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THE OVARIAN INSULIN-LIKE GROWTH FACTORS, A LOCAL AMPLIFICATION MECHANISM FOR STEROIDOGENESIS AND HORMONE ACTION

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Summary—The importance of the ovarian insulin-like growth factors (IGFs) has been suggested by data from numerous laboratories and several approaches in the last several years. In the aggregate, these data indicate that this system could function as an important local amplification mechanism for steroidogenesis and gonadotropin action. Studies supporting this hypothesis have described several interacting components of this autocrine/paracrine system. First, the several types of ovarian cells possess an IGF-response system, which includes receptors for IGFs and an effective intracellular transduction system. The IGFs can promote growth and/or differentiation of ovarian cells, and their predominant actions depend on the nature of the cells and the presence of additional modulating factors. The biochemical events leading to enhanced steroidogenesis are now understood in considerable detail and include induction of several steps in the cAMP-dependent steroidogenic cascade. The second component of the ovarian IGF system comprises hormone-responsive local production of IGFs. Both IGF-I and IGF-II may be secreted; gonadotropins, gonadal steroids and locally produced growth factors can regulate the IGF system at this level. Finally, ovarian cells secrete a heterogeneous and complex family of IGF-binding proteins (IGFBPs). These proteins can impact on multiple ovarian functions in a manner which is generally opposite to that of the IGFs themselves. As is the case for the IGFs, the secretion of these proteins by ovarian cells is regulated by gonadotropins and locally produced ovarian factors. Collectively, these several components provide an integrated, synergistically cooperative local network to promote gonadotropin-dependent growth and differentiation in the ovary.

INTRODUCTION

Shortly after insulin-like growth factors (IGFs) were first purified, the actions of these peptides were examined in ovarian cell culture and found to be important promoters of growth-related endpoints such as ornithine decarboxylase activity [1] and cell number in culture [2]. Within a short period of time, it was also discovered that these peptides were potent amplifiers of gonadotropin action and steroidogenesis *in vitro* (reviewed in Refs [3, 4]). The importance of these effects for ovarian physiology was reinforced by the demonstration that IGFs were also present in the ovary and secreted by ovarian cells. In the course of studies to characterize the IGFs secreted by such cells, the presence of IGF-binding proteins (IGFBPs) was also discovered. More recently, additional information has indicated that several of these proteins are secreted in the ovary and suggested an important role for these factors in ovarian regulation as well. In the paragraphs which follow, we will review the current evidence regarding the nature of these several ovarian components and their interaction with classic ovarian regulators such as gonadotropins and gonadal steroids. From data in these several discrete areas, we will attempt to piece together a physiological construct of the nature of this system and its importance for ovarian physiology. We have aimed for a selective review, emphasizing our own data.

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IGF ACTION IN THE OVARY

The action of IGFs on ovarian cells *in vitro* has now been studied for more than 10 years. Information concerning granulosa cells is more complete, but more limited data indicate comparable actions in theca-interstitial cells [5] and probably luteal cells [6]. The actions of the IGFs are presumably mediated by specific receptors. Three binding sites for IGFs and insulin have

been demonstrated on ovarian cells—the so-called type I IGF receptor [7], the type II IGF/mannose-6-phosphate receptor [8] and the insulin receptor [9]. The importance of type II and insulin receptors for ovarian physiology has not been completely defined. However, the type I receptor is generally agreed to be critical to IGF action. Since these binding sites can be induced by gonadotropins [10] and gonadal steroids [11], such receptors could serve as an important control locus.

After activating their cell surface receptor(s), the insulin-like peptides can promote either granulosa cell replication (and related endpoints) or cellular cytodifferentiation. When the IGFs are used alone in serum-free medium or, particularly, when combined with other growth factors [7, 12], growth-related phenomena predominate. In contrast, in the presence of gonadotropins, steroidogenic responses are more important. In our hands [7], and in those of some other laboratories [13], it has been difficult to demonstrate IGF induction of steroidogenesis in the absence of gonadotropins. However, in other culture systems, these growth factors have been shown to be potent inducers of steroidogenesis when used alone [14]. The interactions of IGFs and gonadotropins on steroidogenesis involve enhancement of both cAMP synthesis [15] and action [16]. The more proximal steps may involve induction of regulatory GTP-binding proteins. Although comparable studies have not been performed in the ovary, to our knowledge, studies in adrenal cells have indicated IGF induction of such proteins [17, 18]. Similar events seem likely to be involved in IGF actions in the ovary. These events, coupled with more distal effects, which enhance cAMP action, lead to induction of key steroidogenic enzymes through mechanisms which entail, at least in part, an increase in the level of mRNA for such enzymes [19–21]. To date, IGF effects on lipoprotein binding as well as processing of cholesterol and cholesterol esters [22], side-chain cleavage activity [14, 20, 21], 17α-hydroxylase [20], and aromatase [13, 19, 23], have been demonstrated.

