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Insulin-like growth factor I affects the intraovarian interleukin-1 system: evidence for suppression of type I interleukin-1 receptor expression and enhancement of secretory phospholipase A_2 expression and activity

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This study assessed the possibility that the intraovarian insulin-like growth factor I (IGF-I) system interacts with the intraovarian interleukin-1 (IL-1) system, the central role of which has been the subject of increasing attention. To this end, whole ovarian dispersates from immature rats were cultured for 48 h in the absence or presence of IGF-I or IGF-binding protein-3 (IGFBP-3), with or without IL-1β. Cellular RNA content was subjected to a solution hybridization, RNase protection assay with gel-purified [^{32}P]-UTP-labelled antisense riboprobes for rat IL-1β, type I IL-1 receptor (IL-1R) and secretory phospholipase A_2 (sPLA$_2$). PLA$_2$ activity in conditioned media was assayed by measuring the release of [^{3}H]-labelled palmitic acid from the *sn-2* position of [^{3}H]-labelled phosphatidylcholine dipalmitoyl (PCDP) substrate. Treatment with IGF-I resulted in a significant ($P < 0.01$) decrease in type I IL-1R transcripts (an effect which was reversed by co-treatment with IL-1β), was without effect on IL-1β transcripts, and significantly ($P < 0.05$) increased sPLA$_2$ gene expression (an effect which was further enhanced by co-treatment with IL-1β). Treatment with IGF-I resulted in a significant increase in extracellular PLA$_2$ activity over untreated control. These observations suggest that IGF-I may down-regulate ovarian IL-1 action by decreasing type I IL-1R gene expression, while up-regulating sPLA$_2$ gene expression and activity. These findings are consistent with a role for IGF-I in suppressing IL-1 actions while promoting the generation of prostaglandins. It is tempting to speculate that IGF-I, an intraovarian regulator concerned with promoting folliculogenesis, may be also entwined with priming the prostaglandin-producing potential in anticipation of subsequent ovulation.

Key words: IGF-I/IGFBP-3/interleukin-1/phospholipase A_2 activity

Introduction

Current views favour the existence of intraovarian regulatory systems, presumed to modulate gonadotrophin action *in situ* in the interest of follicular development, acquisition of dominance and eventual ovulation. In that regard, the insulin-like growth factor I (IGF-I) and interleukin (IL)-1 systems, both replete with ligands, receptors, binding proteins (BPs) and receptor antagonist, were extensively studied.

The main role of IGF-I in the ovary appears to be local amplification of the effects of follicle stimulating hormone (FSH) (Adashi *et al.*, 1992a), thus becoming a determinant of follicular fate. This is based on the following observations: firstly, healthy follicular granulosa cells are IGF-I-replete but IGFBP-4/IGFBP-5-depleted. The reverse pattern exists in atretic follicles (Oliver *et al.*, 1989; Nakatani *et al.*, 1991; Erickson *et al.*, 1992a,b); secondly, FSH-dependent differentiation of granulosa cells entails induction of type I IGF receptors (Adashi *et al.*, 1986, 1988) and reduced accumulation of IGFBP-4 and IGFBP-5 (Adashi *et al.*, 1991, 1993), partially due to induction of their respective endopeptidases (Fielder *et al.*, 1993); and thirdly, IGF-I suppresses, whereas IGFBPs promote, presumably as the result of their IGF-I-sequestering property, follicular apoptosis, in turn leading to an antigonadotrophic effect (Adashi *et al.*, 1992b).

Whereas the IGF-I system is involved primarily in follicular development, intraovarian IL-1β is presumed to be an intermediary in the ovulatory process itself. IL-1β synergizes the ovulatory effect of LH in ex-vivo perfused ovaries (Brännstrom *et al.*, 1993; Takehara *et al.*, 1994). The addition of IL-1 receptor antagonist (IL-1RA) attenuates luetinizing hormone (LH)-induced ovulation under both *ex vivo* (Peterson *et al.*, 1993) and *in vivo* (Simón *et al.*, 1994) circumstances, and IL-1β induces a host of well established ovulation-associated phenomena (Kokia *et al.*, 1992; Hurwitz *et al.*, 1993; Kokia *et al.*, 1993; Ben-Shlomo *et al.*, 1994a,b). Interestingly, a possible interaction of the intraovarian IGF-I and IL-1 systems has not been studied. Such an interaction is known from rabbit articular chondrocytes, wherein IGF-I reduces the effect of IL-1β on the expression and activity of secretory phospholipase A_2 (sPLA$_2$) (Berenbaum *et al.*, 1994; Matsumoto *et al.*, 1994). In vascular smooth muscle IGF-I inhibits IL-1β-induced nitric oxide production (Schini *et al.*, 1994).

