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Localization of prolactin and its receptor messenger RNA in the human decidua

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Abstract. Prolactin (PRL) is known as an anterior pituitary hormone. On the other hand, PRL is also produced in the human decidualized endometrium. The physiological role and site of action of endometrial PRL have not yet been clarified. This study was designed to investigate the localization of PRL receptor (PRL-R) gene-expressing cells in the human decidualized endometrium using in situ hybridization histochemistry. Sense and antisense ^{35}S-labeled RNA probes for human PRL-R mRNA were hybridized with cryostat sections of human decidua, which were obtained from patients undergoing therapeutic abortion at 8–10 weeks of gestation. Hybridization signals for PRL-R mRNA were seen over the decidual cells. No labeled cells were seen in the chorion, amnion, or trophoblast. Comparing the localization of PRL-R gene-expressing cells to that of PRL gene-expressing cells using adjacent sections, their distributions were quite similar. These results indicate that not only PRL but also PRL-R transcripts are located in the decidual cells.
Key words. In situ hybridization; endometrium; pregnancy; prolactin; prolactin receptor.

Prolactin (PRL) is a polypeptide hormone secreted from the anterior pituitary[1]. PRL has also been found in human amniotic fluid in high concentrations[2]. The source of this amniotic fluid PRL has been shown to be maternal decidualized endometrium[3]. The amino acid sequence of endometrial PRL is identical to pituitary PRL, although endometrial PRL mRNA is 150 nucleotides larger than pituitary PRL mRNA[4]. Previous study has shown by in situ hybridization histochemistry (ISH) that the endometrial PRL gene is expressed in the decidual cells[5]. The physiological role of endometrial PRL has not yet been fully elucidated. It has been considered that endometrial PRL has functions analogous to those in the pituitary, but specifically adapted to fetal growth[6]. In particular, because PRL treatment changes the permeability of isolated fetal membranes to water in vitro[7], it has been hypothesized that endometrial PRL plays a role in regulating volume and ion content of the amniotic fluid[6]. A ligand binding study has indicated that PRL-R is located in the human chorion laeve[8]. However, no histochemical evidence showing the exact location of PRL-R in the human utero-placental unit has yet been reported.

Complementary DNA (cDNA) to PRL-R mRNA has already been isolated from the estrogen-treated femal rat liver[9], rabbit mammary gland[10], and human hepatoma and breast cancer libraries[11]. Sequence analysis of these clones has revealed that PRL and growth hormone (GH) receptors (GH-R) form a gene family, as do the hormones themselves[12]. In the human endometrium, Northern blot analysis has shown that the PRL-R gene is expressed in the chorion laeve[11]. However, because it is impossible to separate chorion laeve completely from other endometrial structures, the possibility that decidual cells located close to the chorion express PRL-R cannot be excluded. In the rat decidual tissue, in fact, decidual tissue possesses binding sites for PRL[13]. Therefore, it is necessary to examine the localization of PRL-R positive cells by histochemical techniques such as immunocytochemistry and ISH within the human utero-placental unit. In the present study, we have used ISH and shown that PRL-R gene is expressed within decidual cells.

Materials and methods

Sample collection. Decidual tissues were obtained by curettage from patients undergoing therapeutic abortion at 8–10 weeks of gestation (n = 6). Informed consent for the use of utero-placental tissues was obtained from all patients before the treatment. Tissues were cut with an autoclaved lather blade to approximately 10 mm × 10 mm × 10 mm cubes and immediately frozen on dry ice. These were stored in liquid nitrogen until use.

