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Effects of nonglycosylated and glycosylated prolactin on basal and gonadotropin-stimulated steroidogenesis in chicken ovarian follicles

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ABSTRACT

In galliformes, the circulating isoform of prolactin (PRL) significantly changes during different reproductive states. However, the role of the major isoform (glycosylated PRL [G-PRL]) in ovarian steroidogenesis is unknown. The present study aimed to compare the effects of nonglycosylated (NG-) and G-PRL on basal and gonadotropin-stimulated estradiol (E_2) and progesterone (P_4) production in granulosa cells or follicular walls of chicken of different size class follicles. In the initial experiment, granulosa cells of preovulatory F3-F1 and prehierarchical 6- to 8-mm follicles were incubated for 24 h with different concentrations of NG- or G-PRL (0, 1, 10, 100, or 1,000 ng/mL). In the subsequent experiments, these categorized granulosa cells and follicular walls of prehierarchical 4–6, 2–4, and <2-mm follicles were incubated for 24 h in the absence and presence of 10-ng/mL FSH or LH, or in combination with different concentrations of NG- or G-PRL (10, 100, or 1,000 ng/mL). We observed that lower levels of NG-PRL induced ($P < 0.05$) E_2 and P_4 secretion in granulosa cells of either preovulatory or prehierarchical follicles, but at higher levels, this effect was reduced. In contrast, G-PRL promoted ($P < 0.05$) basal E_2 and P_4 secretion in preovulatory granulosa cells but was inhibitory ($P < 0.05$) in prehierarchical granulosa cells. Results obtained by real-time quantitative PCR (qPCR) demonstrated that these effects were mediated through modulation of the expression of *StAR*, *CYP11A1*, *CYP19A1*, and *3β-HSD*. Furthermore, G-PRL was less potent than NG-PRL in inhibiting FSH- or LH-stimulated E_2 and P_4 production in granulosa cells of preovulatory follicles, whereas NG-PRL enhanced ($P < 0.05$) but G-PRL reduced ($P < 0.05$) FSH-induced P_4 production in those of prehierarchical follicles. In follicular walls from each group of prehierarchical 4–6, 2–4, and <2-mm follicles, NG- and G-PRL had both stimulatory and inhibitory influences on the actions of FSH on E_2 and P_4 secretion, but both suppressed ($P < 0.05$) LH-induced E_2 and P_4 secretion except for the synergistic effects of LH and G-PRL on P_4 secretion by follicular walls of the follicles of 4–6 mm. Taken together, these results suggest that both NG- and G-PRL are biologically active in regulating basal and gonadotropin-stimulated E_2 and P_4 production in chicken ovarian follicles. However, their effects are different depending on the concentration, the type of gonadotropin (FSH or LH), and the stage of follicle development.

1. Introduction

In the laying hen, the main source of sex steroids are ovarian follicles, which consist of primary oocytes in the center surrounded by adjacent granulosa and distant theca

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layers which are the 2 major sites of steroidogenesis [1]. It has been established that both morphologic structure and steroidogenic capability of granulosa and theca layers vary with follicular growth and development [2]. During the prehierarchical stage, theca cells of immature (<8 mm) follicles mainly synthesize androgens and estrogens, whereas granulosa cells are maintained in an undifferentiated state and are steroidogenically inactive [3]. However, once the follicles enter the preovulatory hierarchy, granulosa cells are differentiated and become steroidogenically active [3]. In contrast to the classical 2-cell/2-gonadotropin model for mammalian ovarian steroidogenesis in which theca cells produce androgens as the substrate for estrogen biosynthesis in granulosa cells [4], a 3-cell model has been proposed in avian hierarchical follicles where progesterone (P_4) synthesized by granulosa cells is converted to testosterone (T) in theca interna and T then undergoes aromatization to estradiol (E_2) in theca externa [5]. With the maturation of avian preovulatory follicles, P_4 secretion by granulosa cells increases while theca cells reduce E_2 biosynthesis [6]. The process of ovarian steroidogenesis is primarily under the control of FSH and LH and many locally secreted factors [7,8]. In addition, there is evidence that prolactin (PRL) is also involved in regulating vertebrate ovarian functions [9,10].