The objectives of the current study were to assess the effect of IGF-I on the transcript levels of IL-1β and its cognate type I receptor, as well as on $sPLA_2$ expression and activity, used herein as representative IL-1 end-points.

Materials and methods

Animals and tissue cultures

Immature Sprague–Dawley female rats from Zivic-Miller Laboratories (Zelienople, PA, USA) were killed by CO_2 asphyxiation on day 25 of life. The project was approved by the Institutional Animal Care and Use Committee. Whole ovarian dispersates were prepared and cultured as previously described (Hurwitz *et al.*, 1991).

Reagents and hormones

Phosphatidylcholine, L-α-dipalmitoyl-[2-palmitoyl-9,10–^{3}H(N), PCDP], specific activity 42 Ci/mmol; palmitic acid (9,10^{3}H), specific activity 39 Ci/mmol; and [α-^{32}P]-UTP, specific activity 800 Ci/mmol were purchased from DuPont, New England Nuclear (Boston, MA, USA). The corresponding unlabelled principles and RNase A were purchased from Sigma Chemical Co. (St Louis, MO, USA). McCoy's 5a (serum-free) medium, penicillin-streptomycin solution, bovine-serum albumin (BSA), DNase, Trypan Blue stain, and Moloney Murine Luekemia Virus reverse transcriptase were obtained from Life Technologies Inc (Grand Island, NY, USA). Collagenase (*Clostridium histolyticum*; CLS type I; 144 IU/mg) was from Worthington Biochemical Corp. (Freehold, NJ, USA). Hexane, methanol and 2-propanol were from J.T.Baker Inc (Phillipsburg, NJ, USA). Chloroform was from Fisher Scientific (Fair Lawn, NJ, USA). Random primers (pdN6) were from Pharmacia (Piscataway, NJ, USA). Ampli*Taq* polymerase was from Perkin Elmer (Norwalk, CT, USA). Recombinant human IL-1β (2×10^7 units/mg) was generously provided by Doctors Errol B.De Souza and C.E.Newton, DuPont-Merck Pharmaceutical Co. (Wilmington, DE, USA). IGF-I was from Bachem (Torrance, CA, USA), IGFBP-3 was a generous gift from Dr Christopher Maack of Biogrowth Inc. (Richmond, CA, USA). RNase T1 was from Pharmacia. Ecl136II was from New England Biolabs Inc (Beverly, MA, USA). pCR1000 vector was from Invitrogen (San Diego, CA, USA). T7 RNA polymerase, pGEM7Zf+ and other molecular grade reagents were from Promega (Madison, WI, USA).

Nucleic acid probes

A plasmid containing a 750 bp *Sma*I/*Eco*RI insert of the cDNA encoding rat type II $sPLA_2$ (Ishizaki *et al.*, 1989) was kindly provided by Dr J.Ishizaki from Shionogi Research Laboratories (Osaka, Japan). For the purpose of RNase protection assays, a *Bam*HI fragment was excised and subcloned into pGEM7Zf+. This construct was then linearized with *Ecl*136II and transcribed with T7 RNA polymerase to yield a 535 nt riboprobe which, upon hybridization, was projected to generate a 452 nt protected fragment. The rat IL-1β cDNA was provided by Dr A.Shaw of Glaxo (Geneva, Switzerland). A 222 bp *Pst*I fragment of the original cDNA was sub-cloned into pGEM2. T7-driven transcription of the *Eco*RI-linearized plasmid yielded a 272 nt riboprobe which upon hybridization was projected to generate a 222 nt protected fragment. Rat type I IL-1R probe was generated as previously described by Scherzer *et al.* (1996). The expected lengths for the probe and the resultant protected fragment are 374 and 307 nt respectively. The ribosomal protein L19 (RPL19) probe was generated as previously described (Scherzer *et al.*, 1996). T7-driven RNA polymerase was designed to generate a 234 nt probe capable of protecting a 153 nt segment.

