RAPID COMMUNICATION

Expression of Prolactin-Releasing Peptide in Human Placenta and Decidua

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Abstract. The aims of this study were to determine whether the human placenta and decidua express PrRP gene and whether PrRP regulates PRL secretion from cultured human decidual cells. PrRP gene expression was analyzed by reverse transcription (RT)-PCR, and the level of the gene expression was quantified by a ribonuclease protection assay. PrRP gene expression was detected in both the placenta and decidua. These tissues expressed PrRP mRNA throughout pregnancy and the level of PrRP mRNA expression somewhat increased during midpregnancy. Placental and decidual cells also expressed PrRP mRNA, in vitro. To determine whether PrRP affects decidual PRL secretion, human endometrial stromal cells and decidual cells were cultured and treated with or without 1 nM PrRP31. PrRP31 did not affect PRL secretion in either short or long term incubation. Moreover, the RT-PCR analysis indicated that human decidua does not express the PrRP receptor, hGR3, mRNA. These findings suggest that PrRP produced by the human placenta and decidua does not affect decidual PRL secretion due to a lack of the receptor, and that it may play other roles during pregnancy.

Key words: PrRP, PRL, hGR3, Placenta, Human

(RECENTLY, a hypothalamic peptide, which stimulates PRL secretion, was isolated from a hypothalamic extract utilizing an orphan seven-transmembrane-domain receptor, hGR3, and named PRL-releasing peptide (PrRP) [1]. PrRP cDNA generates at least two peptides, PrRP31 and PrRP20, from the preproprotein. These biologically active peptides are mainly expressed in the hypothalamus and show stronger and more specific PRL-releasing activity compared with known PRL-releasing factors [1].

PRL is an important hormone for the development of the mammary glands and promotion of milk synthesis [2, 3]. Although, PRL is mainly released by the pituitary gland, the human endometrium and decidua also release PRL [4, 5]. This finding suggests that PrRP may be expressed by the human placenta or decidua and may stimulate decidual PRL release. Interestingly, both the human placenta and decidua expressed PrRP mRNA but PrRP did not affect the decidual PRL release in contrast to pituitary PRL.

Materials and Methods

Tissue collection

Normal human placenta and decidua were obtained from women who aborted or delivered at Osaka University Medical School and Suita...
Municipal Hospital. First and second trimester placenta and decidua were obtained from patients undergoing abortion at 7-10 weeks and 17-20 weeks of gestation, respectively. Third trimester placenta and decidua were obtained within a few minutes of normal delivery at 37-41 weeks of gestation. Permission to obtain the tissue was approved by the Human Investigation Committee of Osaka University Graduate School of Medicine and informed consent was obtained from each patient.

**Placental cell culture**

Primary culture of first trimester placenta was performed as reported previously [6]. The cells were suspended in a culture medium (RPMI-1640 containing 10% FBS, 50 μg/ml streptomycin, and 100 U/ml penicillin) at a concentration of 1 × 10^6 cells/ml. They were plated into a 6-multiwell plate and allowed to attach for 2 days. The cells were incubated at 37°C under a humidified atmosphere of 95% air-5% CO₂.

**Decidual cell culture**

Primary culture of first trimester decidua was performed as reported previously [7]. Briefly, minced decidua was treated with 0.25% collagenase and the resulting cell dispersion was filtered through a 38 μm pore size sieves to remove epithelial cells [7]. The cells were then centrifuged through a 20-60% Percoll gradient (gradient 1). Band 1 at a density of about 1.048 contained the PRL producing cells. The cells in band 1 were re-centrifuged through a 40-55% Percoll gradient (gradient 2). Band 1a at a density of about 1.048 contained an almost homogenous population of large round cells. To remove contamination of fibroblastic cells, the cells were cultured in a plastic dish. The remaining cell suspension was homogenous and these cells produced PRL [7]. The cells were suspended in a culture medium (Ham’s F-10 containing 10% FBS, 50 μg/ml streptomycin, 100 U/ml penicillin, 200 U/ml gentamycin, and 10 μg/ml bovine-porcine insulin) at a concentration of 1 × 10^6 cells/ml. They were plated into a 6- or 24-multwell plate and allowed to attach for 2 days. The medium was replaced with fresh medium and experiments were started at this time. The cells were treated with or without 1 μm PrRP31 in the culture medium up to 3 days. We used PrRP31 in this study since Hinuma et al. demonstrated that PrRP31 binds to the receptor and causes biological activities [1]. The supernatants were collected at 0.5, 2, 24, 48, and 72 h and were stored at -20°C until assayed. The cells were incubated at 37°C under a humidified atmosphere of 95% air-5% CO₂.