In chickens and turkeys which display incubation behavior, elevated levels of PRL are associated with ovarian involution as hens incubate their clutch of eggs. Suppression of PRL levels via active or passive immunization against PRL or its major releasing factor (vasoactive intestinal peptide, VIP) blocks incubation behavior [11–14], suggesting that high levels of PRL are a requisite aspect of the behavior. Evidence supports both indirect (inhibition of gonadotrophin release [15–18]) and direct (inhibition of steroidogenesis [19–22]) roles for high levels of PRL suppressing the hypothalamic-hypophyseal-ovarian axis to initiate ovarian regression. However, the effects of PRL are concentration dependent. At lower levels, PRL would appear to be progonadal since passive immunization against PRL reduces large white follicular growth and hence recruitment into the follicular hierarchy in chickens [23]. In addition, photostimulation-induced increase in PRL secretion is associated with the onset of egg laying in parallel with increased ovarian steroid levels [24–26]. However, the effects of PRL on follicular cell steroidogenesis are variable according to the stage of the ovulation cycle, follicle size, and concentration [27]. Consistent with the direct effects of PRL on ovarian functions, the PRL receptor (PRLR) is widely expressed in the follicular hierarchy with higher levels in nonhierarchical follicles [28,29].

During the transition to incubation behavior, hens simultaneously lay eggs while incubating eggs. At this time, the concentration of PRL increases above a threshold value to change from pro-to antigonadal roles consistent with a shift in circulating PRL isoform. Both the absolute and relative amounts of glycosylated (G) to nonglycosylated (NG-) PRL change during this interval with the dominant form being glycosylated PRL (G-PRL) [30,31]. The physiological role of this isoform is unknown, although in mammals, G-PRL has lower biological activity than NG-PRL and is postulated to downregulate PRL action in selective target tissues [32]. Since PRL glycosylation can influence the ligand-binding affinity [33], it is possible that glycosylation may modify the biological actions of PRL in the ovary. Therefore, the present study aimed to examine the effects of NG- and G-PRL on both basal and gonadotropin-stimulated steroid production as well as the expression of steroidogenic enzyme genes in granulosa cells or follicular walls of follicles at different developmental stages in chickens.

2. Materials and methods

2.1. Hormones, chemicals, and reagents

Recombinant human FSH (rhFSH, AFP8468A), ovine LH (oLH, AFP-5551B), nonglycosylated ovine PRL (NG-oPRL, AFP-10692C), and glycosylated ovine PRL (G-oPRL, AFP-5742B) were obtained from National Hormone and Peptide Program (Torrance, CA, USA). A stock solution of each hormone was correspondingly prepared with either PBS at appropriate pH or 0.01-M $NaHCO_3$, and then stored in small aliquots at −80°C. The final concentration of each working solution used for corresponding treatment was prepared with culture medium. Fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium/Nutrient Mixture F12 (DMEM/F12), 0.4% Trypan Blue Solution as well as penicillin and streptomycin mixture were purchased from Invitrogen Life technologies (Carlsbad, CA, USA). Type II collagenase was purchased from Sigma-Aldrich (Oakville, ON, Canada). Trizol reagent and high-capacity cDNA reverse transcription kit were purchased from Invitrogen Life technologies. Power SYBR Green PCR Master Mix was purchased from D-Mark Bioscience (Toronto, ON, Canada). The EIA kits for E_2 and P_4 were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Protein Assay Kit and Bovine Serum Albumin (BSA) were purchased from Bio-Rad Laboratories (Hercules, CA, USA).

2.2. Animals and tissue collection

All experimental procedures using chickens in this study were approved by the Faculty Animal Care Committee of McGill University. White Leghorn hens, 25–35 wk of age and laying actively, were used in all studies described. Hens were fed ad libitum and kept in individual cages under standard conditions at the Poultry Complex of Macdonald Campus Farm, McGill University. The time of oviposition was monitored for each hen using surveillance camera (Lorex corporation, MD, USA), and ovulation was predicted to occur within 15–30 min after oviposition of the previous egg in the laying sequence. Hens, with an egg in the oviduct, were sacrificed by cervical dislocation about 1–4 h before predicted time of a midsequence ovulation. After slaughter, the ovary from each hen was immediately removed and placed into ice-cold 0.9% NaCl solution. According to the diameter and the position in the follicular hierarchy, ovarian follicles were categorized into several groups, including the prehierarchical (<2, 2–4, 4–6, and 6–8 mm) and the 3 largest preovulatory (namely F3-F1; 24–40 mm, F3 < F2 < F1) follicles. After being cleaned of surrounding vascular and connective tissues with fine