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MULTIPLE AND DIFFERENTIAL REGULATION OF OVARIAN PROLACTIN RECEPTOR MESSENGER RNAs AND THEIR EXPRESSION

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Summary: Three predominant prolactin receptor (PRL-R) mRNA species of 9.7, 2.1 and 1.8 kb and two minor species of 4.6 and 2.6 kb were demonstrated in the rat ovary. The long transmembrane form of the ovarian PRL-R is derived from the 9.7 kb and 4.6 kb species. The 2.6 kb species could be a variant form of this receptor with truncated or altered extracellular domain, while the 1.8 and 2.1 kb species correspond to the short transmembrane receptor form with truncated cytoplasmic domain. All five mRNA species were expressed and regulated in a developmental stage-specific manner during gonadotropin-induced ovarian maturation, luteinization and subsequent desensitization by gonadotropin treatment. Coordinate regulation of prolactin receptor binding activities and mRNA levels was observed during gonadotropin-induced heterologous up- and down-regulation of ovarian receptor sites.

Gonadotropins and lactogens of pituitary and placental origin are the primary protein hormones regulating the ovarian functions. Gonadotropins are responsible for the induction of follicular growth, ovulation, and luteinization (1). Prolactin plays an essential role in the formation and maintenance of a functional corpus luteum and also exerts luteolytic actions in several animal species (2). Prolactin actions are initiated through hormone interaction with specific membrane receptors which develop in the granulosa cell simultaneously with LH receptors under the influence of follicular stimulating hormone (FSH), and are further increased thereafter by homologous up-regulation and by the influence of luteinizing hormone (LH) (2). Physicochemical analysis of purified ovarian lactogen receptors has revealed structural heterogeneity (2-5). This was also shown by the identification of cDNA clones coding for distinct ovarian receptor isoforms (6, 7). One of these is a membrane-anchored receptor bearing an extended cytoplasmic domain containing a region with unique sequences, another is a truncated receptor with a short cytoplasmic domain identical to the rat liver receptor, and a third is lacking the transmembrane region that codes for a binding site and has potential for expression as a soluble protein. The *in vivo* model of hormone-induced pubertal development, ovulation and luteinization has been extensively used for studies on the regulation of LH and PRL receptors (2, 8, 9). Administration of human chorionic gonadotropin (hCG) causes a temporary loss of lactogen receptors in luteinized ovaries with maximal receptor expression (9).

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Such heterologous down-regulation of the PRL-R is accompanied by more profound homologous down-regulation of LH/hCG receptors (8). In this study, we have investigated the nature and the induction pattern of PRL-R mRNA species and have correlated changes of the steady-state levels of PRL-R mRNA and the membrane lactogen receptor expression during hormonal induction of the ovarian differentiation and desensitization.

MATERIALS AND METHODS

25-day-old immature female Sprague-Dawley rats were injected subcutaneously (s.c.) with 50 IU of pregnant mare serum gonadotropin (PMSG), a preparation with FSH and LH activities, followed 65 h later by an injection of 25 IU hCG (1st hCG). These treatments will cause follicular development (after PMSG), ovulation and luteinization (after 1st hCG) and markedly induce ovarian PRL and LH/hCG receptors (8, 9). Six days after hCG administration (the time of maximal luteinization) animals received a second s.c. dose of hCG (25 IU) (10). Rats were decapitated and ovaries were removed at various stages of hormonal treatment, rapidly frozen in liquid nitrogen, and stored at -70°C for further mRNA analysis and receptor binding assay.

Total RNA and Poly(A)$^+$ RNA were prepared as previously described (10). α-[^{32}P]dCTP labelled cDNA probes (1-2 x 10^9 cpm/μg) were used to define different species of the ovarian PRL-R mRNA (Fig. 1). These include the PRL-R cDNA coding for the ovarian long form PRL-R (R1 cDNA, 2.1 kb) (6). The rest of the probes are restriction fragments representative of specific domains of R1 cDNA (Fig. 1, above), including EcoRI/NcoI (E/N, 0.71 kb), StuI/HincII (S/H, 0.42 kb), HincII/EcorRI (H/E, 0.44 kb), NcoI/AlwNI (N/A, 0.18 kb), NcoI/StuI (N/S, 0.37 kb), AlwNI/StuI (A/S, 0.19 kb) and AlwNI/EcoRI (A/E, 1.2 kb). Major mRNA species were quantitated by scanning on the AMBIS Radioanalytic Imaging System . Blots were stripped for rehybridization to other probes including 0.6 kb β-actin cDNA probe, which was used to normalize the quantitation of specific mRNA species. Slot blots of RNA samples were subjected to hybridization and quantitation as the Northern blot. PRL and LH/hCG receptor binding capacities were derived from [^{125}I]hGH and [^{125}I]hCG binding studies in ovarian membranes as previously described (11).

