

Ovarian Expression, Cellular Localization, and Hormonal Regulation of Rat Secretory Phospholipase A₂: Increased Expression by Interleukin-1 and by Gonadotropins¹

Izhar Ben-Shlomo,³ Shahar Kol,⁴ Motomu Ando, Kristiina Ruutiainen Altman, Lechoslaw T. Putowski,⁵ Richard M. Rohan,⁶ and Eli Y. Adashi²

Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Maryland School of Medicine, Baltimore, Maryland 21201

ABSTRACT

It has been suggested that ovulation may constitute a cyclic inflammatory-like process and that gonadotropin-inducible intraovarian interleukin (IL)-1, an established mediator of inflammation, may play a central role in this regard. In support of this hypothesis, our group has been able to document the ability of IL-1 to potentially stimulate prostaglandin biosynthesis by cultured rat ovarian cells. Herein we explore the possibility that the prostaglandin-stimulating action of IL-1 is due, in part, to the enhanced expression of ovarian secretory phospholipase-A₂ (sPLA₂). A single sPLA₂ transcript of 1.4 kilobases was noted in all extraovarian tissues of immature rat origin subjected to Northern blot analysis. However, only a barely detectable signal was apparent in ovarian tissue. In contrast, the more sensitive RNase protection assay revealed the unequivocal presence of ovarian sPLA₂ transcripts. Cellular localization studies by way of in situ hybridization documented sPLA₂ transcripts primarily in the granulosa cell of the periovulatory ovary. Molecular probing of untreated cultured whole ovarian dispersates disclosed spontaneous elaboration of sPLA₂ transcripts as early as 20 h after the introduction of cells into culture. Treatment of cultured whole ovarian dispersates with IL-1 β for 48 h produced a 1.7-fold increase (over the value in untreated controls) in the relative expression of sPLA₂ transcripts ($p < 0.01$) along with a 1.7-fold increase in media PLA₂ activity ($p < 0.01$). A more marked increase was documented for IL-1 β -treated cultured isolated granulosa cells (12.5-fold increase, $p < 0.001$). Treatment of whole ovarian dispersates with an IL-1 receptor antagonist (IL-1RA) produced a reduction in the basal expression of sPLA₂ transcripts (55% at the 5 μ g/ml dose level; $p < 0.01$) and PLA₂ activity (40%; $p < 0.01$), thereby suggesting basal endogenous IL-1-like bioactivity. Treatment of cultured whole ovarian dis-

persates with either hCG or FSH led to 2.6-fold ($p = 0.056$) and 3-fold ($p = 0.029$) increases in the abundance of sPLA₂ transcripts, respectively, effects blocked by the concurrent presence of IL-1RA. These observations 1) document the immature rat ovary as a site of sPLA₂ gene expression, 2) localize the relevant transcripts to the postovulatory granulosa cell, 3) confirm the presence of functional secreted ovarian PLA₂ activity, 4) reveal PLA₂ expression to be IL-1- and gonadotropin-dependent, and 5) suggest the existence of endogenous PLA₂-stimulating IL-1-like activity. These findings also suggest that the ability of hCG or FSH to up-regulate ovarian sPLA₂ transcripts may be due, in part, to the endogenous elaboration of IL-like activity.

INTRODUCTION

It has been suggested that ovulation may constitute a cyclic inflammatory-like process [1, 2] and that gonadotropin-inducible intraovarian interleukin (IL)-1 [3], an established mediator of inflammation [4], may play a central role in this regard [5]. Indeed, the mammalian ovary has been shown to be a site of IL-1 production [6–9], reception [8, 10, 11], and action [12–39]. Most importantly, IL-1 has been shown to promote follicular rupture and to synergize with LH in this respect [40, 41]. The IL-1 receptor antagonist in turn was shown to repress follicular rupture [42, 43].

Since prostaglandin biosynthesis constitutes an established corollary of the ovulatory cascade [44], the possibility that IL-1 may modulate ovarian prostanoid accumulation has been investigated [45–47]. In this connection, this laboratory documented the ability of IL-1 β to potentially stimulate prostaglandin (PGE₂ > PGF_{2 α}) biosynthesis by cultured whole ovarian dispersates from immature rats [45]. In order to begin elucidation of the molecular mechanisms underlying this phenomenon, we explored the possibility that the IL-1-stimulated biosynthesis of ovarian prostaglandins is due, in part, to the enhancement of ovarian phospholipase-A₂ (PLA₂) activity. This enzyme, which is the initial and event-limiting step in the eicosanoid biosynthetic cascade [48], is a member of a heterogeneous family of enzymes consisting of two main classes of proteins: a secretory, low molecular weight species (14 kDa, sPLA₂) and a cytosolic, high molecular weight (85–110 kDa, cPLA₂) species. The former is further subclassified into digestive group I (synthesized and secreted mainly by the pancreas) and nondigestive group II (synthesized and secreted by many cell types). This paper will focus on the ovarian expression, cellular localization, and hormonal regulation of the group II sPLA₂ variety. Special emphasis will be placed on examining the possibility that ovarian PLA₂ expression is IL-1 dependent and the possibility that IL-1 may be an intermediary in the prostaglandin-stimulating property of gonadotropins [44].

Accepted February 28, 1997.

Received June 12, 1996.

¹This work was supported in part by NIH grants HD-19998 and HD-30288 (E.Y.A.). I.B.-S. is the recipient of a Lalor Foundation Fellowship, an International Fellowship Award from the Israeli Medical Association, a Fulbright Fellowship, and a Harfe'a Charitable Trust Award. S.K. is the recipient of a 1994 Merck Senior Fellow Award of the Endocrine Society and an American Physician Fellowship Award. K.R.A. is the recipient of a Finnish Culture Foundation Award, a Fogarty International Fellowship, and a Lalor Foundation Fellowship. L.T.P. is the recipient of a Fulbright Fellowship. I.B.-S. and S.K. have contributed equally to the conception and execution of this work.

²Correspondence: Eli Y. Adashi, Division of Reproductive Sciences, Departments of Obstetrics and Gynecology, University of Utah School of Medicine, ARUP II, Mailbox #20, 546 Chipeta Way, Salt Lake City, UT 84108. FAX: (801) 585-9256; e-mail: EADASHI@HSC.UTAH.EDU

³Current address: Department of Obstetrics and Gynecology, Haemek Medical Center, Afula, Israel.

⁴Current address: Department of Obstetrics and Gynecology, Rambam Medical Center, Haifa, Israel.

⁵Current address: 2nd Clinic of Surgical Gynecology, Lublin, Poland.

⁶Current address: Department of Surgery, Children's Hospital, 300 Longwood Avenue, Boston, MA 02115

MATERIALS AND METHODS

Animals

Immature Sprague-Dawley female rats from Zivic-Miller Laboratories (Zelienople, PA) were killed by CO₂ asphyxiation. The project was approved by the Institutional Animal Care and Use Committee.

Hormones

Recombinant human IL-1 β (2×10^7 U/mg) was generously provided by Drs. Errol B. De Souza and C.E. Newton, DuPont-Merck Pharmaceutical Co. (Wilmington, DE). A recombinantly expressed preparation of the naturally occurring human IL-1 receptor antagonist (IL-1RA) was generously provided by Dr. Daniel E. Tracey, The Upjohn Co. (Kalamazoo, MI). Highly purified hCG (CR-127, 14 900 IU/mg) was generously provided by Dr. R.E. Canfield through the Center for Population Research, NICHD, NIH (Bethesda, MD). Ovine FSH (oFSH; NIH-FSH-S17; FSH potency equal to 20 U/mg; LH activity of 0.04 NIH-LH-S1U/mg) was generously provided by the National Pituitary Agency, National Institute of Diabetes and Digestive and Kidney Diseases, NIH (Bethesda, MD). Equine CGs were from Sigma Chemical Company (St. Louis, MO).