RNase protection assay

RNA was extracted from cultured cells with RNAZOL-B (Tel Test, Friendswood, TX, USA) according to the manufacturer's protocol. The riboprobes were prepared and purified as previously described (Kol *et al.*, 1996). Solution hybridization RNase-protection products were electrophoresed as previously described (Kol *et al.*, 1996). Gels were exposed to a phosphor screen (Molecular Dynamics, Sunnyvale, CA, USA) and digitized data were analyzed with Image Quant software (Molecular Dynamics). The hormonally-independent RPL19 signal was used to normalize for possible variations in RNA loads.

Cell-free enzyme assays

Extracellular $sPLA_2$ activity was determined by measuring the release of [^{3}H]-labelled palmitic acid from the *sn-2* position of [^{3}H]-labelled PCDP substrate. Whole ovarian dispersates (1.5×10^6 viable cells/dish) were initially cultured as described for 48 h in the absence or presence of the specified treatments. Media were then collected, and a 300 ml aliquot was incubated with 5 mM PCDP inclusive of 25×10^3 c.p.m. of labelled material for 1h at 37°C in a total assay volume of 1 ml. The assay buffer consisted of 5% methanol and 2 mM $CaCl_2$. The enzymatic reaction was terminated with 7% formic acid (final pH 3.5), the resultant products being extracted using ethyl acetate. After the latter solvent was evaporated in a Speed-Vac Centrifuge (Savant Instruments Inc, Farmingdale, NY, USA), the residues were resuspended in 250 μl hexane/isopropanol/acetic acid, 95/5/.025 by volume. The resultant products were then fractionated by high pressure liquid chromatography (HPLC) using a Waters Apparatus, WISP 710B (Milford, MA, USA) on a normal phase diol column (LiChrosorb Diol, 10 μm) using a concave gradient of hexane/isopropanol (95:5–60:40) at 2 ml/min. The column was previously calibrated with authentic [^{3}H] palmitic acid. Radiolabelled palmitic acid was detected and quantified by on-line scintillation counting with a Radiomatic Flow Detector (Packard Instrument Co, CT, USA). The ratio of labelled product to labelled substrate (25×10^3 c.p.m.) was used to calculate the rate of enzymatic conversion (pmol/10^5 cells/h), corrected to substrate availability in the reaction mixture, and product recovery.

Statistical analysis

Except as noted, each experiment was replicated a minimum of three times. Data points are presented as mean ± SE and statistical significance determined by Student's *t*-test as calculated using Statview 512+ for Macintosh (Brain Power Inc, Calabasas, CA, USA).

Results

IL-1β and type I IL-1R gene expression in whole ovarian dispersates: effect of treatment with IGF-I and IGFBP-3

To determine the effect of treatment with IGF-I and IGFBP-3 on in-vitro IL-1β and type I IL-1R gene expression, whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in the absence or presence of IGF-I (50 ng/ml) or IGFBP-3 (10 mg/ml). At the conclusion of this period, total RNA was extracted and subjected to RNase protection assay with [^{32}P]-UTP-labelled antisense riboprobes corresponding to IL-1β, type I IL-1R and RPL19. As shown (Figure 1), treatment with IGF-I produced a modest, albeit significant ($P < 0.01$), decrease in type I IL-1R gene expression. IGF-I was without effect on the level of IL-1β transcripts. Similarly, treatment with IGFBP-3 was without effect on the level of IL-1β and