RESULTS AND DISCUSSION

Northern blot analyses using 2.1 kb R1 cDNA probe coding for the ovarian long form PRL-R revealed five mRNA species of 9.7, 4.6, 2.6, 2.1 and 1.8 kb in the luteinized rat ovary (Fig. 1, below). The predominant 9.7 kb and the minor 4.6 kb species were recognized by all probes shown in Fig. 1. The 1.8 and 2.1 kb major forms were recognized by probes containing regions corresponding to the 5' extracellular domain, transmembrane region or adjacent part of the intracellular domain (probes E/N, N/A, A/S, A/E), and specifically recognized by a PCR-generated probe corresponding to sequences of 3' 75 bp coding region and adjacent 3' 185 bp non-coding region (nucleotides 856-1116) unique to the short transmembrane form of rat ovarian and liver PRL-R (data not shown). These two species were not recognized by the 0.42 kb probe (S/H) corresponding to the cytoplasmic unique area of the long form ovarian PRL-R or by probe H/E bearing the 3' sequences. The 2.6 kb was only recognized by the transmembrane and cytoplasmic probes (S/H and H/E) but not by probe E/N containing most of the extracellular domain. From these results, we could deduce that the long form of the ovarian PRL-R is derived from the 9.7 kb and the 4.6 kb species. The two distinct species of 1.8 and 2.1 kb code for the short form PRL-R protein bearing a truncated cytoplasmic domain with differences most likely in the length of the non-coding regions. The 2.6 kb species could represent

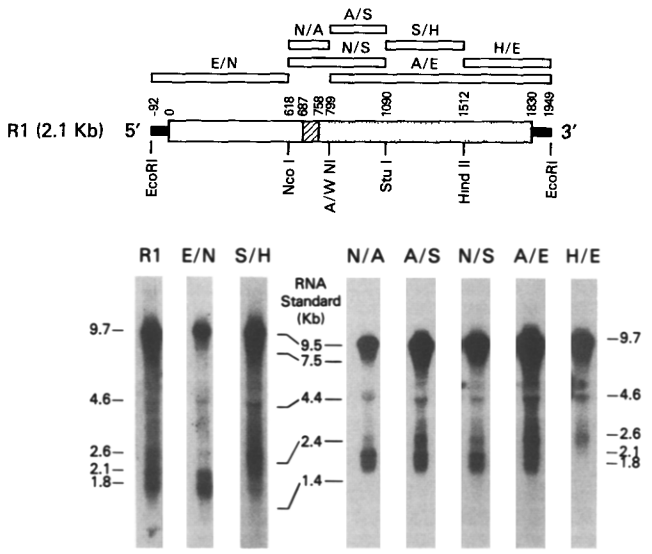

Fig. 1. **Identification of different species of ovarian prolactin receptor mRNA in rat ovaries using probes derived from the long form of ovarian cDNA (R1 cDNA).** The upper panel shows R1 cDNA and restriction fragments of R1 cDNA used as probes. The open area represents the extracellular domain of the PRL-R cDNA; the cross-hatched portion, the transmembrane region; the stippled area, the cytoplasmic domain and the darker filled ends, the 5' and 3' untranslated regions. The lower panel shows the Northern blot analyses of poly $(A)^+$ RNA from luteinized rat ovaries with mRNA species revealed by hybridization with probes shown in the upper panel.

a variant form of the receptor bearing a largely truncated extracellular domain or an altered extracellular domain derived from 5' alternatively spliced sequence which was not recognized by the extracellular domain probe (E/N). Although we have characterized a cDNA coding for a putative soluble form of the ovarian PRL-R (R2 cDNA) containg 150 amino acids of the extracellular domain including the signal peptide (6), we were not able to identify the corresponding message, suggesting low abundance or rapid turn-over of this species.