OVARIAN IGF SECRETION

The study of ovarian IGF levels and secretion has been a particular emphasis of our own laboratory and has entailed measurement of ovarian IGF concentrations in follicular fluid, IGF levels in media conditioned by cultured ovarian cells and demonstration of mRNA for the IGFs in ovarian samples.

Regarding levels of IGF-I in follicular fluid, our studies have shown that this peptide is easily demonstrable in follicular fluid [24, 25], and that concentrations in follicular fluid from preovulatory or gonadotropin-stimulated follicles [25, 26] are higher than those in serum or small ovarian follicles. In related studies, we found that follicular fluid from well-differentiated follicles stimulated steroidogenesis by cultured granulosa cells, and that a monoclonal antibody to IGF-I significantly inhibited this effect [27]. These data indicate that IGFs are important contributors to the steroidogenic milieu in the preovulatory follicle.

Studies of IGF-I production by cultured granulosa cells have provided a more direct demonstration of ovarian cellular secretion than is possible through analysis of follicular fluid. In addition, such studies have allowed a more detailed examination of the regulation of IGF secretion by hormones and other putative ovarian regulators. These studies have indicated that granulosa cells from immature porcine follicles secrete IGF-I for up to 10 days in culture [25]. Under these circumstances, IGF-I levels are enhanced by FSH and LH in a manner which appears to be cAMP-dependent [28]. Estradiol is also a stimulator of IGF-I production, and this steroid enhances the effects of FSH on IGF-I [28]. In addition to the effects of these classic ovarian regulators, IGF-I secretion was enhanced by GH but not prolactin [29] as well as by epidermal growth factor (EGF) but not transforming growth factor-β (TGF-β) [30]. More recent studies [31] have indicated that granulosa cells from more highly differentiated follicles produced higher levels of IGF-I in culture and are more hormonally responsive than those from immature follicles which have been used for most of our experiments.

The importance of hormone-dependent IGF production for granulosa cell function *in vitro* has also been examined with a monoclonal antibody to IGF-I [27]. Under these culture conditons, FSH, estradiol and GH alone and in various combinations, elicit a significant stimulation of progesterone production; the steroidogenic stimulus provided by these hormones was inhibited by approx. 50% by the monoclonal antibody [27]. Such studies provide the most direct evidence to date regarding a local autocrine amplifying role of the IGFs in hormone-dependent steroidogenesis.

While data regarding IGF-I is most abundant in porcine and rat ovarian systems, the predominant granulosa cell-secreted IGF in humans appears to be IGF-II [32]. Recent studies from our laboratory [33, 34], have indicated that IGF-II is also abundant in porcine follicular fluid and secreted by porcine ovarian cells. Granulosa cells from immature follicles do not appear to secrete this peptide; however, IGF-II can be detected in medium conditioned by granulosa or theca cells from more highly differentiated follicles (J. S. Mondschein and J. M. Hammond, unpublished). Although hormonal regulation of IGF-II secretion in the pig ovary remains to be completely elucidated, it is clearly different from that of IGF-I. Our studies *in vivo* have failed to show an increase of ovarian IGF-II with either GH or gonadotropin stimulation [35]. *In vitro*, preliminary studies also suggest that IGF-II production is not enhanced by the pituitary and steroid hormones previously shown to increase IGF-I levels (J. S. Mondschein and J. M. Hammond).

As a further indication of the biosynthetic capacity for IGFs in the ovary, we and others have analyzed the mRNA for these peptides in ovarian cells. Our studies, in the pig, have indicated that the mRNA for IGF-I and IGF-II are easily measurable in whole ovarian homogenates (S. E. Samaras and J. M. Hammond, unpublished). Using dissected ovarian components as well as *in situ* hybridization, we have found that IGF-I mRNA is heavily enriched in the membrana granulosa, but also expressed in corpora lutea. In contrast, IGF-II mRNA is apparently concentrated in theca cell preparations.

OVARIAN IGFBPSs

Our immunoassays of IGF levels in follicular fluid and conditioned medium have routinely involved chromatography which demonstrated IGFBPs in these samples as well [24, 25]. More recently, data from numerous laboratories (for a review, see Ref. [36]) have indicated that these proteins represent a heterogeneous family of discrete gene products with variable tissue expression, hormonal regulation and the potential for either positive or negative effects on the growth and/or differentiation of various tissues. Three IGFBPs have been cloned and sequenced and a recommended nomenclature applied (IGFBP-1, IGFBP-2 and IGFBP-3) [37]. Each of these IGFBPs is present and secreted in the ovary under some circumstances [38–40]. In addition, other lower molecular weight IGFBPs have been identified [41]. Recent studies from Ling *et al.* [42] have indicated that these proteins inhibit many aspects of gonadotropin action in cultured rat granulosa cells. In many respects, these results are similar to those obtained with a monoclonal to IGF-I [27, 42]. Thus, their principal mode of action may be the sequestration and inactivation of locally produced IGFs. However, there appear to be some qualitative and quantitative differences in the actions of the IGFBPs and the antibody which cannot be readily explained by this mechanism [42].