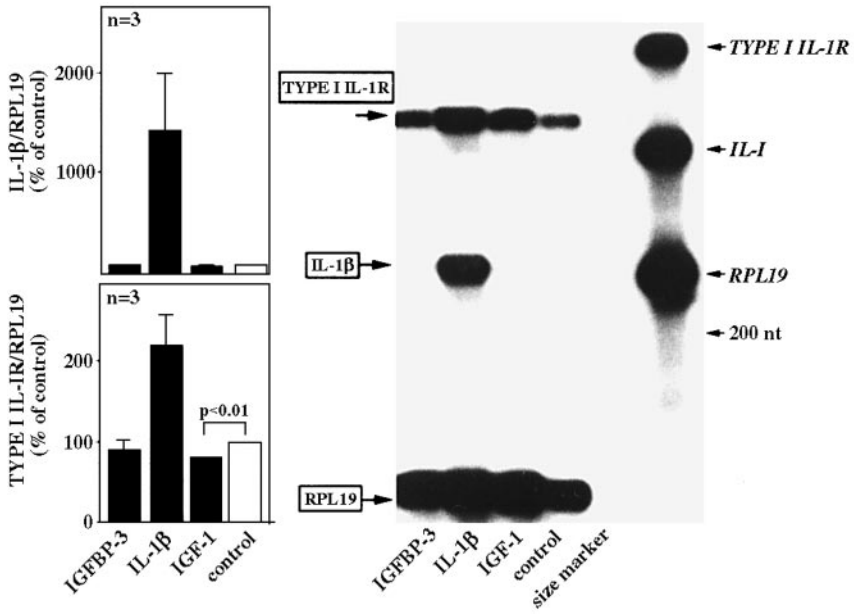

Figure 1. Interleukin (IL)-1β and type I IL-1R gene expression in whole ovarian dispersates cultured in the presence of insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3. Results of RNase protection assay using antisense riboprobes corresponding to rat IL-1β, type I IL-1R and RPL19. The left two panels present the mean ± SE of three independent experiments. Some error bars are too small to be visible. The right panel shows a single representative autoradiograph. The full-length riboprobes are labelled in italics, and the protected fragments in bold lettering. A lane with RNA from IL-1β-treated cells is shown as a positive control.