Reagents

McCoy's 5a medium (without serum), penicillin-streptomycin solution, L-glutamine, trypan blue stain (0.4%), and BSA were obtained from Gibco-BRL Life Sciences (Gaithersburg, MD). Collagenase (Clostridium histolyticum; CLS type I; 144 U/mg) was from Worthington Biochemical Corp. (Freehold, NJ). DNase (bovine pancreas), phosphatidylcholine L- α -1-stearyl-2-arachidonyl, pepsin, and RNase A were obtained from Sigma. 5-Bromo-4-chloro-3-indolyl phosphate, nitro blue tetrazolium, digoxigenin-UTP, and anti-digoxigenin polyclonal antibody *Fab* fragments were from Boehringer-Mannheim Biochemical Products (Indianapolis, IN). [α -³²P]UTP (800 Ci/mmol), [α -³²P]dCTP (3000 Ci/mmol), and phosphatidylcholine L- α -1-stearyl-2-arachidonyl [arachidonyl-5,6,8,9,11,12,14,15-³H(N)] (42 Ci/mmol) were from DuPont New England Nuclear Research Products (Boston, MA). Agarose was from FMC BioProducts (Rockland, ME) and formamide was from Fisher Biotech (Fairlawn, NJ).

In Vivo Treatment Paradigm

Intact 25-day-old female rats were injected s.c. with 15 IU of eCG. Ovulation was triggered by hCG (15 IU) 48 h later.

Tissue Culture Procedures

Whole ovarian dispersates from immature rats were prepared and cultured as previously described in 60×12 -mm (1.5×10^6 cells) or 30×10 -mm (0.5×10^6 cells) dishes [49]. Briefly, these cells were maintained at a temperature of 37°C within a water-saturated atmosphere of 95% air: 5% CO₂ in 1 ml of serum-free McCoy's 5a medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100 μ g/ml streptomycin sulfate. All reagents were dissolved in sterile culture media. All treatments were added in 50- μ l aliquots. Pure granulosa cells from immature (25–27 days old) rats were prepared by repeated follicular puncturing and were cultured (1.5×10^6 cells/60 \times 12-mm dish)

as previously described [50]. Briefly, these cells were cultured under the same conditions as described above for the whole ovarian dispersates.

Nucleic Acid Probes

A 750-base pair (bp) *Sma* I/*Eco*RI insert of the cDNA encoding rat type II sPLA₂ [51] was kindly provided by Dr. J. Ishizaki (Shionogi Research Laboratories, Osaka, Japan). For the purpose of Northern blotting, the entire insert was excised with *Sma* I/*Eco*RI, purified, and used as a template for random priming with [α -³²P]dCTP according to the manufacturer's recommendations (Pharmacia, Piscataway, NJ). For RNase protection assays, a 452-bp *Bam*HI fragment was excised and subcloned into pGEM7Zf+ (Promega, Madison, WI). This latter construct was then linearized with *Ecl*136II (New England Biolabs, Beverly, MA) and transcribed with T7 RNA polymerase (Promega) in the presence of [α -³²P]UTP to yield a 535-nucleotide (nt) riboprobe that, upon hybridization, was projected to generate a 452-nt protected fragment.

To correct for possible differential RNA loadings, transcripts for either RPL19 or cyclophilin, both of which are constitutively expressed and presumptively hormone-independent genes, were quantified as internal standards. Cyclophilin mRNA [52] has been used to normalize RNA loading in a variety of experimental systems, including those utilizing ovarian material [53–55]. A plasmid containing a 117-bp *Pst* I to *Bam*HI fragment of the rat cyclophilin cDNA was generously provided by Dr. Sergio R. Ojeda (Oregon Regional Primate Center, Beaverton, OR). This plasmid was linearized with *Eco*RI and transcribed with T7 RNA polymerase to create a 169-nt riboprobe designed to protect a 117-nt fragment. The preparation and employment of the RPL19 probe were as described elsewhere [11].

RNA Extraction and Analysis

RNA of cultured cells and of tissues was extracted with RNAzol-B (Tel Test, Friendswood, TX) according to the recommendations of the manufacturer. RNase protection assays were carried out as previously described [6].

RNase Protection Assay

Linearized DNA templates were transcribed with T7 RNA polymerase to specific activities of 800 Ci/mmol [α -³²P]UTP (sPLA₂) or 160 Ci/mmol [α -³²P]UTP (cyclophilin or RPL19). The riboprobes were gel purified in an effort to eliminate transcribed products shorter than the full-length probes. The assay was performed as previously described [6]. Gels were exposed to XAR film (Eastman Kodak, Rochester, NY) for varying lengths of time with intensifying screens. To generate quantitative data, gels were also exposed to a phosphor screen (Molecular Dynamics, Sunnyvale, CA). The resultant digitized data were analyzed with Image Quant Software (Molecular Dynamics). The hormonally independent RPL19 (or cyclophilin) mRNA signal was used to normalize the sPLA₂ mRNA data for possible variation in RNA loading. Specifically, the net signal of the sPLA₂ or cPLA₂ protected band (respective background subtracted)/net RPL19 signal ratio was calculated for each sample.

The relative intensity of protected RNA bands was quantified by densitometric scanning with a computing densitometer (Molecular Dynamics) and normalized relative to

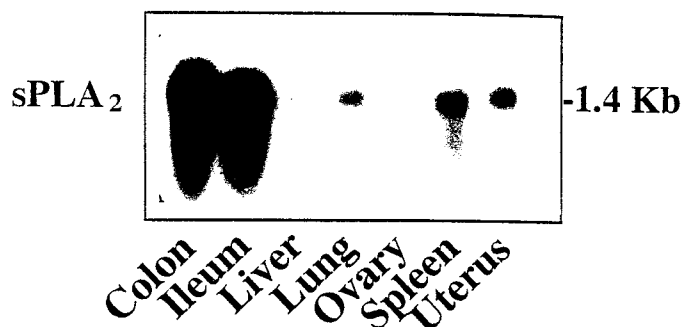


FIG. 1. Tissue distribution of sPLA₂ transcripts in the postovulatory rat: Northern blot analysis. Total RNA (20 µg) extracted from the indicated tissues of eCG/hCG-treated immature rats was subjected to Northern blot analysis employing a ³²P-labeled DNA probe corresponding to rat sPLA₂ as described in *Materials and Methods*.

the cyclophilin or RPL19 transcripts, as detailed for individual experiments. Northern blot analysis was carried out as previously described [55].

Cell-Free PLA₂ Activity Assay of Media Conditioned by Cultured Ovarian Cells

Whole ovarian dispersates were initially cultured as described for 72 h in the absence or presence of the specified treatments. Media were then transferred into 12 × 75-mm glass tubes, and media PLA₂ activity was determined by measuring the release of ³H-labeled arachidonic acid from the sn-2 position of ³H-labeled phosphatidylcholine substrate. The 1-ml reaction volume contained conditioned medium (300 µl), 2.5 × 10⁴ cpm of ³H-labeled phosphatidylcholine L-α-1-stearyl-2-arachidonyl (20 µl), 2.5 µM of unlabeled phosphatidylcholine (20 µl), 5% methanol, 2 mM CaCl₂ (20 µl), and 100 µM Hepes buffer (640 µl). After a 2-h incubation at 37°C, the reaction was terminated by the addition of 100 µl of 7% formic acid. Reactions were extracted twice with 3 ml of ethyl acetate. Extracts were dried, resuspended in isopropanol:hexane:acetic acid (5:95:0.025), and separated by HPLC (Waters, Milford, MA) on a normal-phase diol column (LiChrosorb Diol, Waters, 10 µm) using a concave gradient of hexane:isopropanol (95:5 to 60:40) for 30 min at 2 ml/min. Radiolabeled arachidonic acid and other major prostanoic products (PGE₂ and PGF_{2α}) were detected and quantified by online scintillation counting with a Radiometric Flow Detector (Packard Instrument Co., Meriden, CT).