Multiple and differential regulation of various PRL-R mRNA species were observed during the gonadotropin-induced differentiation. The 9.7 kb species was the only detectable species in immature ovaries and appears to be of major importance during the early follicular development and ovarian maturation since it is the predominant ovarian form and follows closely the changes of PRL-R binding activity. The 2.6 kb, minor but clearly discernable band, was the earliest induced species (at 9 h of PMSG treatment). The induction of the 1.8 and 4.6 kb forms was observed at 24-48 h [Figs. 2 (A, B)]; while the 2.1 kb species was induced only after 9-24 h of the 1st hCG treatment [Fig. 2 (C, E) and 3]. The sequential induction of these mRNA species reflects a developmental stage-specific expression of the PRL-R gene. The 1st hCG treatment caused rapid down-regulation of these forms within one hour and minimal levels were reached at 3 h for the 1.8 kb and at 9 h for the 9.7 kb with a rapid recovery at 6 h for the former and at 24 h for the latter [Fig. 2 (C, D) and 3]. Subsequently all forms increased over the next 4 days reaching peak values at the time of maximal luteinization [Fig. 2 (E,F) and 3]. Following the 2nd hCG dose the 9.7 kb mRNA declined markedly at 3 h reaching the lowest level at 9 h followed by minor recovery at 24 h [Fig. 2 (E,F,G,H) and 3], while

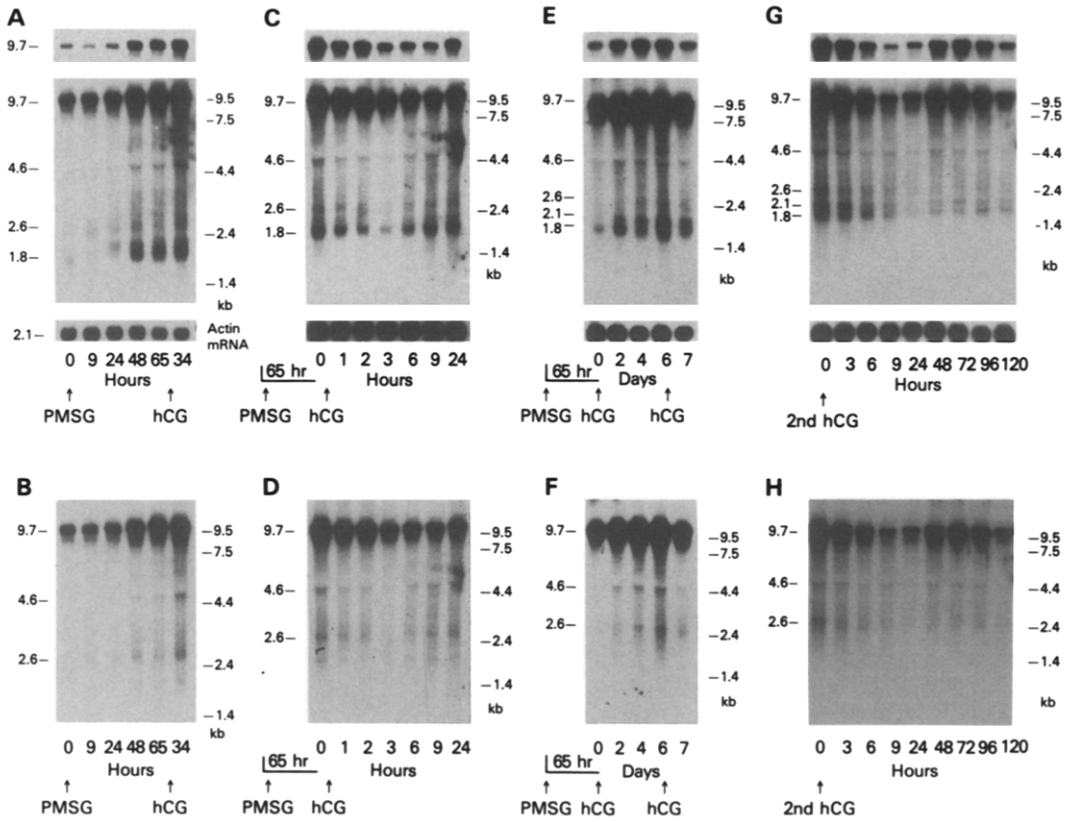

Fig. 2. **Autoradiograms of Northern blot analyses of PRL-R mRNA during follicular development, superovulation and luteinization.** Hybridization was first performed with the R1 cDNA probe (upper panel, A,C,E, and G) and subsequently rehybridized to the 0.42 kb StuI/Hinc II probe (unique cytoplasmic region of R1) after stripping the blots (lower panel, B,D,F and H). Blots are: (A) and (B), 0 (control) 9, 24, 48 and 65 h following PMSG treatment and 34 h after 1st hCG dose; (C) and (D) 1-24 h after the 1st hCG dose; (E) and (F) 2, 4, 6 days after 1st hCG dose and one day (day 7) after 2nd hCG dose; (G) and (H) 3-120 h after 2nd hCG. RNA standards are on the right, and sizes of mRNA species on the left side of each blot. On the top of the upper panel are shown shorter exposure of the 9.7 kb species. The corresponding β-actin mRNA is also shown on the bottom of each upper panel blot.

