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Research Article

Evidence of a role for prolactin as regulators of ovarian follicular development in goose

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ABSTRACT

Background: Prolactin (PRL) regulates development and reproduction, and its effects are mediated by the prolactin receptor (PRLR). In order to clarify the role of PRLR and PRL in the process of follicular development in the goose ovary, the level of PRLR mRNA expression in the ovary and follicles of the Sichuan white goose was determined, as well as the PRL concentration in ovarian follicles.
Results: The level of PRLR mRNA in the hierarchical follicles (HFs) initially increased, and subsequently decreased, whereas PRLR expression was initially low and later increased in postovulatory follicles (POFs). The level of PRLR mRNA expression was the highest in the F4 follicles, and lowest in the F1 follicles in all of the examined follicles. Compared with the level of PRLR mRNA expression in the small white follicles (SWFs), the level of PRLR mRNA was 2.86- and 1.44-fold higher in the F4 and small yellow follicles (SYFs), respectively ($P < 0.05$). The level of PRLR mRNA expression in the F4 follicles was highest ($P < 0.05$) in HFs. The highest PRL concentration in all of the examined samples was observed in SYFs and F1, with concentration of 6162 mLU/g and 6197 mLU/g, respectively. The PRL concentration in SYFs was significantly higher compared with SWFs ($P < 0.05$).
Conclusions: The change of PRL concentration was similar to the PRLR mRNA expression level in preovulatory follicles. These results suggest that the PRL mediated by the PRLR plays a stimulatory role in the SWF to SYF transition.

1. Introduction

Prolactin (PRL) is a polypeptide hormone that is produced primarily in the anterior pituitary gland, and belongs to the prolactin/growth hormone family [1]. The PRL hormone is involved in a wide range of physiological processes in vertebrates, including metabolism, energy balance, and immunoregulation, and is essential for animal reproduction [2,3]. The effects of PRL are mediated by the prolactin receptor (PRLR) [4]. The binding of the PRL polypeptide to the PRLR activates intracellular signaling cascades, such as the JAK2/STAT5 signaling pathway, and regulates the expression of its various target genes [5,6]. In birds, the PRL is involved in the regulation of gonadal development and egg laying [7,8]. Previous studies have shown that PRL can suppress reproduction and induce nesting behavior in birds. The PRL hormone suppresses FSH-induced aromatase expression and estradiol production, and stimulates FSH-induced progesterone production in granulose cells [9]. However, recent studies have shown that PRL plays an important role in promoting follicular development [10], and that PRLR knockdown suppresses reproduction in female mice, indicating that PRLR may regulate reproduction [11]. The expression level of PRLR increases during the development of the mouse ovary, which suggested that PRLR was critical to ovarian function and fertility. The expression of PRLR increased at 12 h after treatment using pregnant mare serum gonadotrophin. It suggests that PRLR may affect follicular development and maturation [12]. The PRLR protein is expressed in oocytes, and prenatal mouse follicles treated with PRL have demonstrated an increased rate of oocyte maturation [13]. Thus, PRL plays an important dual role in animal reproductive processes. However, the level of PRLR gene expression and the concentration of PRL in the ovarian follicles of geese were unclear during the follicular development. The aim of our study was to determine the expression profile of the PRLR and the concentration of PRL in the ovarian follicles of Sichuan white geese. Our findings increase our understanding of the molecular mechanisms of follicular development and ovulation in geese.

2. Materials and methods

2.1. Experimental geese and tissue collection

Five healthy female Sichuan white geese were selected randomly from a local breeding farm and were conducted in accordance with the

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guidelines used at the farm. All of the geese were fed under uniform standard management in conditions of nature light. The geese were killed by exsanguinations to obtain ovaries along with the ovarian follicles. The follicles were separated from the ovary and weighed to identify the small white follicles (SWFs), small yellow follicles (SYFs), hierarchical follicles (from F5 to F1), postovulatory follicles (from POF1 to POF4), and ovary. All of the follicles were operated transversely along the stigma to completely eliminate the yolk material. The follicular membranes were washed with the ice-cold sterile saline, paying attention to ensuring that there was no adherent yolk material.

2.2. Extraction of total RNA and reverse transcription PCR

Total RNA was prepared from the geese hierarchical follicles by the Trizol reagent method (Takara Bio Inc., Dalian, China), and then stored at -80°C until analysis. cDNA was synthesized using PrimeScript® RT reagent Kit (Takara Bio Inc., Dalian, China), according to the manufacturer's instruction. Briefly, the 10 μL reaction consisted of 2.0 μL of total RNA, 2.0 μL of 5 × PrimeScript® Buffer, 0.5 μL of PrimeScript® RT Enzyme Mix, 0.5 μL of Random 6-mers, 0.5 μL of oligo dT Primer, and 4.5 μL of RNase Free H_2O. Thermal cycling was performed with 15 min at 37°C, and then 5 s at 85°C. According to the reported CDS sequence of *Anser anser* (accession number: DQ209271) and Anas platyrhynchos (accession number: KC183720), gene-specific primers were designed by using Primer Premier 5.0, and then synthesized commercially by Shanghai Sangon (Shanghai, China). Primers for amplifying PRLR and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) were as follows: PRLR forward primer: 5′-GCCTTTATCCTACCACCAGTTCC-3′ and PRLR reverse primer 5′-GATCCTCGCTGTCCTCTACCTCT-3′, GAPDH forward primer: 5′-GTGGTGCAAGAGGCATTGCTGAC-3′ and GAPDH reverse primer: 5′-GCTGATGCTCCCATGTTCGTGAT -3′. The 25 μL reaction consisted of 0.5 μL of cDNA, 4 μL of 2.5 mM deoxynucleoside triphosphate (dNTP) Mix, 1 μL of 20 μM of PCR forward primer and PCR reverse primer, 2.5 μL of 10 × PCR Buffer, 0.25 μL of 5U/μL Taq™ (Takara Bio Inc., Dalian, China), and 16.75 μL sterile Milli-Q water. Thermal cycling was performed with an initial denaturation step of 5 min at 95°C, followed by 35 cycles of 30 s at 95°C, 55°C for 30 s, and then 72°C for 30 s, and then a final extension at 72°C for 10 min.