In Situ Hybridization

Methodological details conformed to those previously described [11]. Briefly, ovaries were removed, dissected free of surrounding tissues, fixed in 10% buffered formalin, and embedded in paraffin wax. Sections (4–6 µm thick) were mounted on aminopropyltriethoxy-silane-coated glass slides and baked overnight at 60°C. Wax was removed with xylene and ethanol, and slides were rinsed prior to treatment with pepsin (Sigma; 4 mg/ml in 0.2 N HCl) at room temperature for 13 min. Slides were rinsed again and fixed with 2.7% paraformaldehyde for 5 min.

Riboprobes were synthesized with digoxigenin-UTP (Boehringer-Mannheim). Riboprobes (8 ng/µl) in hybridization buffer (50% v:v formamide, 5% w:v dextran sulfate, double-strength saline-sodium citrate [SSC; single-strength SSC is 0.15 M sodium chloride and 0.015 M sodium citrate], 0.1 mM EDTA, 150 µg/ml *Escherichia coli* tRNA,

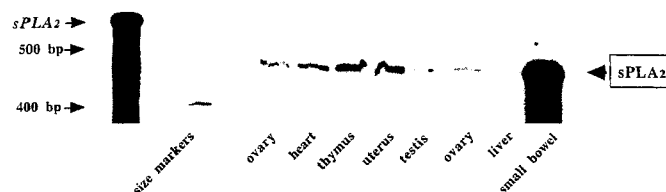


FIG. 2. Tissue distribution of sPLA₂ transcripts in the immature rat: RNase protection assay. Total RNA (20 µg) extracted from the indicated tissues of immature rats was subjected to an RNase protection assay using a ³²P-labeled rat sPLA₂ antisense RNA as described in *Materials and Methods*.

1 mM Tris-HCl, pH 7.3) were added to each sample. Slides were covered, heated to 95°C for 15 min in a humidified chamber, and hybridized at 47°C overnight. After hybridization, slides were rinsed in 4-strength SSC and double-strength SSC and treated with 150 µg/ml RNase A (Sigma, London, UK) in double-strength SSC at 37°C for 30 min [30].

Slides were then washed, preincubated in blocking buffer (100 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Boehringer-Mannheim blocking reagent), and incubated with alkaline phosphatase-conjugated anti-digoxigenin polyclonal antibody Fab fragments (Boehringer-Mannheim) at 1:500 dilution in blocking buffer for 5 h. Unbound antibody was removed by washing in 0.1 M maleic acid (pH 7.5), 150 mM NaCl for 30 min. Bound antibody was detected by incubation in 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 50 mM MgCl₂ containing 375 µg/ml nitroblue tetrazolium and 187.5 µg/ml 5-bromo-4-chloro-3-indolyl-phosphate (Boehringer-Mannheim) in the dark overnight. Reactions were stopped with double-distilled H₂O, and the slides were dried and mounted in aqueous mounting medium.

Data Analysis

Except as noted, each experiment was replicated a minimum of three times. Data points are presented as mean ± SE, and statistical significance was determined by ANOVA (Fisher's Protected Least Significance Difference) and Student's *t*-test. Statistical values were calculated using Statview 512+ for Macintosh (Brain Power, Inc., Calabasas, CA). Median effective concentrations (EC₅₀) and their 95% confidence interval were calculated from the respective linear log-dose/effect curves.

RESULTS

Tissue Distribution of sPLA₂ Transcripts in the Immature Rat

The relative expression of sPLA₂ in the ovary, colon, ileum, liver, lung, spleen, and uterus of immature rats after *in vivo* treatment with eCG/hCG was determined by Northern blot analysis as described in *Materials and Methods*. As shown in Figure 1, a single sPLA₂ transcript of 1.4 kilobases (kb) was noted in all tissues studied, proving particularly prominent in the colon and ileum. The lung, spleen, and uterus displayed a less intense signal. A further decrease in signal intensity was noted for the liver. Only a faint signal was evident in ovarian tissue collected 24 h post-hCG (i.e., 10 h after induced ovulation). These findings suggest that, in relative terms, whole ovarian tissue (as distinct from specific cellular populations thereof) is not a major site of sPLA₂ gene expression. The more sensitive RNase protection assay revealed the unequivocal presence of sPLA₂ transcripts (Fig. 2) in ovarian material from un-

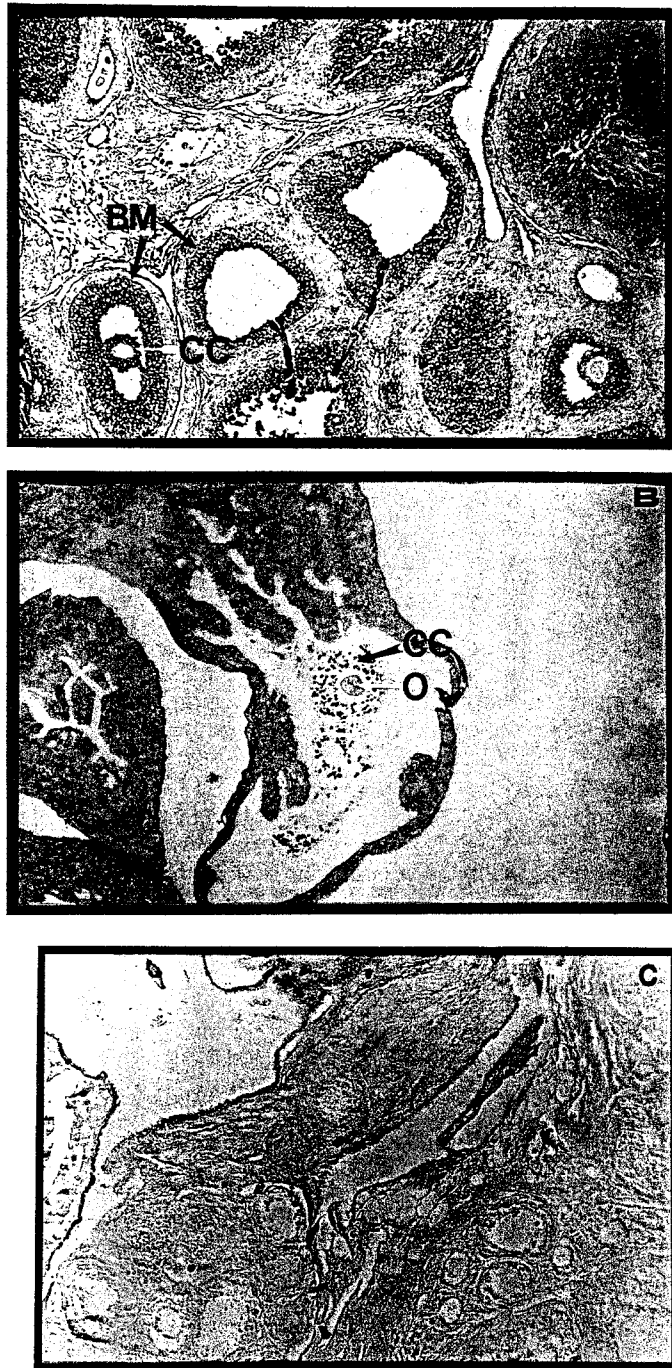


FIG. 3. Cellular localization of *sPLA₂* transcripts: in situ hybridization studies. Ovaries were obtained from eCG/hCG-treated immature rats. The resultant ovarian and tubal material was processed for in situ hybridization using a digoxigenin-labeled rat *sPLA₂* antisense RNA as described in *Materials and Methods*. A) Section through an early postovulatory ovary (17 h post-hCG). B) Section through the postovulatory fallopian tube. C) Section probed by sense probe. BM, basement membrane; CC, cumulus cells; O, oocyte. A, C: $\times 100$; B: $\times 200$ (reproduced at 75%).

treated rats. Of all tissues studied, the small bowel showed the most prominent signal. A substantial *sPLA₂* signal was also noted in the thymus, the uterus, and the heart (but not liver).