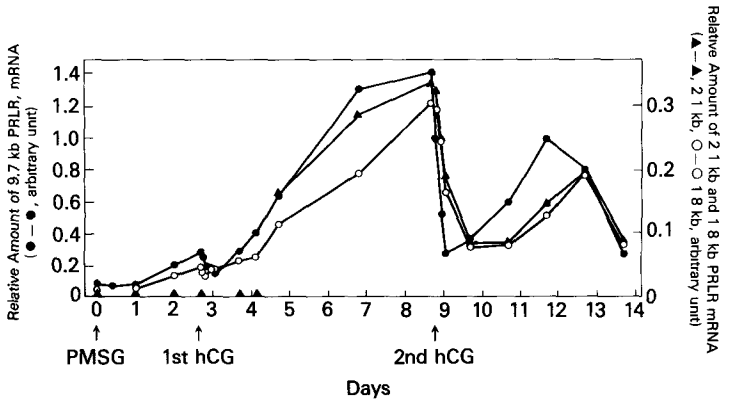

Fig. 3. **Profiles of 9.7, 2.1 and 1.8 kb PRL-R mRNA species derived from Northern blots hybridized with R1 cDNA.** Values were derived from closely agreeing duplicate blots normalized by the corresponding β-actin mRNA, and expressed in arbitrary units.

the 1.8 and 2.1 kb species showed slower reduction and recovery than the 9.7 kb species, suggesting that the long form and the short form of PRL-R may be involved in different functions. It has been reported that only the long form PRL-R was able to transduce the stimulatory effect of prolactin on the milk protein gene transcription (12), while the short form of the receptor with truncated intracellular domain was postulated to act as transport or clearance receptor (5). Similar changes were observed for the minor 2.6 and 4.6 kb species after the 2nd hCG dose [Fig. 2 (G,H)]. The reduction was followed by partial recovery of all forms and levels maintained until 72-96 h of 2nd hCG with a marked fall thereafter.

The overall pattern of changes in PRL-R mRNA and membrane PRL-R induced by hormonal treatments at different stages of ovarian maturation is shown in Fig. 4. The profile derived from the slot blot analysis of ovarian PRL-R mRNA represents integrated changes of various mRNA forms, with each species contributing to the extent of their abundance (Fig. 4, above). Low PRL-R mRNA levels were observed in immature ovaries and no significant changes were observed within 24 h of PMSG treatment (Fig. 4, above), while the receptor binding activity was elevated significantly (from 144±31 to 234±19 fmol/mg protein, $p<0.02$, n=3). This suggested that factors other than mRNA levels such as receptor unmasking and receptor recycling may contribute to the observed differences during this early follicular development. Thereafter increases of mRNA levels and further increases of the receptor binding activity were observed at 65 h of PMSG treatment. The subsequent hCG administration (the 1st hCG) caused a moderate down-regulation at 9 h of both PRL-R mRNA (by