Probe preparation. cDNAs corresponding to PRL-R and PRL mRNA were kindly donated from Dr. P. A. Kelly, McGill University, Montreal, Canada and Dr. K. Nakajima, Mie University, Tsu, Japan, respectively. Their characterizations are described elsewhere[11,14]. A

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612 base pair fragment (bases 1–612) from PRL cDNA and a 200 base pair fragment (bases 1313–1512) from PRL-R cDNA were subcloned into a pBluescript SK II (+) transcription vector and amplified in *E. coli* (XLI-Blue). Then the vectors were linearized and ^{35}S-labeled sense and antisense RNA probes were transcribed using T3 and T7 RNA polymerases. Following transcription, probes for PRL mRNA were hydrolyzed to approximately 200 bases according to procedures described elsewhere[15].

Tissue preparation and in situ hybridization. Protocols were essentially the same as those described previously[16,17]. Ten µm thick frozen sections were cut, mounted onto organosaline-coated slides, fixed with paraformaldehyde and hybridized with sense or antisense probe (7×10^5 cpm/section) for 24 h at 50 °C. Then sections were treated with ribonuclease A (20 µg/ml), dehydrated through a graded series of ethanols, dried, dipped into Kodak NTB3 photographic emulsion, and exposed for 2–4 weeks at 4 °C. Sections were then developed with Kodak D-19 developer, fixed, and counterstained with hematoxylin-eosin solution.

Results and discussion

In the sections that were hybridized to an antisense probe for PRL-R mRNA, dense concentrations of silver grains over specific cells were observed (figs 1A and 2A). Such specific signals were not seen in the section that received a sense probe for PRL-R mRNA (fig. 1B). These results indicate that signals were derived from the specific hybridization of the antisense probe.

Hybridization signals were distributed in the stromal tissues of the endometrium (figs 1A and 2A). Decidual cells were strongly labeled. Labeled cells were exclusively located in the region close to the chorion laeve (Ch). No hybridization signals were seen in the Ch, amnion (Am), endometrial gland or capillary endothe-