Cellular Localization of *sPLA₂* Transcripts: In Situ Hybridization Studies

To establish the identity of the somatic ovarian cell population responsible for *sPLA₂* gene expression, ovaries

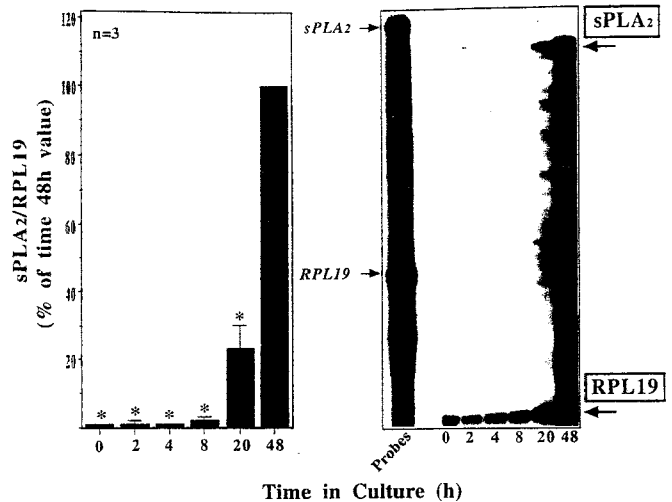


FIG. 4. *sPLA₂* gene expression by cultured whole ovarian dispersates: time dependence. Whole ovarian dispersates (1.5×10^6 viable cells/ 60×12 -mm dish) were cultured for the duration indicated (up to 48 h), in the absence of any treatment, in 3 ml of serum-free media. Total cellular RNA was extracted and subjected to RNase protection assay using a 32 P-labeled rat *sPLA₂* antisense RNA as described in *Materials and Methods*. The samples were simultaneously probed with 32 P-labeled RPL19 antisense RNA to normalize for possible variation in RNA loading. The intensity of RNA bands was quantified as described in *Materials and Methods*. The left panel depicts the mean \pm SE of three experiments. The right panel reflects a single representative experiment. * Statistically significant ($p < 0.05$) vs. 48-h value.

were obtained from eCG-primed/hCG-triggered immature rats. The resultant ovarian (and tubal) material was processed for in situ hybridization as described in *Materials and Methods*. Follicles in various stages of development were apparent (Fig. 3A) in a section from an early postovulatory ovary (17 h post-hCG). In this section, expression of *sPLA₂* was localized to membrana granulosa cells with minimal staining of the theca-interstitial cell compartment. Cumulus granulosa cells were *sPLA₂* positive as well. Within the membrana granulosa, the gradient of expression proved more intense toward the antrum. Modestly intense labeling was also apparent in the early corpus luteum. A section through the postovulatory fallopian tube (Fig. 3B) disclosed an ovulated oocyte surrounded by expanded (mucified) *sPLA₂*-positive cumulus cells, thereby suggesting that the *sPLA₂*-positive cumulus mass is associated with mature follicles destined to ovulate. A control ovarian section labeled with the sense *sPLA₂* probe (Fig. 3C) displayed no staining.

Expression of *sPLA₂* Gene by Cultured Whole Ovarian Dispersates: Time Dependence

To determine whether ovarian *sPLA₂* gene expression occurs in vitro, whole ovarian dispersates were cultured for the duration indicated (up to 48 h) in the absence of any treatment. As shown in Figure 4 ($n = 3$), an appreciable signal corresponding to *sPLA₂* transcripts was first noted 20 h into the experiment. A further increase was noted at the 48-h time point. The duration of all subsequent experiments was thus kept at 48 h to provide a standard reference point for the detection of possible regulatory effects.

Expression of *sPLA₂* Gene by Cultured Whole Ovarian Dispersates: Effect of Treatment with IL-1 β

To evaluate the dependence of ovarian *sPLA₂* transcripts on IL-1 β , whole ovarian dispersates were cultured for 48

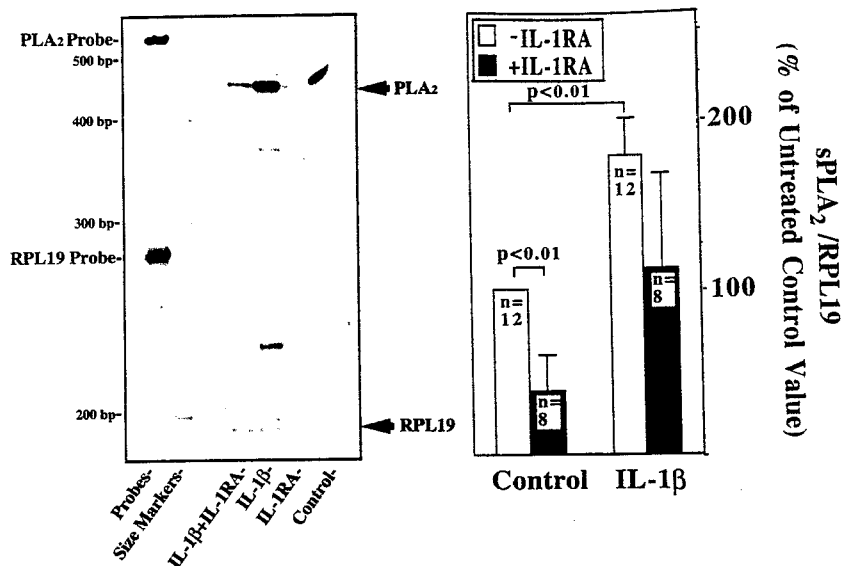


FIG. 5. Expression of sPLA₂ gene by cultured whole ovarian dispersates: effect of treatment with IL-1β. Whole ovarian dispersates (1.5×10^6 viable cells/60 × 12-mm dish) were cultured in 3 ml of serum-free media for 48 h in the absence or presence of IL-1β (50 ng/ml), with or without IL-1RA (5000 ng/ml). The resultant RNA samples were subjected to a liquid hybridization/RNase protection assay using a ³²P-labeled rat sPLA₂ antisense RNA as described in *Materials and Methods*. Simultaneous probing was carried out with a ³²P-labeled rat RPL19 antisense RNA probe to normalize for possible variation in RNA loading. The intensity of RNA bands was quantified as described in *Materials and Methods*. The right panel depicts the mean ± SE of the indicated number of experiments. The left panel reflects a single experiment.

h in the absence or presence of exogenously provided IL-1β (50 ng/ml), with or without IL-1RA (5000 ng/ml). As shown in Figure 5, treatment with IL-1β produced a 1.74-fold increase in the relative expression of sPLA₂ transcripts over the value in untreated controls ($p < 0.01$). Importantly, this effect was reversed by IL-1RA, albeit without achievement of statistical significance in light of substantial inter-experimental variability. Treatment with IL-1RA led to a 55% decrease in the basal level of expression of ovarian sPLA₂ transcripts ($p < 0.01$), a phenomenon described in greater detail in Figure 8. These observations support the view that ovarian sPLA₂ transcripts are IL-1 dependent and that the IL-1 effect is receptor mediated.

PLA₂ Activity in Media Conditioned by Cultured Whole Ovarian Dispersates: Effect of Treatment with IL-1β

To further confirm the IL-1 dependence of ovarian sPLA₂, we set out to examine the effect of treatment with IL-1β on PLA₂ activity in media conditioned by cultured whole ovarian dispersates. Whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml), with or without IL-1RA (5000 ng/ml). At the conclusion of this experimental period, the media were collected and subjected to a cell-free PLA₂ activity assay as described in *Materials and Methods*. PLA₂ activity as detected in media conditioned by cultured ovarian cells is composed primarily, if not exclusively, of the sPLA₂ variety (unpublished results). As shown in Figure 6, treatment with IL-1β resulted in a 75% increase ($p < 0.01$) in PLA₂ activity. The concurrent presence of IL-1RA completely blocked the IL-1β effect ($p < 0.01$), reducing PLA₂ activity to a level 65% that of untreated controls. Provision of IL-1RA by itself produced a significant ($p < 0.01$) decrease (40%) in basal PLA₂ activity, thereby suggesting neutralization of endogenous IL-1 input.

