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Regulation of the Genes for Insulin-like Growth Factor (IGF) I and II and Their Receptors by Steroids and Gonadotropins in the Ovary

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Insulin-like growth factors (IGFs) I and II are two single-chain polypeptide hormones that are structurally related to each other and to proinsulin. Among the large number of growth factors involved in ovarian physiology, IGF-I and IGF-II are considered to be important progression factors for ovarian follicular development. To explore the ovarian expression of IGF-I, IGF-II and their receptor genes, a solution hybridization/RNase protection assay, was used. IGF-I mRNA was seen in the granulosa cells, and IGF-II mRNA in the theca-interstitial compartment. To study the hormonal regulation of the IGF-I and IGF-II gene, immature (21-day-old) hypohysectomized rats were treated with FSH (10 μg/day),GH (150 μg/day) and diethylstilbestrol (DES subcutaneous implant/5 days). Estrogen differentially regulated ovarian IGF-I and IGF-II gene expression. In concert with GH, estrogen up-regulated ovarian IGF-I mRNA, but significantly decreased hepatic IGF-I gene expression. Both IGF receptors (type I and type II) as well as the insulin receptor gene, were expressed in both ovarian cells. The expression of the type I IGF receptor gene (but not the type II IGF gene) was up-regulated by FSH and estrogen *in vivo*. In conclusion, these studies may serve to better understand the auto paracrine role of IGF, and their receptors in the pathophysiology of follicle recruitment, oocyte maturation and potentially embryo development.

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INTRODUCTION

IGF-I and IGF-II are 70 amino acid peptides structurally related to insulin and known for their ability to promote growth and differentiation [1]. Although traditionally viewed as a hepatic product, it is now well recognized that IGF-I may also be synthesized in a variety of extra-hepatic sites wherein it may play auto/paracrine roles [1]. The IGF-I gene consists of at least six exons. IGF-I gene expression is increasingly complex. It is now well established that exons 1 and 2 encode alternate leader sequences, and that exon 5 and part of exon 6 encode alternate E-peptides [2].

A growing body of information agrees with the notion that the murine granulosa cells are a site of IGF-I production, reception and action [3].While the potential role of IGF-I in ovarian physiology has been extensively studied, relatively limited attention has been paid to IGF-II. Radioligand receptor assays have documented the existence of both type I and type II IGF receptors in murine granulosa cell preparations [4]. Whereas type I IGF receptors are clearly in a position to amplify gonadotropin-supported differentiation, the role of type II IGF/mannose-6-phosphate receptors remains uncertain. Similarly, little is known about the hormonal regulation of the type II IGF receptor. In contrast, both FSH and LH have been shown to up-regulate rat granulosa cell type I IGF receptor [3].

MATERIAL AND METHODS

Immature (21–23 days old) intact and hypophysectomized Sprague–Dawley female rats, received 100 μl s.c. injections of either vehicle (0.05 M phosphate buffer, pH 8.4) or vehicle-carried FSH (10 μg/rat/day)

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twice daily for 2.5 days. In a separate series of experiments, immature hypophysectomized female rats received a s.c. DES-containing silastic implant for a total of 5 days.

Extraction of total RNA

Whole ovaries, granulosa and theca-interstitial cells were homogenized in 4 M guanidinium isothiocyanate and the total RNA purified over a cushion of CsCl. The precipitate RNA was resuspended in sterile water, quantified by absorbance at 260 nm, and the integrity of the RNA assessed by visual inspection of the ethidium bromide stained 28S and 18S rRNA bands after electophoresis through 1.25% /2.2 M formaldehyde gels.

Solution hybridization/RNase protection assay

Rat IGF-I, IGF-II, type I IGF receptor, type II IGF/mannose-6-phosphate receptor and insulin receptor riboprobes were prepared as previously described [5–7].

Solution hybridization/RNase protection assays were performed as previously described [8]. Briefly, transcribed riboprobe were labeled using [^{32}P]uridine triphosphate according to the manufacturer's instructions (Promega Biotech). After transcription, 1 μg DNase I was added, the mixture incubated for 15 min at 37°C, and the labeled riboprobes recovered by ethanol precipitation. 20 μg of total RNA were hybridized with 400,000 cpm of each riboprobe for 16 h at 45°C in 75% formamide/0.4 M NaCl, followed by digestion with 40 μg/ml RNase A and 2 μg/ml RNase T1. Protected hybrids were isolated by ethanol precipitation and separated on an 8% polyacrylamide/8M urea denaturing gel. The intensity of protected RNA was quantified by scanning densitometry.