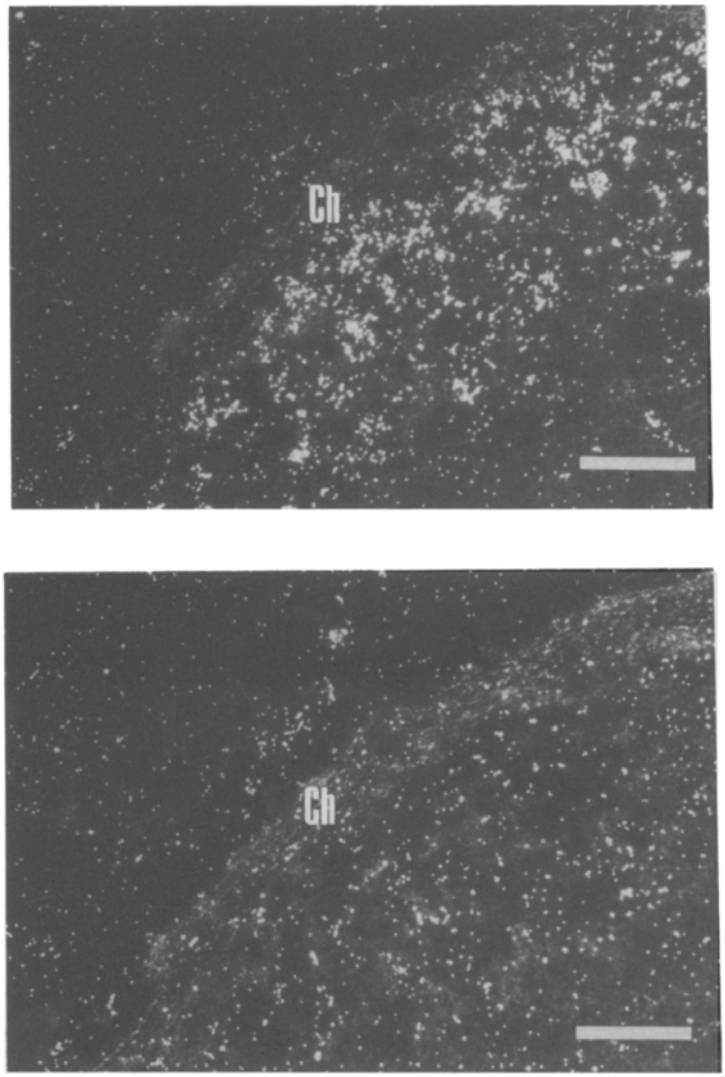

Figure 1. Dark-filled photomicrographs of adjacent sections of human decidualized endometrium (9 weeks of gestation). Section in *A*) received a ^{35}S-labeled antisense RNA probe for PRL-R mRNA (700,000 cpm/section), whereas section in *B*) received a sense probe for PRL-R mRNA (700,000 cpm/section). Both sections were hybridized simultaneously at 50 °C for 24 h and were exposed for 4 weeks at 4 °C for autoradiography. Note that significant hybridization signal is seen only in the sections that received an antisense probe. Scale bar length = 100 µm. Ch = chorion.

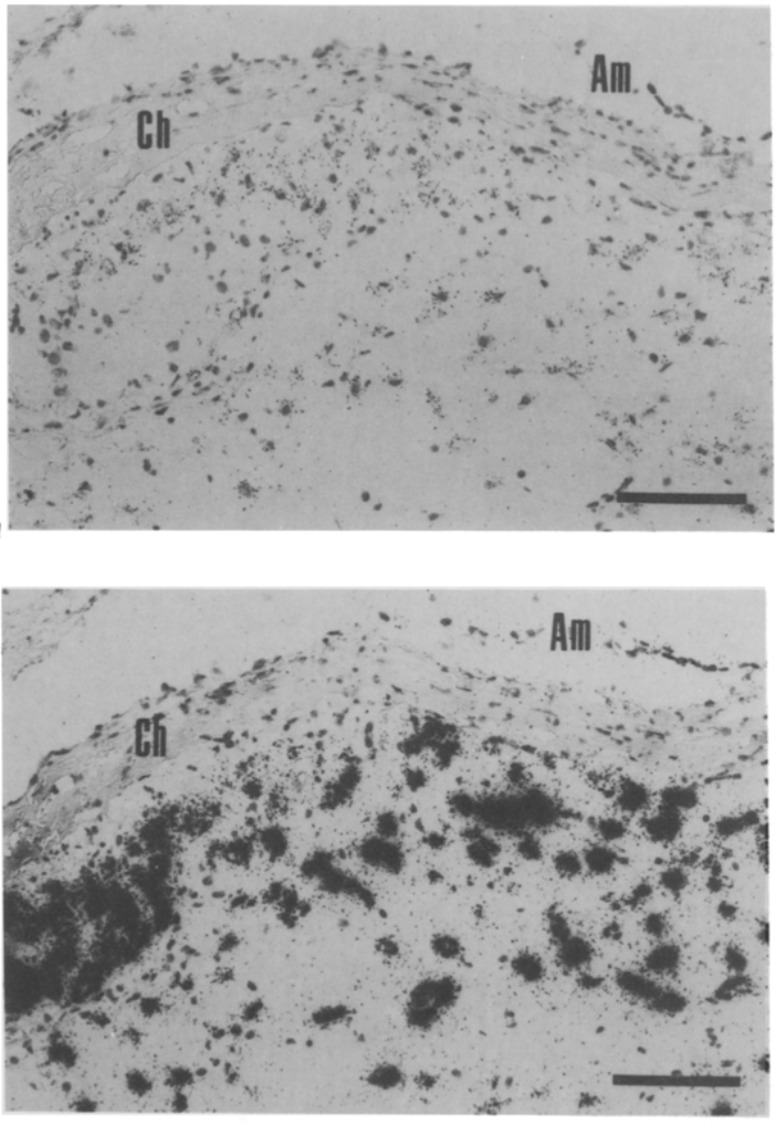

Figure 2. Photomicrographs of adjacent sections of human decidualized endometrium (9 weeks of gestation) with a higher magnification. Section in *A*) received an antisense probe for PRL-R mRNA, whereas section in *B*) received an antisense probe for PRL mRNA. As with probes for PRL-R mRNA, ^{35}S-labeled RNA probe for PRL mRNA (700,000 cpm/section) was applied to the section *B*. Both sections were hybridized simultaneously at 50 °C for 24 h and were exposed for 4 weeks at 4 °C for autoradiography. Note that labeled cells were exclusively located close to the chorion in both sections. Scale bar length = 100 µm. Ch = chorion; Am = amnion.