Expression of sPLA₂ Gene by Cultured Isolated Granulosa Cells from Immature Rats: Effect of Treatment with IL-1β

To confirm the localization of ovarian sPLA₂ transcripts to the granulosa cell (as noted by in situ hybridization) and to confirm their IL-1 dependence, isolated granulosa cells were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml). As shown in Figure 7 ($n = 3$), faint sPLA₂

transcripts were noted in the absence of treatment. However, treatment with IL-1β resulted in marked (12.5-fold increase) induction ($p < 0.001$) of sPLA₂ transcripts.

Expression of sPLA₂ Gene by Cultured Whole Ovarian Dispersates: Effect of Treatment with IL-1RA

To examine the possible dependence of ovarian sPLA₂ gene expression on endogenous IL-1-like activity, whole ovarian dispersates were cultured for 48 h under serum-free conditions in the presence of increasing concentrations (0–5000 ng/ml) of IL-1RA. As shown in Figure 8 ($n = 3$), provision of increasing concentrations of IL-1RA produced dose-dependent decrements in the expression of protected sPLA₂ transcripts with an apparent EC_{50} of 79 ng/ml (40–158 ng/ml, 95% confidence interval). The first significant ($p < 0.05$) decrement was noted at the 50 ng/ml dose level. Although the IL-1RA effect did not reach a formal maximum at the 5000 ng/ml dose level, an 80% reduction of

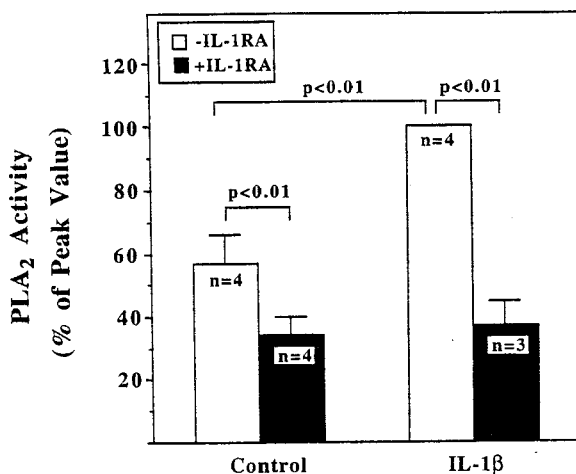
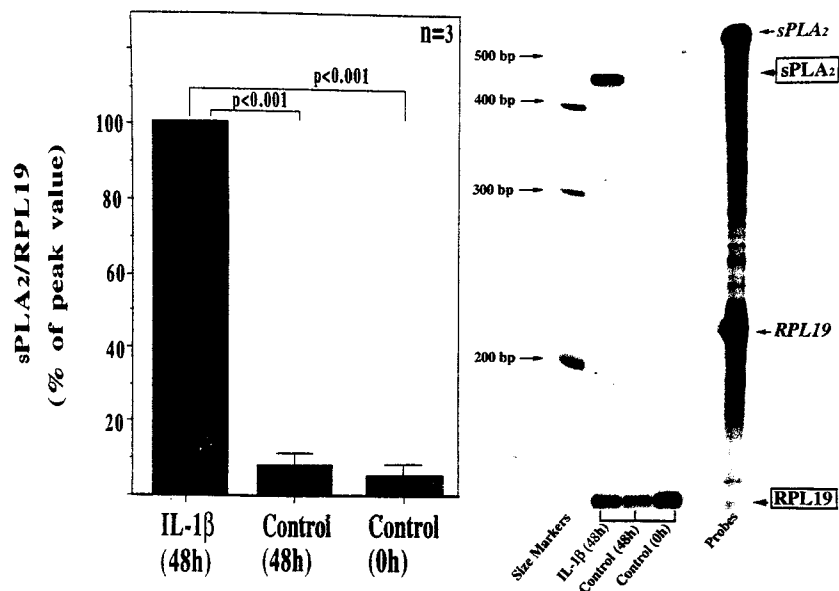


FIG. 6. PLA₂ activity in media conditioned by cultured whole ovarian dispersates: effect of treatment with IL-1β. Whole ovarian dispersates (0.5×10^6 viable cells/30 × 10 mm-dish) were cultured in 1 ml of serum-free media for 48 h in the absence or presence of IL-1β (50 ng/ml), with or without IL-1RA (5000 ng/ml). At the conclusion of the experimental period, the media were collected and subjected to a cell-free PLA₂ activity assay as described in *Materials and Methods*. Data represent the mean ± SE of the indicated number of independent experiments.

FIG. 7. Expression of sPLA₂ gene by cultured isolated granulosa cells: effect of treatment with IL-1 β . Isolated granulosa cells (1.5×10^6 viable cells/60 \times 12-mm dish) were cultured for 48 h in 3 ml of serum-free media in the absence or presence of IL-1 β (50 ng/ml). The resultant RNA samples were subjected to a liquid hybridization/RNase protection assay using a ³²P-labeled rat sPLA₂ antisense RNA as described previously [6]. Simultaneous probing was carried out with a ³²P-labeled rat RPL19 RNA probe to normalize for possible variation in RNA loading. The intensity of RNA bands was quantified as described in *Materials and Methods*. The left panel depicts the mean \pm SE of three experiments. The right panel reflects a single representative experiment.



sPLA₂ transcript abundance was noted ($p < 0.01$). These data suggest that the basal level of expression of sPLA₂ by cultured whole ovarian dispersates is partially contingent upon stimulation by endogenous IL-1-like activity.

Expression of PLA₂ Gene by Cultured Whole Ovarian Dispersates: Effect of Treatment with Gonadotropins

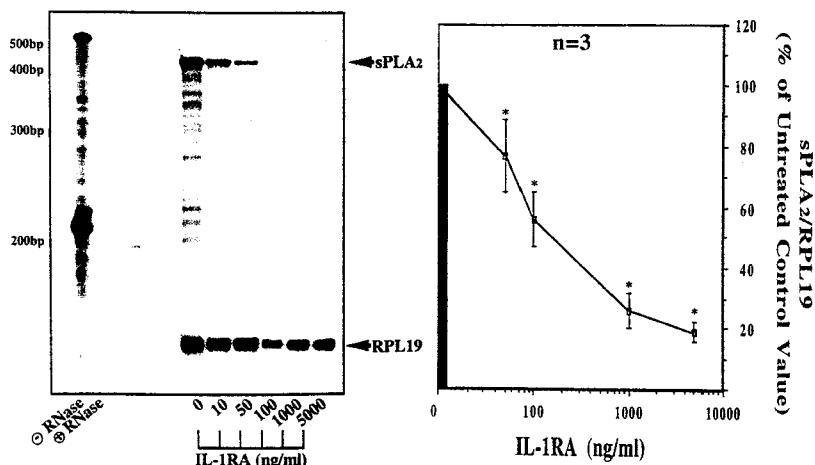
To examine the role of gonadotropins in the regulation of ovarian sPLA₂ gene expression and to explore the possible intermediary role of IL-1 in this regard, whole ovarian dispersates were cultured for 48 h in the absence or presence of FSH (2 mIU/ml) or hCG (10 ng/ml), with or without IL-1RA (5000 ng/ml). As shown in Figure 9 ($n = 5$), treatment with either FSH or hCG led to an increase in sPLA₂ transcripts (3- and 2.6-fold, respectively). However, only the FSH-induced increase was significant ($p = 0.029$); the hCG-induced increase was not ($p = 0.056$). Interestingly, the FSH and hCG effects were reversed by the concurrent presence of IL-1RA ($p < 0.01$ and < 0.05 , respectively). These findings suggest that the ability of hCG or FSH to up-regulate ovarian sPLA₂ transcripts is due, if only in part, to the endogenous elaboration of IL-1-like activity.