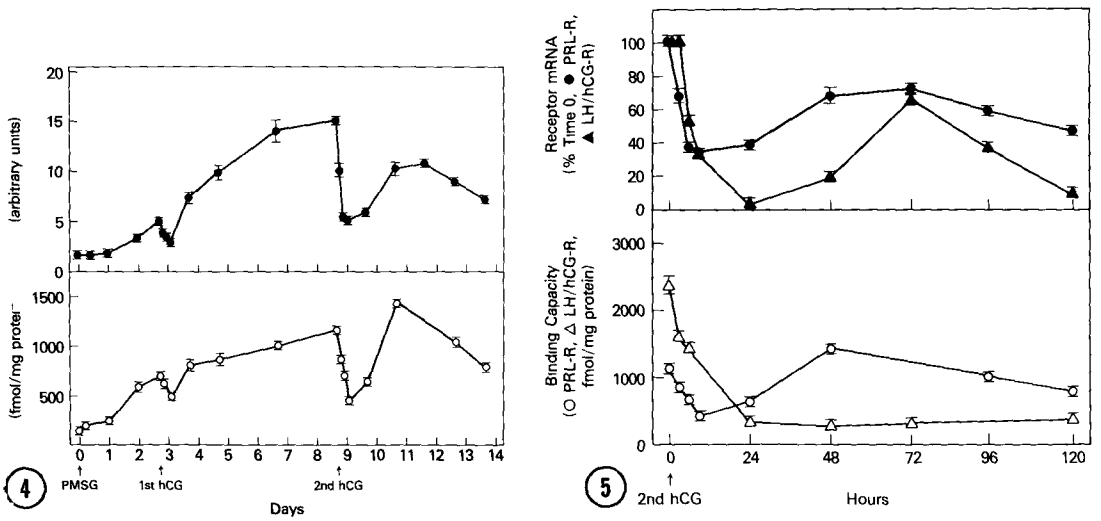

Fig. 4. **Changes of PRL-R mRNA steady-state levels and membrane receptors in gonadotropin-treated rat ovaries.** The upper panel shows slot blot analyses of ovarian poly $(A)^+$ RNA using 2.1 kb R1 cDNA probe. The lower panel shows the [^{125}I]hGH binding profile of the membrane preparations from various stages of ovarian development induced by gonadotropin treatment. Each point is the mean ± SE, n=3.

Fig. 5. **Comparative studies of the modulation of ovarian PRL-R and LH/hCG membrane receptor expression and their corresponding receptor mRNAs after administration of desensitizing dose of hCG to rats at the time of maximal luteinization.** The upper panel shows changes of the prolactin and LH/hCG receptor mRNA. Each point was in triplicate and normalized by the duplicate blot hybridized with β-actin probe. Data are expressed as % of controls (at time zero). The lower panel shows corresponding changes of receptor binding capacities. Each point is the mean ± SE of n=3.

43%) and receptor binding activity (by 39%) followed by a rapid recovery at 24 h. The transient loss of both the PRL-R and its mRNA at this stage of ovarian development has not been previously reported although an *in vivo* ovulatory dose of hCG was shown to cause down-regulation of the ovarian LH/hCG receptor contents as well as receptor mRNA levels (10). In the subsequent up-regulation occurring from 24 h after the first hCG dose to the time of maximal luteinization the mRNA levels showed a faster and more pronounced increase than those in membrane receptor binding activity (increased by 100% for mRNA vs. 30% for binding activity). However, changes in the total membrane receptor population appears to follow more closely the receptor mRNA levels, since we previously observed that the gonadotropin-induced increase of exposed lactogen receptors in ovarian membranes was relatively slower and less marked than that of the cryptic particulate lactogen receptors that were unmasked by detergent treatment of ovarian membranes (12). The subsequent administration of a desensitizing dose of hCG (2nd hCG) caused an abrupt reduction of receptor mRNA levels (by 65% at 9 h) and receptor binding sites (by 62% at 9 h), which was followed by partial recovery at 24 h and maximal recovery at 48-72 h with a gradual decline thereafter. The down-regulation induced by the 2nd hCG dose was shown to be more pronounced and lasted longer than that at the early stage. These differences were probably accounted for by the ovarian cell types and/or the levels of LH/hCG receptors present at different stages. The secondary loss of both receptor binding and mRNA levels following their brief recovery from the second down-regulation could be due to advanced luteolysis of corpora lutea. The sustained loss of LH/hCG receptor induced by the second hCG may result in a reduced responsiveness of the corpus luteum to produce progesterone and lead to functional luteolysis, followed by structural luteolysis charateristic of the involution and degeneration of the corpus luteum (13). The earlier recovered PRL-R may be also involved in the PRL-controlled lysis of corpora lutea (2), while its subsequent loss may result from the membrane regression following the functional luteolysis. The correlation of the PRL-R mRNA and the PRL-R binding activity regulated by the heterologous gonadotropins was well demonstrated from above results. However, it remains to further clarify whether changes of the steady-state levels of PRL-R mRNA are related to the altered gene transcription or mRNA turn-over and how the gonadotropin regulates the expression of PRL-R gene on the mRNA level. The PRL-R and its mRNA levels displayed less profound heterologous down-regulation and more rapid recovery than the homologous down-regulation of LH/hCG receptors following the 2nd hCG dose (Fig.5). The concomitant loss of LH and PRL receptors in the ovary reflected a functional association between the two receptors (9). The down-regulation of LH and PRL receptor mRNAs also suggests that a common mechanism may be involved in the regulation of the two receptor mRNAs following the interaction of the trophic hormone with the membrane LH/hCG receptor. cAMP has been shown to mediate the LH/hCG actions in gonadal cells (14), and therefore could be involved in the heterologous regulation of PRL-R and its mRNA by modulation of gene transcription and/or mRNA turnover.

In conclusion, we have demonstrated the presence of five PRL-R mRNA species in luteinized rat ovaries, and shown that the long form ovarian PRL-R species is predominantly derived from the 9.7 kb species, while the short transmembrane form of PRL-R is derived from the 1.8 and 2.1 kb species. All PRL-R mRNA species were subjected to differential up- and down-regulation by gonadotropins, with coordinate changes on surface membrane lactogen receptors.

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