**RNA isolation and reverse transcription (RT)-PCR**

Total RNA was isolated from the human placenta, decidua, placental cells, and decidual cells as described previously [8]. Four μg of total RNAs was reverse transcribed with M-MLV reverse transcriptase using random hexamer primers. The resulting cDNAs were subjected to PCR amplification. PCR primers for PrRP, sense: 5’-GAGATCCGCACCTCTGACAT-3’ and antisense: 5’-CGACATAGCACCAGCGCTTCCA-3’, were designed based on the nucleotide sequences of human PrRP. The condition for the PCR reaction was 94°C for 5 min for 1 cycle; 94°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min for 35 cycles; and 72°C for 5 min. PCR primers for PrRP receptor (hGR3), sense: 5’-GCACTCTCGCTCGCTCACCGTGA-3’ and antisense: 5’-CGTAAGGGGTGAGATGCCTG-3’, were designed based on the nucleotide sequences of hGR3. Amplification was performed with the following profiles: 94°C for 2 min for 1 cycle; 94°C for 30 sec, 56°C for 30 sec, and 72°C for 1 min for 35 cycles; and 72°C for 5 min. The product of PrRP RT-PCR was confirmed as following. The PCR product was electrophoresed on a 1.2% low-melting point agarose gel. Then, the gel slice containing DNA fragments of 159 bp was phenol/chloroform extracted and was precipitated with ethanol. The DNA was subcloned into pGEM-T vector and transformed in DH5α. Recombinant clones were selected at random and the plasmid DNA was purified with a plasmid purification kit. Plasmid DNA containing the PCR products were amplified by PCR using T7 and SP6 primers. The nucleotide sequence was determined by the PRISM BigDye Terminator cycle sequencing kit and AB PRISM TM 310 genetic analyzer (Perkin Elmer Applied Biosystems, UK).
Ribonuclease protection assay (RPA)

RPA was carried out using an RPA kit (Ambion Inc., Austin, TX). The plasmid containing human PrRP cDNA was linearized with restriction enzyme Not I and PrRP cRNA was generated by in vitro transcription with T7 RNA polymerase. 32P-labeled α-UTP was incorporated into the PrRP cRNA. Human pTRI-GAPDH cDNA was used as an internal control and transcribed in a similar manner. Ten μg of total RNA was hybridized with 5 × 10^4 cpm of a RNA probe at 42°C for 48 h. Non-hybridized RNA was digested by a mixture of RNase A/T1. Protected fragments were separated on a 5% acrylamide gel, then dried and exposed to X-ray film at -80°C. Bands corresponding to protected fragments were quantified by densitometry [8].

Enzyme immunoassay (EIA)

The PRL concentration in the medium was measured by an automated immunoassay system, Serono Diagnostics SR1 immunoassay system (Serono Diagnostics Jogel, Philadelphia, PA). The range of sensitivity in this assay was 0.5-150 ng/ml.

Statistical analysis

The data were analyzed for homogeneity of variance with Bartlett's test. The data were analyzed by analysis of variance for a completely randomized design following Dunnett's test. A p value less than 0.05 was considered statistically significant.

Results and Discussion

Human stromal cell culture

Endometrial tissue was obtained from normally menstruating women and a stromal cell culture was prepared as described previously [9]. Briefly, the tissue was collected in DMEM and rinsed in calcium- and magnesium-free PBS (PBS-). Then, the tissue was incubated with 0.1% collagenase (Worthington Biochemical, Lakewood, NJ) and 0.01% DNAse-1 (Sigma Chemical Co., St. Louis, MO) on a shaking platform at 37°C. The digested tissue was filtered through a 100 μm mesh. The stromal cells were separated from the epithelial cells using a modification of the selective attachment [10] and selective trypsinization methods [11]. The stromal cells were seeded in 75-cm² polystyrene flasks and incubated until confluence in a culture medium of DMEM containing 10% FBS, 10 nM of estradiol, and 1 µM of medroxy-progesterone acetate (culture medium) for 3 to 4 days. The cells were trypsinized and either subpassaged or plated at a density of 2 × 10^5 in 24-well culture plates.

The cells were incubated in the culture medium for 13 days to allow cell differentiation, and then treated with or without 1 μM PrRP31 in the culture medium up to 3 days. The supernatants were collected at 0.5, 2, 24, 48, and 72 h and were stored at -20°C until assayed.