DISCUSSION

IL-1 has been implicated as a mediator in the cascade of events leading to ovulation [5]. In this context, IL-1 has been shown to be a potent promoter of the generation of ovarian prostanoids [42, 45–47]. Although the latter activity is undoubtedly attributable, at least in part, to stimulation of the inducible isoform of prostaglandin endoperoxide synthase (unpublished results), other mechanisms may also be at play. In this paper we explore the possibility that the IL-1-stimulated biosynthesis of ovarian prostaglandins is also due, in part, to the enhancement of ovarian PLA₂ activity. Specifically, we set out to document the ovarian expression, cellular localization, and hormonal regulation of type II secretory PLA₂, the presumed dominant PLA₂ species in the mammalian ovary (unpublished results).

PLA₂ is known to catalyze the hydrolysis of the *sn*-2 fatty acyl chain of phospholipids. Cleavage of the *sn*-2 arachidonic acid residue of phospholipids provides the substrate for the biosynthesis of eicosanoids, thereby giving rise to potent proinflammatory mediators (i.e., prostaglandins and leukotrienes). Consequently, it is the release of

FIG. 8. Expression of sPLA₂ gene by cultured whole ovarian dispersates: effect of treatment with IL-1RA. Whole ovarian dispersates (1.5×10^6 viable cells/60 \times 12-mm dish) were cultured in 3 ml of serum-free media for 48 h in the presence of increasing concentrations of IL-1RA. The resultant RNA samples were subjected to a liquid hybridization/RNase protection assay using ³²P-labeled rat sPLA₂ antisense RNA as previously described [6]. Simultaneous probing was carried out with a ³²P-labeled antisense RNA probe to normalize for possible variation in RNA loading (RPL19 in one experiment and cyclophilin in two experiments). The intensity of RNA bands was quantified as described in *Materials and Methods*. The right panel depicts the mean \pm SE of three experiments. The left panel reflects a single representative experiment. * Statistically significant ($p < 0.05$) vs. value for untreated control.



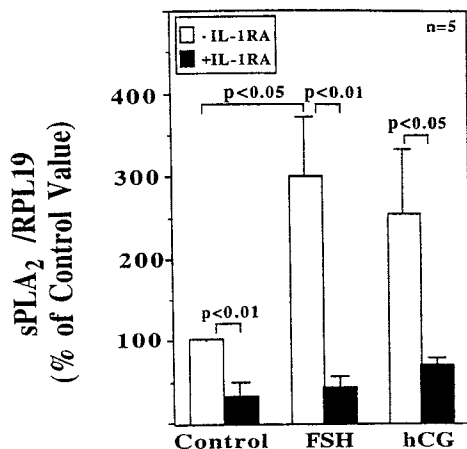


FIG. 9. Expression of sPLA₂ gene by cultured whole ovarian dispersates: effect of treatment with gonadotropins. Whole ovarian dispersates (1.5×10^6 viable cells/60 \times 12-mm dish) were cultured in 3 ml of serum-free media for 48 h in the absence or presence of FSH (2 mIU/ml) or hCG (10 ng/ml) with or without IL-1RA (5000 ng/ml). The resultant RNA samples were subjected to a liquid hybridization/RNase protection assay using a ³²P-labeled rat sPLA₂ antisense RNA as described in *Materials and Methods*. Simultaneous probing was carried out with a ³²P-labeled rat RPL19 antisense RNA probe to normalize for possible variation in RNA loading. The intensity of RNA bands was quantified as described in *Materials and Methods*. The bar graph depicts the mean \pm SE of five experiments.

arachidonic acid that is considered to be the rate-limiting event in the eicosanoid production cascade [48].

Initial efforts at establishing the ovarian expression of sPLA₂ by Northern blot analysis revealed that, as compared to other tissues, intact whole ovarian material from postovulatory rats is not a major site of sPLA₂ expression (Fig. 1). It is likely that this observation reflects the highly compartmentalized nature of ovarian sPLA₂ expression, whose predominant localization to the postovulatory membrana granulosa and cumulus layer was subsequently revealed by in situ hybridization technology (Fig. 3). However, sPLA₂ expression by whole ovarian material (Fig. 4), by IL-1-treated cultured whole ovarian dispersates (Fig. 5), or by IL-1-treated cultured isolated granulosa cells (Fig. 7) proved vigorous when probed by sensitive RNase protection technology.

To evaluate the possible dependence of ovarian sPLA₂ on IL-1 β , whole ovarian dispersates were cultured in the absence or presence of IL-1 β (Fig. 5). Treatment with IL-1 β proved up-regulatory to ovarian sPLA₂ transcripts, thereby strongly suggesting IL-1-dependence. Moreover, the IL-1 β effect appeared to be receptor mediated in that the concurrent presence of IL-1RA markedly attenuated the IL-1 β effect (Fig. 5). Taken together, these observations support the view that ovarian sPLA₂ transcripts are IL-1 dependent and that the IL-1 effect under study is receptor mediated. Comparable conclusions could be drawn from the study of PLA₂ activity (Fig. 6). In these respects, our findings are in keeping with those reported for several extraovarian sites [56, 57].

It is also of interest that IL-1 β proved capable of stimulating sPLA₂ expression in cultured granulosa cells. This confirms previous demonstrations of the ability of IL-1 to directly alter rat granulosa cell function [13, 14, 16, 17, 20, 30] and further supports the presence of IL-1-induced actions, and existence of IL-1 receptors on this cell type [11]. Other endpoints of ovarian IL-1 such as changes in cellular morphology [25], stimulation of proteoglycan biosynthesis

[33], prostaglandin biosynthesis [45], and nitric oxide synthase activity [58] require the entire ovarian cellular complement. Induction of these events by IL-1 may occur in or require the intermediacy of other cell types. It is noteworthy that the in vitro induction of prostaglandin biosynthesis by IL-1 requires the whole ovary [45], whereas IL-1 will increase sPLA₂ expression in isolated granulosa cells (Fig. 7). Thus increased sPLA₂ expression is not sufficient for full production of prostaglandins but undoubtedly also requires the IL-1-induced expression of prostaglandin endoperoxide synthase (unpublished results).

To further examine the possible dependence of ovarian sPLA₂ gene expression on IL-1, we set out to examine the role of endogenous IL-1-like activity. The presence of endogenous IL-1 activity in cultured whole ovarian dispersates has been suggested by the ability of IL-1RA to decrease the basal expression of some IL-1-induced effects [37] and has been confirmed by the detection of IL-1 β transcripts and IL-1 bioactivity (unpublished results). Indeed, provision of increasing concentrations of IL-1RA produced dose-dependent decrements in the relative expression of sPLA₂ transcripts by whole ovarian dispersates, thereby suggesting that the basal level of expression of sPLA₂ is partially contingent upon stimulation by endogenous IL-1-like activity (Fig. 8). Similar data are displayed in Figures 5 and 9. It is noteworthy that the addition of 50 ng/ml IL-1 β could increase sPLA₂ expression only 1.74-fold, thereby suggesting that the endogenous production of IL-1-like activity is sufficient to induce more than 50% of the possible maximal expression.