2.3. Construction of PRLR and GAPDH cDNA plasmid

The recombinant plasmids containing PRLR and GAPDH cDNA were termed pPRLR and pGAPDH, respectively. The constructs were prepared from total RNA in the hierarchical follicles of the goose ovaries during the egg-laying stage, and the complementary double stranded cDNA fragments were subcloned into the pMD®19-T Vector (Takara Bio In, Dalian, China) as described.

2.4. qRT-PCR with SYBR Green I chemistry

The qRT-PCR was performed on the first strand cDNA using the Bio-Rad CFX Real-time PCR Detection System and software (BIO-RAD, California, USA) with SYBR® Premix Ex Taq™ (Takara Bio Inc., Dalian, China). Briefly, the 50 μL reaction consisted of 1 μL of cDNA, 25 μL of SYBR® Premix Ex Taq™ (2 × concentration), 2 μL of 20 μM of PCR forward primer and PCR reverse primer, and 22 μL of nuclease-free water. Thermal cycling was performed with an initial denaturation step of 10 s at 94°C, followed by 39 cycles of 5 s at 94°C, and 55°C for 30 s, and then a final extension at 72°C for 10 s. For generation of the standard curves, the pPRLR and pGAPDH standards were also run.

2.5. Determination of PRL concentration in each follicle

The PRL concentration was measured using the Goose Prolactin ELISA Kit (Beijing Gersion Bio-Technology Co. Ltd., Beijing, China). Following manufacturers' instructions, 0.1 g follicular samples were added to 1.0 mL saline and homogenized and then the supernatant was extracted. The standards and samples were added to the Microelisa Stripplate in two replicates and three replicates for each sample. For the set standard wells and the testing sample wells, 50 μL standard was added to the standard well and 10 μL testing sample was added to the testing sample well. Next, 40 μL Sample Diluent was added to the testing ample wells; nothing was added to the Blank wells. Then, 100 μL of HRP-conjugate reagent was added to each well, covered with an adhesive strip and incubated for 60 min at 37°C. Each well was aspirated and washed, repeating the process five times. Then, 50 μL of chromogen solution A and 50 μL of chromogen solution B were added to each well; the wells were gently mixed and incubated for 15 min at 37°C, protected from light. Then 50 μL Stop Solution was added to each well, and the Optical Density (OD) at 450 nm was read using a microtitre plate reader within 15 min.

2.6. Statistical analysis

Threshold and Ct (threshold cycle) values were determined automatically by the Bio-Rad CFX Real-time PCR Detection software, using default parameters. The relative level of expression for PRLR was calculated relative to GAPDH (the normalizer) using the $2^{-\Delta\Delta Ct}$ method. The level of PRLR mRNA expression and the concentration of PRL was expressed as the mean of three means ± SD. The abundance of PRLR in the SWF of geese was assigned a value of 1. All data were analyzed using SAS statistical software for Windows (SAS Institute Inc., Cary, NC, USA). The data were analyzed by one-way ANOVA followed by Duncan's test. Differences were considered to be significant at $P < 0.05$.

3. Results

The RT-PCR analysis showed that the PRLR and GAPDH mRNAs were present in the hierarchical follicles during the egg-laying stages. The RT-PCR products for the PRLR and GAPDH mRNAs were 175 and 86 bp, respectively (Fig. 1), which corresponded to the predicted size for each, indicating an acceptable level of specificity for the qRT-PCR method. The qRT-PCR results showed that the expression of the PRLR mRNA in the hierarchical follicles increased during the early stages of follicular development, and subsequently decreased. The level of PRLR expression was the highest in the F4 follicles, and was lowest in F1 follicles. Except for the F1 follicles, the level of PRLR mRNA in the hierarchical follicles

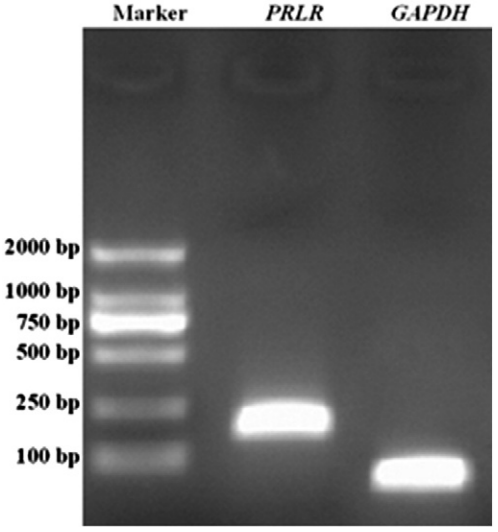

Fig. 1. Electrophoresis photograph of RT-PCR products for GAPDH and PRLR in the hierarchical follicles of Sichuan white goose ovary. The amplicons of 86 bp GAPDH, and 175 bp PRLR were separated on 15 g agarose L^{-1} gels, stained with ethidium bromide, examined with ultraviolet light and visualized with a Gel-Pro Imager (Media Cybernetics, Maryland, USA). A 2000 bp molecular weight marker (M) was used.