Our own studies have focused on the nature of IGFBPs in the porcine ovary and their physiological regulation. The nature of the IGFBPs has been examined by ligand blotting after resolving these proteins on polyacrylamide gels as a function of molecular size, by immunoprecipitation strategies, and by hybridization analyses of ovarian RNA. Collectively, these observations have indicated that IGFBP-3 and IGFBP-2 are the main forms of IGFBP in porcine follicular fluid and cell conditioned medium [40]. IGFBP-3 is generally more abundant than IGFBP-2. Under some circumstances, however, lower molecular weight forms (29 and 22 kDa) are clearly apparent. These forms are not recognized by antibodies to either of the aforementioned IGFBPs or to human IGFBP-1, the form which has received greatest attention in the human ovary. Hybridization analysis with cDNA probes to IGFBP-2 and IGFBP-3 also gives a strong signal in porcine ovary, while no signal can be detected with probes to human IGFBP-1 (S. E. Samaras and J. M. Hammond, unpublished).

Our approach to the physiological regulations of the IGFBPs paralleled, in large measure, that described above for analysis of immunoreactive IGF-I. Comparing follicular fluid from large and small follicles, we found that small follicles had approximately twice the total IGFBP activity [43]. IGFBP-3 was the predominant IGFBP in both types of follicular fluid, and its concentrations were virtually identical in the two classes of follicles. However, there were substantially higher levels of IGFBP-2 and unidentified low molecular weight forms in immature follicles. When follicular fluid from hormone-treated animals was analyzed, we found that GH treatment significantly enhanced IGFBP activity, whereas gonadotropin treatment diminished IGFBP activity in follicular fluid [35]. Studies of cultured granulosa cells

have indicated that these cells are capable of secreting each of the IGFBP forms identified in follicular fluid. The amount and type of IGFBP activity secreted depends on the follicle of origin of the granulosa cells, the length of time in culture, and the complement of hormones and growth factors to which the cells are exposed *in vitro* [40]. Particularly dramatic changes in culture were occasioned by treatment of granulosa cells with FSH and TGF-β which inhibited IGFBP secretion with a predominant action on IGFBP-3. In contrast, estradiol and EGF enhanced the production of this IGFBP.

SUMMARY AND CONCLUSIONS

Evidence reviewed in preceding sections provides a strong argument for the ovarian IGFs as important intraovarian regulators. Much of the evidence presented has relied on *in vitro* systems which allow easier experimental access. In such systems, effects of the IGFs have been shown on virtually every aspect of ovarian function examined to date. In addition, these studies have shown hormonally regulated IGF production and abrogation of hormone effects when locally produced IGF was sequestered with a monoclonal antibody.

Compelling proof for operation of these mechanisms *in vivo* remains elusive. However, a number of correlative studies support this possibility. In particular, our own studies with monoclonal antibodies have suggested that IGFs are critical to the steroidogenic milieu of the preovulatory follicle [27]. Other studies from our laboratory have indicated that IGF levels correlate with follicular development [25, 26] and, in some strains, with increased birth rate [44]. Finally, administration of GH, presumably by increasing ovarian IGF levels, has been found to promote ovulation induction in women [45, 46].

The central theme in ovarian IGF research to date has been the role of these peptides as an amplification system for gonadotropin action. The data generated indicates that this role can be fulfilled by multiple interlocking mechanisms: (1) FSH and FSH-stimulated steroids increase IGF-I receptor levels; (2) FSH and IGFs have a synergistic interaction on the cAMP-dependent steroidogenic cascade; (3) gonadotropins promote production of IGF by cultured granulosa cells, particulary in the presence of estradiol; and (4) FSH decreases the secretion of the inhibitory IGFBPs. Follicles which are able to develop these mechanisms should have a substantial competitive advantage in growth, development and ultimately ovulation.

Despite the wealth of information about this system, there are some challenging complexities which remain to be elucidated. These include the relative importance of IGF-I and IGF-II for ovarian physiology and the variation in expression of these two growth factors amongst species, the identification and function of all the IGFBPs, the interaction between the IGF system and other ovarian growth factors, and expression and action of IGFs in thecal and luteal compartments of the ovary. Progress in these areas will be required before a comprehensive understanding of the role of the IGF system in ovarian physiology can be achieved.

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