To establish that the phenomena discussed above are not limited to the transcriptional level, we also examined the IL-1 dependence of PLA₂ activity in media conditioned by whole ovarian dispersates. These observations (Fig. 6) revealed IL-1 β to be a potent stimulator of ovarian sPLA₂ activity, an effect eliminated by the concurrent presence of IL-1RA. Importantly, the provision of IL-1RA by itself produced a significant ($p < 0.01$) decrease (40%) in basal sPLA₂ activity, suggesting partial neutralization of endogenous IL-1 input. Taken together, these findings suggest that the IL-1 dependence of ovarian sPLA₂ is apparent at both the transcript and protein levels. As IL-1 may be cytotoxic to whole ovarian dispersates of rat origin [25, 31], it is possible that the PLA₂ activity detected in the media may also reflect, in part, released cytosolic PLA₂ (cPLA₂).

Since the induction of intraovarian IL-1 β gene expression is gonadotropin dependent [3, 6], we further examined the role of gonadotropins in the regulation of ovarian sPLA₂ expression and explored the possible intermediary role of IL-1 in this regard. Whole ovarian dispersates were cultured in the absence or presence of FSH or hCG, with or without IL-1RA. Interestingly, treatment with either FSH or hCG led to an increase in ovarian sPLA₂ transcripts (Fig. 9), although the hCG effect was of borderline significance. Importantly, these latter effects were reversed by the concurrent presence of IL-1RA, suggesting that the ability of hCG and FSH to up-regulate ovarian sPLA₂ transcripts is due, if only in part, to the endogenous elaboration of IL-1-like activity. It is therefore tempting to speculate that periovulatory eicosanoid production in the ovary is regulated by gonadotropins via IL-1, whose ability to up-regulate sPLA₂ expression and activity has been demonstrated herein.

ACKNOWLEDGMENT

The authors wish to thank Ms. Cornelia T. Szmajda for her invaluable assistance in the preparation of this manuscript.

REFERENCES

- Espey LL. Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. *Biol Reprod* 1994; 50: 233–238.
- Espey LL. Ovulation as an inflammatory reaction—a hypothesis. *Biol Reprod* 1980; 22:73–106.
- Hurwitz A, Ricciarelli E, Botero L, Rohan RM, Hernandez ER, Adashi EY. Endocrine- and autocrine-mediated regulation of rat ovarian (theca-interstitial) interleukin-1 gene expression: gonadotropin-dependent preovulatory acquisition. *Endocrinology* 1991; 129:3427–3429.
- Dinarello CA, Wolff SM. The role of interleukin-1 in disease. *N Engl J Med* 1993; 328:106–113.
- Ben-Shlomo I, Adashi EY. Interleukin-1 as a mediator in the ovulatory sequence: evidence for a meaningful role of cytokines in ovarian physiology. *Curr Opin Endocrinol Diabetes. Current Science Series* 1994; 3:187–192.
- Hurwitz A, Loukides J, Ricciarelli E, Botero L, Katz E, McAllister JM, Garcia JE, Rohan R, Adashi EY, Hernandez ER. Human intraovarian interleukin-1 (IL-1) system: highly compartmentalized and hormonally-dependent regulation of the genes encoding IL-1, its receptor, and its receptor antagonist. *J Clin Invest* 1992; 89:1–8.
- Polan ML, Loukides JA, Honig J. Interleukin-1 in human ovarian cells and in peripheral blood monocytes increases during the luteal phase: evidence for a midcycle surge in the human. *Am J Obstet Gynecol* 1994; 170:1000–1007.
- Simón C, Frances A, Piquette G, Polan ML. Immunohistochemical localization of the interleukin-1 system in the mouse ovary during follicular growth, ovulation, and luteinization. *Biol Reprod* 1994; 50: 449–457.
- Brännström M, Norman RJ, Seamark RF, Robertson SA. Rat ovary produces cytokines during ovulation. *Biol Reprod* 1994; 50:88–94.
- Deyerle KL, Sims JE, Dower SK, Bothwell MA. Pattern of IL-1 receptor gene expression suggests role in noninflammatory processes. *J Immunol* 1992; 149:1657–1665.
- Scherzer WJ, Ruutinen-Altman KSL, Putowski LT, Kol S, Adashi EY, Rohan RM. 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* 1996; 3:131–140.
- Gottschall PE, Uehara A, Hoffmann ST, Arimura A. Interleukin-1 inhibits follicle stimulating hormone-induced differentiation in rat granulosa cells *in vitro*. *Biochem Biophys Res Commun* 1987; 149:502–509.
- Gottschall PE, Katsuura G, Hoffmann ST, Arimura A. Interleukin-1: an inhibitor of luteinizing hormone receptor formation in cultured rat granulosa cells. *FASEB J* 1988; 2:2492–2496.
- Gottschall PE, Katsuura G, Dahl RR, Hoffmann ST, Arimura A. Discordance in the effects of interleukin-1 on rat granulosa cell differentiation induced by follicle-stimulating hormone or activators of adenylate cyclase. *Biol Reprod* 1988; 39:1074–1085.
- Fukuoka M, Mori T, Taii S, Yasuda K. Interleukin-1 inhibits luteinization of porcine granulosa cells in culture. *Endocrinology* 1988; 122: 367–369.
- Gottschall PE, Katsuura G, Arimura A. Interleukin-1 β is more potent than interleukin-1 α in suppressing follicle-stimulating hormone-induced differentiation of ovarian granulosa cells. *Biochem Biophys Res Commun* 1989; 163:764–770.
- Gottschall PE, Katsuura G, Arimura A. Interleukin-1 suppresses follicle-stimulating hormone-induced estradiol secretion from cultured ovarian granulosa cells. *J Reprod Immunol* 1989; 15:281–290.
- Fukuoka M, Yasuda K, Taii S, Takakura K, Mori T. Interleukin-1 stimulates growth and inhibits progesterone secretion in cultures of porcine granulosa cells. *Endocrinology* 1989; 124:884–890.
- Fukuoka M, Taii S, Yasuda K, Takakura K, Mori T. Inhibitory effects of interleukin-1 on luteinizing hormone-stimulated adenosine 3',5'-monophosphate accumulation by cultured porcine granulosa cells. *Endocrinology* 1989; 125:136–143.
- Kasson BG, Gorospe WC. Effects of interleukins 1, 2 and 3 on follicle-stimulating hormone-induced differentiation of rat granulosa cells. *Mol Cell Endocrinol* 1989; 62:103–111.
- Nakamura Y, Kato H, Terranova PF. Interleukin-1 α increases thecal progesterone production of preovulatory follicles in cyclic hamsters. *Biol Reprod* 1990; 43:168–173.
- Yasuda K, Fukuoka M, Taii S, Takakura K, Mori T. Inhibitory effects of interleukin-1 on follicle-stimulating hormone induction of aromatase activity, progesterone secretion, and functional luteinizing hormone receptors in cultures of porcine granulosa cells. *Biol Reprod* 1990; 43:905–912.
- Hurwitz A, Payne DW, Packman JN, Andreani CL, Resnick CE, Hernandez ER, Adashi EY. Cytokine-mediated regulation of ovarian function: interleukin-1 inhibits gonadotropin-induced androgen biosynthesis. *Endocrinology* 1991; 129:1250–1256.
- Sjögren A, Holmes PV, Hillensjö T. Interleukin-1 α modulates luteinizing hormone stimulated cyclic AMP and progesterone release from human granulosa cells *in vitro*. *Hum Reprod* 1991; 7:910–913.
- Hurwitz A, Hernandez ER, Payne DW, Dharmarajan AM, Adashi EY. Interleukin-1 is both morphogenic and cytotoxic to cultured rat ovarian cells: obligatory role for heterologous, contact-independent cell-cell interaction. *Endocrinology* 1992; 131:1643–1649.
- Barak V, Yanai P, Treves AJ, Roisman I, Simon A, Laufer N. Interleukin-1: local production and modulation of human granulosa luteal cells steroidogenesis. *Fertil Steril* 1992; 58:719–725.
- Fukuoka M, Yasuda K, Fujiwara, Hideharu K, Mori T. Interactions between interferon γ , tumour necrosis factor α , and interleukin-1 in modulating progesterone and oestradiol production by human luteinized granulosa cells in culture. *Hum Reprod* 1992; 7:1361–1364.
- Fukuoka M, Yasuda K, Emi N, Fujiwara H, Iwai M, Takakura K, Kanzaki H, Mori T. Cytokine modulation of progesterone and estradiol secretion in cultures of luteinized human granulosa cells. *J Clin Endocrinol & Metab* 1992; 75:254–258.
- Fukuoka M, Yasuda K, Taii S, Mori T. Synergistic actions of cytokines and growth factors in enhancing porcine granulosa cells growth. *Endocrinol Jpn* 1992; 39:277–288.
- Gorospe WC, Spangelo BL. Interleukin-6 production by rat granulosa cells *in vitro*: effects of cytokines, follicle-stimulating hormone, and cyclic 3',5'-adenosine monophosphate. *Biol Reprod* 1993; 48:538–543.
- Ellman C, Corbett JA, Misko TP, McDaniel M, Beckerman KP. Nitric oxide mediates interleukin-1-induced cellular cytotoxicity in the rat ovary: a potential role for nitric oxide in the ovulatory process. *J Clin Invest* 1993; 92:3053–3056.
- Hurwitz A, Dushnik M, Solomon H, Ben-Shitrit A, Finci-Yeheskel Z, Milwidsky A, Meyer M, Adashi EY, Yagel S. Cytokine-mediated regulation of rat ovarian function: interleukin-1 stimulates the accumulation of 92-kD gelatinase. *Endocrinology* 1993; 132:2709–2714.
- Kokia E, Hurwitz A, Ben-Shlomo I, Adashi EY, Yanagishita M. Receptor mediated stimulatory effects of IL-1 β on hyaluronic acid and proteoglycan biosynthesis by cultured rat ovarian cells: role for heterologous cell-cell interactions. *Endocrinology* 1993; 133:2391–2394.
- Pitzel L, Jarry H, Wuttke W. Effects and interactions of prostaglandin F_{2 α} , oxytocin, and cytokines on steroidogenesis of porcine luteal cells. *Endocrinology* 1993; 132:751–756.
- Hurwitz A, Finci-Yeheskel Z, Dushnik M, Milwidsky A, Ben-Shitrit A, Yagel S, Adashi EY, Mayer M. Cytokine-mediated regulation of rat ovarian function: interleukin-1 inhibits plasminogen activator activity through the induction of plasminogen activator inhibitor-1 (PAI-1). *Mol Cell Endocrinol* 1994; 101:307–314.
- Ben-Shlomo I, Adashi EY, Payne DW. The morphogenic/cytotoxic and prostaglandin-stimulating activities of interleukin-1 β in the rat ovary are nitric oxide-independent. *J Clin Invest* 1994; 94:1463–1469.
- Kokia E, Ben-Shlomo I, Adashi EY. The ovarian action of IL-1 is receptor-mediated: reversal by a naturally occurring interleukin-1 receptor antagonist. *Fertil Steril* 1994; 63:176–181.
- Chun SY, Eisenhauer KM, Kubo M, Hsueh AJ. Interleukin-1 β suppresses apoptosis in rat ovarian follicles by increasing nitric oxide production. *Endocrinology* 1995; 136:3120–3127.
- Spicer LJ, Alpizar E. Effects of cytokines on FSH-induced estradiol production by bovine granulosa cells *in vitro*: dependence on size of follicle. *Domest Anim Endocrinol* 1994; 11:25–34.
- Brännström M, Wang L, Norman RJ. Ovulatory effect of interleukin-1 β on the perfused rat ovary. *Endocrinology* 1993; 132:399–404.
- Takehara Y, Dharmarajan AM, Kaufman G, Wallach EE. Effect of interleukin-1 β on ovulation in the *in vitro* perfused rabbit ovary. *Endocrinology* 1994; 134:1788–1793.
- Peterson CM, Hales HA, Hatasaka HH, Mitchell MD, Rittenhouse L, Jones KP. Interleukin-1 β (IL-1 β) modulates prostaglandin production and the natural IL-1 receptor antagonist inhibits ovulation in the optimally stimulated rat ovarian perfusion model. *Endocrinology* 1993; 133:2301–2306.
- Simón C, Tsafiriri A, Chun S-Y, Piquette GN, Dang W, Polan ML. Interleukin-1 receptor antagonist suppresses hCG-induced ovulation in the rat. *Biol Reprod* 1994; 51:662–667.
- Tsafiriri A, Lindner HR, Zor U, Lamprecht SA. Physiological role of