lium (fig. 2A). No hybridization signals were detected in the trophoblast either (data not shown). Figure 2B shows an example of ISH detection of antisense probe for PRL mRNA using a section adjacent to that shown in figure 2A. The sections shown in figures 2A and 2B were hybridized simultaneously and exposed for 4 weeks. In the sections that received an antisense probe for PRL mRNA, labeled cells were also exclusively located around the region close to the chorion laeve. Again, no hybridization signals were seen in the Ch, Am, endometrial gland or capillary endothelial cells. Hybridization signals for PRL mRNA were much stronger than those for PRL-R mRNA. As with PRL-R, the sense probe for PRL mRNA did not show any significant hybridization signal (data not shown).

The results of the present study demonstrate that hybridization signals are located in the decidual cells in the human endometrium when sections were hybridized to an antisense probe for PRL-R mRNA. Although PRL-R cDNA contains regions of similarity with GH-R[12,18], the nucleotide sequence of the cDNA region (bases 1313–1512) used in the present study does not contain any region homologous to GH-R cDNA[18]. Therefore, it is likely that hybridization signals detected in the present study show the localization of PRL-R mRNA in the decidual cells.

Previous studies reported the localization of PRL-R in the chorion laeve using ligand binding[8] and Northern blot analysis[11]. In the present study, only decidual cells showed hybridization signals, whereas no signal was detected in the chorion. In particular, only decidual cells located close to the chorion laeve showed hybridization signal. These results of the present study indicate that decidual cells specifically express the PRL-R gene. One

possible explanation of this discrepancy is that different kinds of PRL-R are expressed in the chorion. We used a cDNA region located within the cytoplasmic domain of the long form of PRL-R cDNA. This region is lacking in the short form of PRL-R mRNA[12]. Therefore, although the short form of PRL-R has not been found in human tissues, the possibility that it is expressed in the chorion cannot be excluded. The other possibility is that previous results indicating that PRL-R is located in the chorion laeve[8,11] could be the consequence of inevitable contamination by decidual tissue. This is also possible because it is rather difficult to separate chorion laeve completely from the adjacent decidual tissue which expresses the PRL-R gene.

As shown in figures 2A and 2B PRL and PRL-R gene are expressed in the decidual cells. Comparing the distribution of PRL-R gene-expressing cells to that of PRL gene-expressing cells in the adjacent section (figs 2A and 2B), the distributions are quite similar. Both genes are expressed exclusively in the cells located close to the chorion. These results indicate that the secretion and synthesis of PRL could be regulated in part by PRL itself. Furthermore, these results also suggest that PRL acts locally on the uterus as well as regulating volume and content of the amniotic fluid[6].

PRL has been shown to suppress the immune response and be involved in the maintenance of T-cell immunocompetence[19]. It is detected in endometrium after day 23 of the menstrual cycle and its production increases as decidualization spreads throughout the endometrial stroma[20]. This process proceeds precisely at the time when implantation would normally occur. Moreover, decidual PRL secretion is low in infertile women with a luteal phase defective endometrium[21]. Because of these results, several investigations have indicated that PRL plays a role in human implantation by preventing immune rejection of the blastocyst and fetus[6,22]. The fact that human decidual tissue expresses the PRL-R gene, as shown in the present study, supports such an autocrine/paracrine role for decidual PRL. However, further study is necessary to clarify the role of endometrial PRL in implantation.

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