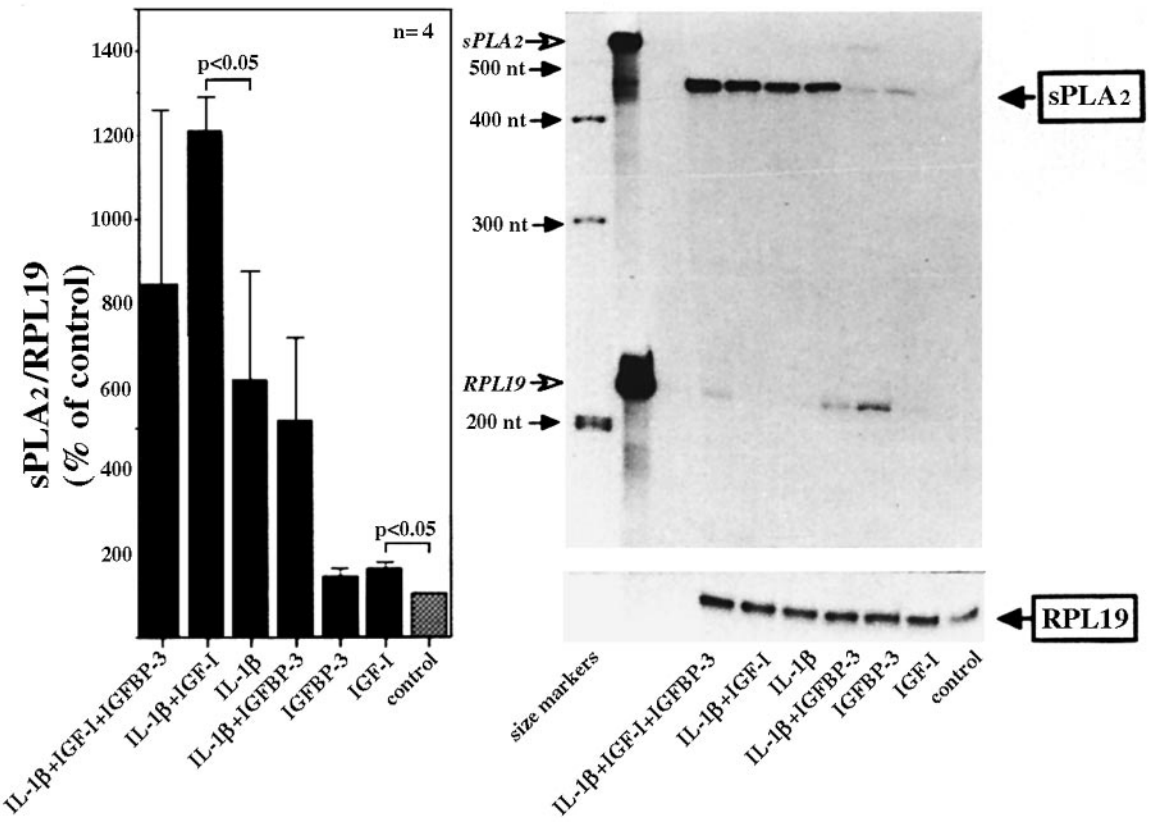

Figure 2. Effect of culture with insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 on the basal and interleukin (IL)-induced levels of secretory phospholipase A_2 ($sPLA_2$) gene expression by whole ovarian dispersates. Results of RNase protection assay using antisense riboprobes corresponding to rat $sPLA_2$ and RPL19. The left panel depicts in bar graph form the mean ± SE of four independent experiments. The right panel shows a single representative autoradiograph. The full-length riboprobes are labelled in italics. The corresponding protected fragments are depicted in bold lettering.

type I IL-1R transcripts. These observations suggest that IGF-I may be capable of down-regulating ovarian IL-1 action by decreasing the expression of type I IL-1 receptors. An additional set of experiments suggests that maximally stimulatory concentrations of IL-1β are capable of reversing the IGF-I-induced effect on type I IL-1R gene expression (not shown).

Effect of treatment with IGF-I and IGFBP-3 on basal and IL-1β-induced levels of $sPLA_2$ gene expression

To determine the effect of treatment with IGF-I and IGFBP-3 on in-vitro $sPLA_2$ gene expression, and the ability of IL-1β to induce $sPLA_2$ transcripts, whole ovarian dispersates were cultured as above. Total RNA was extracted and subjected to an RNase protection assay with [^{32}P]-labelled rat antisense riboprobes corresponding to $sPLA_2$ and RPL19. As shown (Figure 2), treatment with IGF-I produced a modest, albeit significant ($P < 0.05$), increase in $sPLA_2$ gene expression. Treatment with IGFBP-3 did not change the basal levels of $sPLA_2$ transcripts. Co-treatment with IGF-I and IL-1β resulted in a significant ($P < 0.05$) increase in $sPLA_2$ transcripts compared to treatment with IL-1β alone. The addition of IGFBP-3 reversed that effect, while IGFBP-3 itself did not affect the IL-1β-induced $sPLA_2$ gene up-regulation. These observations suggest that in IL-1β-stimulated IGF-I-replete conditions cellular $sPLA_2$ gene expression increases, beyond the level induced by IL-1β alone.

Effect of treatment with IGF-I on basal and IL-1β-induced ovarian extracellular PLA_2 activity

To determine whether the observed IGF-I-induced increase in $sPLA_2$ transcripts is reflected also in extracellular PLA_2 activity, whole ovarian dispersates were cultured as above. At the conclusion of this period, media were collected and subjected to a cell-free PLA_2 activity assay as described, with PCDP as substrate. As shown in Figure 3, treatment with IGF-I resulted in a significant ($P < 0.005$) increase in extracellular PLA2 activity over untreated controls. Co-treatment with IL-1β and IGF-I resulted in a modest increase in extracellular PLA_2 activity, compared to treatment with IL-1β alone. However, this increase did not reach statistical significance ($P = 0.086$). These observation document the ability of IGF-I to induce an increase in extracellular PLA_2 activity.