RESULTS

To assess IGF-I gene expression, total RNA (20 μg) from the ovaries of immature rats were analyzed. As in liver [8], three IGF-I mRNA [322 and 297 (exon 2) and 242 (exon 1) bases long] were identified. A 500 bp transcript corresponding with the IGF-II mRNA was seen as well. To determine if ovarian IGF-I and IGF-II gene expression were cell-type specific, RNA samples from isolated granulosa and theca-interstitial cells were used. IGF-I mRNA was exclusively seen in the granulosa cells and IGF-II mRNA primarily in the theca-interstitial compartment.

To evaluate the role of estrogen in the regulation of ovarian IGF-I and IGF-II gene expression, immature hypophysectomized rats with a DES-s.c. silastic implant were studied. Estrogen treatment resulted in a 2-fold increase in the abundance of IGF-I transcripts and decrease in IGF-II mRNA, relative to untreated controls.

Given the pivotal role of growth hormone (GH) in the regulation of hepatic IGF-I gene expression, we studied the possibility that ovarian IGF-I gene expression may also be GH dependent. Whereas treatment of hypophysectomized rats with ovine GH by itself resulted in a 5-fold increase in hepatic IGF-I gene expression, limited inhibitory effect was observed in the ovary. In contrast, combined treatment with oGH and DES resulted in a 3-fold increase in the abundance of IGF-I mRNA.

To assess the ovarian expression of the type I IGF, type II IGF and insulin receptor genes in the immature intact rat, total RNA from whole ovarian material, as well as from freshly isolated granulosa and theca-interstitial cells were used. Single transcripts corresponding to type I IGF receptor, type II IGF receptor and insulin receptor mRNA were identified in whole ovary, and both ovarian cells.

Hypophysectomy of immature rats led to significant changes in the abundance of transcripts corresponding to type I IGF receptor in whole ovary. Treatment with FSH produced a 4-fold increase in the abundance of the steady state levels of the type I IGF receptor mRNA as compared with vehicle treated controls. Systemic treatment with DES resulted in a 3.7-fold increase in the relative representation of the type I IGF receptor mRNA (but not for the type II IGF and insulin receptor) relative to vehicle-treated control.

DISCUSSION

In this study we present evidence that the genes encoding the IGFs and their receptors are expressed in the rat ovary. Our findings establish that the immature rat ovary is a site of IGF-I and IGF-II production, reception and action. Cellular localization suggests that the rat granulosa cells and theca-interstitial compartment are a site of IGF-I and IGF-II gene expression, respectively. As such, these two peptides may play a role in ovarian physiology in an auto/paracrine manner.

As in liver [8], ovarian IGF-I gene generates different 5′-UT mRNA variants, resulting from an alternative use of leader exons 1 and 2. The demonstration of estrogen-augmented granulosa cell IGF-I gene expression suggests that the promotion of IGF-I production may be part of granulosa cell differentiation. As such, locally produced IGFs acting in concert with gonadotropins and gonadal steroids may play an important regulatory role in the course of folliculogenesis.

These observations also provide evidence that ovarian IGF-I and IGF-II gene expression may be differentially regulated by estrogens. The ability of estrogen to attenuate theca-interstitial IGF-II gene expression is in keeping with the inhibitory nature of estrogens at the level of this cell type as exemplified by the supression of gonadotropin-supported androgen biosynthesis [9].

The present observations document the ovarian granulosa and theca-interstitial cells as sites of type I IGF receptor, type II IGF/mannose-6-phosphate receptor and insulin receptor gene expression. Moreover, our studies reveal a differential pattern of hormonal regulation, wherein the ovarian type I IGF receptor (but not the type II IGF/mannose-6-phosphate receptor or insulin receptor) displays gonadotropins as well as estrogen dependence. Indeed, treatment of immature hypophysectomized rats with FSH leads to a 4-fold increase in the abundance of type I IGF receptor. Given the central role of FSH in the promotion of follicular development, the demonstration of FSH-enhanced type I IGF receptor suggests that the acquisition of IGF responsiveness may be part and parcel of granulosa cell ontogeny. Our present findings further reveal the apparent estrogen dependence of ovarian type I IGF receptor in the rat. Given that FSH plays a central role in the promotion of ovarian estrogen biosynthesis, it is tempting to speculate that the ability of FSH to up-regulate type I IGF receptor gene expression may be indirectly mediated by estrogens. In conclusion, these studies may serve to better understand the auto/paracrine role of IGFs and their receptors in the pathophysiology of follicle recruitment, oocyte maturation and potentially embryo development.

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