To determine whether the human placenta and decidua express PrRP mRNA, total RNAs were extracted from the placenta and decidua and RT-PCR for PrRP was performed. As shown in Fig. 1A, both placenta and decidua expressed PrRP mRNA throughout pregnancy. Sequence analysis indicated that the structure of the PCR product completely corresponded with known PrRP cDNA (data not shown). These results suggest that the RT-PCR for PrRP is specific, and that the hypothalamus, placenta, and decidua all express the same PrRP mRNA. RPA analysis for PrRP was performed to determine the gestational profile of PrRP gene expression in the placenta and decidua during pregnancy. These tissues expressed PrRP mRNA throughout pregnancy and the levels somewhat increased at midpregnancy (Fig. 1B). To determine whether trophoblast and decidual cells express PrRP mRNA, primary cultures of placental and decidual cells were performed. Total RNAs were isolated from these cells and RT-PCR for PrRP was performed. Both placental and decidual cells expressed PrRP mRNA (Fig. 2). This finding suggests that, at least, decidual cells express PrRP mRNA since the decidual cell culture contained a homogeneous population of decidual cells [7], although the placental cell culture contains heterogeneous populations of cells including trophoblast cells.

Dopamine and TRH regulate PRL release from the pituitary gland, but they do not regulate decidual
Fig. 1. Expression of PrRP mRNA in the human placenta and decidua. (A) Total RNAs were extracted from the placenta and decidua on the 1st, 2nd, and 3rd trimester of pregnancy and RT-PCR analysis for PrRP was performed. The PCR products (159 bp) were separated on 1.2% agarose gel and visualized by ethidium bromide staining. The left side of lanes shows a ladder marker. (B) Ten µg of total RNAs were extracted from the placenta and decidua on the 1st, 2nd, and 3rd trimester of pregnancy and ribonuclease protection assay for PrRP was performed. The intensity of the PrRP mRNA band was normalized to that of the corresponding human pTRI-GAPDH band. The data shown are from a representative experiment. The experiment was performed three times, and similar results were obtained.

Fig. 2. Expression of PrRP mRNA in cultured placental cells and decidual cells. Cells (4 × 10^6) were plated in 6-well plates and incubated for 2 days. Total RNAs were extracted from cultured placental cells (lane 2) and decidual cells (lane 3), and RT-PCR analysis for PrRP was performed. The PCR products (159 bp) were separated on 1.2% agarose gel and visualized by ethidium bromide staining. Lane 1 shows a ladder marker.

Fig. 3. Effect of PrRP on PRL secretion by cultured human stromal cells. Cells (2 × 10^5) were plated in 24-well plates and incubated for 13 days to allow cell differentiation. Cells were then treated with or without 1 µM PrRP31 up to 3 days. The medium was collected and assayed for PRL concentration. Each value represents the mean ± SEM of 4 wells. The data shown are from a representative experiment. The experiment was performed three times, and similar results were obtained.
PRL release [12]. There are PRL-RF and PRL-IF which specifically regulate decidual PRL release [13, 14]. These findings suggest that regulation of PRL release is different between the pituitary gland and decidua. Therefore, we tried to examine whether PrRP regulates decidual PRL release. Endometrial stromal cells differentiate and start to release PRL under specific conditions [10]. The cells were cultured without treatment for 13 days to allow cell differentiation, then treated with or without 1 μM PrRP31, which causes maximum stimulation of PRL release by pituitary cells, up to 72 h [1]. However, PrRP did not affect PRL release from the differentiated stromal cells (Fig. 3). Moreover, 1 pM PrRP31 did not affect PRL release from a primary culture of decidual cells (data not shown). To determine whether PrRP does not affect decidual PRL release due to a lack of PrRP receptor, hGR3, total RNA was extracted from the human decidua and cultured decidual cells and a RT-PCR analysis for hGR3 was performed. The human decidua and decidual cells did not express hGR3 mRNA (data not shown). Fujii et al. reported that the distributions of PrRP and PrRP receptor mRNAs do not always overlap [15]. These findings suggest that PrRP produced by the placenta and decidua does not regulate PRL release due to a lack of the receptor, and that PrRP may play other physiological roles during pregnancy. For example, it is possible that PrRP produced by the placenta regulates fetal PRL release from the fetal pituitary gland.

In conclusion, we showed that human placental and decidual cells express PrRP mRNA and that PrRP does not regulate decidual PRL release due to a lack of receptor in the decidua. However, the physiological roles of PrRP produced by the placenta and decidua remain to be determined.

References