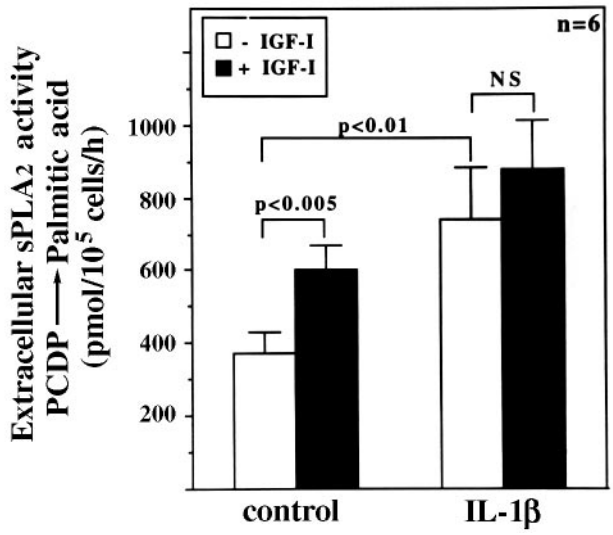

Figure 3. Effect of culture with insulin-like growth factor (IGF)-I on basal and interleukin (IL)-1β-induced ovarian extracellular phospholipase A_2 (PLA_2) activity. Results of cell-free PLA_2 activity assay as described, with phosphatidylcholine dipalmitoyl (PCDP) as substrate and palmitic acid as product. The graph depicts the mean ± SE of six independent experiments.

Discussion

Herein we documented the ability of IGF-I to inhibit the basal expression of type I IL-1R in cultured whole ovarian dispersates of rat origin, but not the IL-β-induced increase of these transcripts. In contrast, IGF-I increases the basal, as well as the IL-1β-induced levels of $sPLA_2$ expression, as well as the PLA_2 activity in conditioned media. Interestingly, all these changes are not dependent upon the expression of IL-β transcripts, which IGF-I was not found to affect.

A large body of information now supports the view that IGF-I may be involved in the coordination of follicular development, its main role being the amplification of gonadotrophin action. Follicular growth is associated with a decrease in the local concentration of IGF-I binding protein, while follicular atresia involves increased IGFBP availability. Further regulation of the level of IGFBP is affected by IGF-I itself, which controls their expression and proteolytic degradation (Grimes and Hammond, 1994).

IL-1 on the other hand, may be the centrepiece of an intraovarian regulatory loop concerned with the genesis and maintenance of the preovulatory cascade of follicular events, leading to ovulation itself. In this regard, Takehara *et al.* (1994) and Simón *et al.* (1994) showed that an IL-1 vacuum (created by treating rats and rabbits with the naturally occurring IL-1 receptor antagonist) blocks ovulation.

As the framework of the system of specific growth factors is being unravelled, the next logical step is to deduce the possible interactions between these systems. In this regard, IGF-I was shown to enhance angiotensin II production, which is typically associated with the event of ovulation, in ex-vivo perfused rabbit ovaries (Yoshimura *et al.*, 1996). The authors suggested that proper follicular development, made possible by adequate IGF-I levels, also entails acquisition of the ability to produce angiotensin II which is needed in the process of ovulation.

It is tempting to speculate that IGF-I inhibits type I IL-1R expression in the interest of protecting the developing follicles from ovulation-associated events triggered prematurely by the IL-1 system. However, once a critical local concentration of IL-1 is reached, such as that which is built-up after the midcycle gonadotrophin surge, it overrides any IGF-I inhibitory effect, possibly with the aim of committing the follicle to the rapid sequence of cellular events, which characterizes ovulation. In this regard, the notable ability of an IGF-I-replete environment to increase the ability of ovarian cells to produce $sPLA_2$ suggests that healthy follicles, while being prevented from entering a premature cascade of events leading to ovulation, acquire the ability to produce large amounts of

$sPLA_2$ in anticipation of timely entry into the ovulatory cascade. By no means, are the changes in $sPLA_2$ production capacity the single event concerned with prostaglandins production in the rat ovary. Hedin *et al.* (1987) have unequivocally shown that the synthesis of prostaglandins, that is increased by human chorionic gonadotrophin (HCG) in rats before ovulation, is associated with a transient induction of prostaglandin endoperoxide synthase (PGS). Hence, the eventual levels of prostaglandins produced in the follicle during ovulation depend upon more factors than $sPLA_2$ levels alone. Taken together, IL-1 and IGF-I may cooperate in up-regulating $sPLA_2$ concentration, thereby establishing the ground for subsequent outpouring of prostaglandins, triggered by the ovulation-associated increase in PGS levels.