- prostaglandins in the induction of ovulation. Prostaglandins 1972; 2: 1-10.
45. Kokia E, Hurwitz A, Ricciarelli E, Tedeschi C, Resnick CE, Mitchell MD, Adashi EY. Interleukin-1 stimulates ovarian prostaglandin biosynthesis: evidence for heterologous contact-independent cell-cell interaction. Endocrinology 1992; 130:3095-3097.
 46. Brännström M, Wang L, Norman RG. Effects of cytokines on prostaglandin production and steroidogenesis of incubated preovulatory follicles of the rat. Biol Reprod 1993; 48:165-171.
 47. Townson DH, Pate JL. Regulation of prostaglandin synthesis by Interleukin-1 in cultured bovine luteal cells. Biol Reprod 1994; 51:480-485.
 48. Dennis EA. Diversity of group types, regulation, and function of phospholipase A₂. J Biol Chem 1994; 269:13057-13060.
 49. Hernandez ER, Jimenez JL, Payne DW, Adashi E. Adrenergic regulation of ovarian androgen biosynthesis is mediated via β_2 -adrenergic theca-interstitial cell recognition sites. Endocrinology 1988; 122: 1592-1602.
 50. Adashi EY, Resnick CE, Svoboda ME, Van Wyk JJ. Somatomedin-C synergizes with follicle-stimulating hormone in the acquisition of progesterin biosynthetic capacity. Endocrinology 1985; 116:2135-2142.
 - Ishizaki J, Ohara O, Nakamura E, Tamaki M, Ono T, Kanda A, Yoshida N, Teraoka H, Tojo H, Okamoto M. cDNA cloning and sequence determination of rat membrane-associated phospholipase A₂. Biochem Biophys Res Commun 1989; 162:1030-1036.
 52. Danielson PE, Forss-Petter S, Brow MA, Calavetta L, Douglass J, Milner RJ, Sutcliffe JG. p1B15: a cDNA clone of the rat mRNA encoding cyclophilin. DNA 1988; 7:261-267.
 53. Mercado M, Shimasaki S, Ling N, DePaolo L. Effects of estrous cycle stage and pregnancy on follistatin gene expression and immunoreactivity in rat reproductive tissues: progesterone is implicated in regulating uterine gene expression. Endocrinology 1993; 132:1774-1781.
 54. Michel U, McMaster JW, Findlay JK. Regulation of steady-state follistatin mRNA levels in rat granulosa cells *in vitro*. J Mol Endocrinol 1992; 9:147-156.
 55. Putowski LT, Choi DS, Mordacq J, Scherzer WJ, Mayo KE, Kiefer MC, Adashi EY, Rohan RM. *In vivo* hormonal regulation of insulin-like growth factor binding protein-5 mRNA in the immature rat ovary. J Soc Gynecol Invest 1995; 2:735-742.
 56. Oka S, Arita H. Inflammatory factors stimulate expression of group II phospholipase A₂ in rat cultured astrocytes. J Biol Chem 1991; 266: 9956-9960.
 57. Konieczkowski M, Sedor JR. Cell-specific regulation of type II phospholipase A₂ expression in rat mesangial cells. J Clin Invest 1993; 92: 2524-2532.
 58. Ben-Shlomo I, Kokia E, Jackson MJ, Adashi EY, Payne DW. Interleukin-1 β 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 1994; 51:310-318.