaries. Each value is the mean ± SE (fmol per ovary, $n = 3$). The lower panel shows the quantitation of LH receptor mRNAs expressed as dpm/ovary normalized to the corresponding β-actin mRNA. (●—●) represents 5.8 kb LH receptor mRNA; (▲—▲) represents 2.6 + 2.3 kb LH receptor mRNA. Bars indicate mean ± SE of triplicate or mean ± range of duplicate blot analyses. Similar profiles were observed with mRNA/actin ratios.

stage-specific actions of gonadotropins indicate the complexity of the regulatory mechanisms operating in gonadal target cells. Low concentrations of hCG cause up-regulation of LH receptors in granulosa cells of preovulatory follicles [1] and in Leydig cells of the testis [18]. In contrast, high doses of hCG cause profound down-regulation of LH receptors and desensitization of adenylate cyclase in luteinized cells of pseudopregnant rat ovaries and mature Leydig cells, but up-regulate LH receptors in the fetal population of Leydig cells [6,18]. LH and FSH receptors are coupled to the guanyl nucleotide regulatory protein, Gs, and exert their actions mainly through cAMP-dependent mechanisms [3,19]. Cyclic AMP is a transcriptional regulator and may play diverse roles through activation of specific regulators at different stages of differentiation. Elucidation of the gene structure of the LH receptor will permit more detailed analysis of homologous and heterologous receptor regulation at the transcriptional level, and the definition of changes that result from mRNA processing and/or degradation at individual stages of ovarian development. These studies have demonstrated that gonadotropin-induced expression, up-regulation and down-regulation of LH receptors are highly correlated with the levels of LH receptor mRNA in the ovary.

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