In summary, we have presented novel evidence for an interaction between the ovarian IL-1 and IGF-I systems, which may be instrumental in the sequence of events concerned with properly timed ovulation achieved by adequately developed dominant follicles.

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References

Adashi, E.Y., Resnick, C.E., Svoboda, M.E. *et al.* (1986) Follicle-stimulating hormone enhances somatomedin-C binding to cultured rat granulosa cells: evidence for cAMP-dependence. *J. Biol. Chem.*, **261,** 3923–3926.

Adashi, E.Y., Resnick, C.E., Svoboda, M.E. *et al.* (1988) *In vivo* regulation of granulosa cell somatomedin-C/insulin-like growth factor-I receptors. *Endocrinology*, **122,** 1383–1390.

Adashi, E.Y., Resnick, C.E., Hurwitz, A. *et al.* (1991) Ovarian granulosa cell-derived insulin-like growth factor binding proteins: modulatory role of follicle-stimulating hormone. *Endocrinology*, **128,** 754–760.

Adashi, E.Y., Resnick, C.E., Ricciarelli, E. *et al.* (1992a) Local tissue modification of follicle stimulating hormone action. In Genazzani, A.R., Petraglia, F. (eds), *Hormones in Gynecological Endocrinology.* Parthenon Publishing Group, Carnforth, UK, pp. 255.

Adashi, E.Y., Resnick, C.E., Ricciarelli, E. *et al.* (1992b) Granulosa cell-derived insulin-like growth factor (IGF) binding proteins are inhibitory to IGF-I hormonal action: evidence derived from the use of a truncated IGF-I analog. *J. Clin. Invest.*, **90,** 1593–1599.

Adashi, E.Y., Resnick, C.E., Tedeschi, C. *et al.* (1993) A kinase-mediated regulation of granulosa cell-derived insulin-like growth factor binding proteins: disparate response sensitivities of distinct IGFBP species. *Endocrinology*, **132,** 1463–1468.

Ben-Shlomo, I., Kokia, E., Adashi, E.Y. *et al.* (1994a) Interleukin-1β stimulates nitrite production in the rat ovary: evidence for heterologous cell–cell interaction and for insulin-mediated regulation of the inducible isoform of nitric oxide synthase. *Biol. Reprod.*, **51,** 310–318.

Ben-Shlomo, I., Adashi, E.Y. and Payne, D.W. (1994b) The morphogenic/cytotoxic and prostaglandin-stimulating activities of interleukin-1β in the rat ovary are nitric oxide-independent. *J. Clin. Invest.*, **94,** 1463–1469.

Berenbaum, F., Thomas, G., Poiraudeau, S. *et al.* (1994) Insulin-like growth factors counteract the effect of interleukin 1β on type II phospholipase A_2 expression and arachidonic acid release by rabbit articular chondrocytes. *FEBS Lett.*, **340,** 51–55.

Brännstrom, M., Wang, L. and Norman, L. (1993) Ovulatory effect of interleukin-1β on the perfused rat ovary. *Endocrinology*, **132,** 399–404

Erickson, G.F., Nakatani, A., Ling, N. *et al.* (1992a) Cyclic changes in insulin-like growth factor binding protein-4 messenger ribonucleic acid in the rat ovary. *Endocrinology*, **130,** 625–636.

Erickson, G., Nakatani, A., Ling, N. *et al.* (1992b) Localization of insulin-like growth factor-binding protein-5 messenger ribonucleic acid in rat ovaries during the estrous cycle. *Endocrinology*, **130,** 1867–1878.

Fielder, P.J., Pham, H., Adashi, E.Y. *et al.* (1993) Insulin-like growth factors (IGFs) block FSH-induced proteolysis of IGF-binding protein-5 (BP-5) in cultured rat granulosa cells. *Endocrinology*, **133,** 415–418.

Grimes, R.W. and Hammond J.M. (1994) Proteolytic degradation of insulin like growth factor (IGF)-binding protein-3 by porcine ovarian granulosa cells in culture: regulation by IGF-I. *Endocrinology*, **134,** 337–343.

Hedin, L., Gaddy-Kurten, D., Kurten, R. *et al.* (1987) Prostaglandin endoperoxide synthase in rat ovarian follicles: content, cellular distribution, and evidence for hormonal induction preceding ovulation. *Endocrinology*, **121,** 722–731.

Hurwitz, A., Payne, D.W., Packman, J.N. *et al.* (1991) Cytokine-mediated regulation of ovarian function: Interleukin-1 inhibits gonadotropin-induced androgen biosynthesis. *Endocrinology*, **129,** 1250–1256.

Hurwitz, A., Dushnik, M., Solomon, H. *et al.* (1993) Cytokine-mediated regulation of rat ovarian function: interleukin-1 stimulates the accumulation of 92-kD gelatinase. *Endocrinology*, **132,** 2709–2714.

Ishizaki, J., Ohara, O., Nakamura, E. *et al.* (1989) cDNA cloning and sequence determination of rat membrane-associated phospholipase A_2. *Biochem. Biophys. Res. Commun.*, **162,** 1030–1036.

Kokia, E., Hurwitz, A., Ricciarelli, E. *et al.* (1992) Interleukin-1 stimulates ovarian prostaglandin biosynthesis: evidence for heterologous contact-independent cell–cell interaction. *Endocrinology*, **130,** 3095–3097.

Kokia, E., Hurwitz, A., Ben-Shlomo, I. *et al.* (1993) Receptor-mediated stimulatory effect of IL-1β on hyaluronic acid and proteoglycan biosynthesis by cultured rat ovarian cells: role for heterologous cell–cell interactions. *Endocrinology*, **133,** 2391–2394.

Kol, S., Ben-Shlomo, I., Adashi, A.Y. *et al.* (1996) Simplified riboprobe purification using translucent straws as gel tubes. *Genet. Anal. Biomol. Eng.*, **12,** 129–132.

Matsumoto, T., Tsukazaki, T., Enomoto, H. *et al.* (1994) Effects of interleukin-1β on insulin-like growth factor-I autocrine/paracrine axis in cultured rat articular chondrocytes. *Ann. Rheum. Dis.*, **53,** 128–133.

Nakatani, A., Shimasaki, S., Erickson, G.F. *et al.* (1991) Tissue-specific expression of four insulin-like growth factor-binding proteins (1,2,3, and 4) in the rat ovary. *Endocrinology*, **129,** 1521–1529.

Oliver, J.E., Aitman, T.J., Powell, J.F. *et al.* (1989) Insulin-like growth factor I gene expression in the rat ovary is confined to the granulosa cells of developing follicles. *Endocrinology*, **124,** 267–2679.

Peterson, C., Hales, H., Hatasaka, H. *et al.* (1993) Interleukin-1β (IL-1β) modulates prostaglandin production and the natural IL-1 receptor antagonist inhibits ovulation in the optimally stimulated rat ovarian perfusion model. *Endocrinology*, **133,** 2301–2306.

Scherzer, W.J., Ruutiainen-Altman, K.S.L., Putowski, L.T. *et al.* (1996) Detection and *in vivo* hormonal regulation of rat ovarian type I and type II interleukin-1 receptor mRNAs: increased expression during the periovulatory period. *J. Soc. Gynecol. Invest.*, **3,** 131–140.

Schini, V.B., Catovsky, S., Schray-Utz, B. *et al.* (1994) Insulin-like growth factor I inhibits induction of nitric oxide synthase in vascular smooth muscle cells. *Circ. Res.*, **74,** 24–32.

Simón, C., Tsafriri, A., Chun, S. *et al.* (1994) Interleukin-1 receptor antagonist suppresses human chorionic gonadotropin-induced ovulation in the rat. *Biol. Reprod.*, **51,** 662–667.

Takehara, Y., Dharmarajan, A., Kaufman, G. *et al.* (1994) Effect of interleukin-1β on ovulation in the *in vitro* perfused rabbit ovary. *Endocrinology*, **134,** 1788–1793.

Yoshimura, Y., Aoki, N., Sueoka, K. *et al.* (1996) Interactions between insulin-like growth factor-I (IGF-I) and the renin-angiotensin system in follicular growth and ovulation. *J. Clin. Invest.*, **98,** 308–316.

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