Detection and In Vivo Hormonal Regulation of Rat Ovarian Type I and Type II Interleukin-1 Receptor mRNAs: Increased Expression During the Periovulatory Period

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OBJECTIVE: *To study the expression, localization, and in vivo hormonal regulation of type I and type II interleukin-1 (IL-1) receptors in the rat ovary.*

METHODS: *Segments of the cDNAs for rat type I and type II IL-1 receptors were cloned and used as probes in RNase protection assays and in situ hybridization. Tissues obtained from immature rats and hormonally treated rat ovaries were examined.*

RESULTS: *Type I IL-1 receptor (IL-1R(1)) was ubiquitously expressed in rat tissues, including granulosa cells prepared from immature ovaries, whereas type II IL-1 receptor (IL-1R(2)) expression was restricted to macrophages, thymus, and lung. Hypophysectomy and subsequent treatment with FSH and/or diethylstilbestrol did not alter significantly the abundance of IL-1R(1) transcripts in the whole ovary. However, the relative amount of ovarian IL-1R(1) transcripts increased 7.3-fold 6 hours after the administration of hCG to pregnant mare serum gonadotropin–primed immature rats. During this time, IL-1R(1) mRNA was localized primarily in the granulosa cells. The increased expression of IL-1R(1) persisted 24 hours after hCG administration but declined to baseline by 48 hours. Ovarian expression of IL-1R(2) mRNA was observed only before ovulation in amounts that were approximately 70-fold lower than IL-1R(1).*

CONCLUSION: *The increased intraovarian expression of IL-1R(1) in granulosa cells during the periovulatory period implies that this cell type has a heightened receptivity to IL-1 and provides further indirect evidence that this cytokine is involved in the ovulatory process.* (J Soc Gynecol Invest 1996;3:131–9)

KEY WORDS: Receptors, interleukin-1, gene expression, ovary.

The thesis that ovulation constitutes a cyclic inflammatory process[1] and the recognition that interleukin-1 (IL-1) mediates inflammation in multiple tissues[2] has led to the hypothesis that intraovarian IL-1 plays a central role in the ovulatory cascade.[3,4] This hypothesis is supported by several lines of evidence. First, various components of the IL-1 system are expressed in human[5,6] and mouse[7] ovaries. Second, the in vitro administration of IL-1β activates several established components of the preovulatory cascade, including collagenase activity,[8] proteoglycan and hyaluronic acid production,[9] and prostaglandin biosynthesis.[10] Finally, IL-1β induces ovulation in ex vivo perfused rat[11] and rabbit[12] ovaries, whereas IL-1 receptor antagonist reduces the number of hCG-stimulated ovulations ex vivo[13] and in vivo.[14]

In the pregnant mare serum gonadotropin (PMSG)-hCG-primed immature rat, the abundance of ovarian IL-1β mRNA increases before ovulation.[15] However, nothing is known about the ovarian expression of the other components of the IL-1 system in the rat model. Although the in vitro actions of IL-1β imply the presence of functional receptors, it is unknown which type of IL-1 receptor is present in rat ovaries or if receptor expression is hormonally regulated.

Two different high-affinity IL-1 receptors (originally designated as the T cell [type I] and B cell [type II] receptors after

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the hematopoietic cell lineages, in which each type is predominantly expressed[16]) have been identified as the products of separate genes.[17–19] The two receptors are differentially expressed in various tissues and cell types.[19–21] Type I IL-1 receptor may be the sole transducer of IL-1 action,[22] whereas type II IL-1 receptor may be a membrane-bound IL-1 binding protein (decoy receptor) that inhibits biological function.[23,24]

It was our objective to study the expression, localization, and in vivo hormonal regulation of type I and type II interleukin-1 receptors in the rat ovary. At the outset of this study, the two different IL-1 receptors had been cloned only from human[18,19] and mouse.[17,19] Therefore, sequences corresponding to the rat type I and type II IL-1 receptors were isolated through the use of trans-species polymerase chain reaction (PCR) primers with the intent of using the corresponding cDNAs in highly sensitive RNase protection assays. Our findings demonstrate that type I is the predominant form of IL-1 receptor expressed in the immature rat ovary and that the abundance of the RNA for this receptor is substantially increased in the periovulatory time period.

MATERIALS AND METHODS

Animals and Cellular Preparations

Immature (25–28 days old) pituitary-intact and hypophysectomized Sprague-Dawley female rats were purchased from Zivic-Miller Laboratories (Zelienople, PA) and provided with food and water (or saline for hypophysectomized animals) ad libitum. All procedures were approved by the University of Maryland at Baltimore Institutional Animal Care and Use Committee. Animals were killed by asphyxiation with CO_2 and cervical dislocation at the indicated times. All tissues were obtained from immature female rats except testes, which were from 60-day-old males, and ovaries from hormonally-treated rats. Tissues for RNA extraction were collected, frozen immediately on dry ice, and stored at −70C. For collection of granulosa cells, six to eight ovaries were placed in 1 mL of ice-cold McCoy's 5a medium (Gibco BRL, Gaithersburg, MD) in a 35-mm culture dish. Each ovary was held in place with forceps and punctured repeatedly (ten to 20 times) with a 27-gauge needle. The resultant cellular suspension of extruded cells was removed by pipette and concentrated by centrifugation.

In Vivo Hormone Treatments

Diethylstilbestrol (DES) and PMSG were obtained from the Sigma Chemical Co. (St. Louis, MO). Ovine FSH (NIH-oFSH-S17; FSH potency equal to 20 NIH-FSH-S1 U/mg; LH activity 0.04 NIH-LH-S1 U/mg; PRL activity less than 0.1% by weight) was generously provided by the National Pituitary Agency, Pituitary Hormone Distribution Program, NIDDK. Human chorionic gonadotropin (hCG; CR-127; 14,900 IU/mg) was generously provided by Dr. R. E. Canfield through the Center for Population Research, National Institutes of Health (Bethesda, MD). Established paradigms[25] were used to influence the proportion and size of preantral and antral ovarian follicles. The treatments consisted of hypophysectomy (Hx), hypophysectomy and FSH (Hx + FSH), hypophysectomy and DES (Hx + DES), and hypophysectomy, FSH, and DES (Hx + FSH + DES). Rats were hypophysectomized by the supplier at 21 days of age. Diethystilbestrol (approximately 10 μg) was administered via a subcutaneous (sc) Silastic (Dow Corning, Midland, MO) implant that was inserted at the time of hypophysectomy. Ovine FSH was administered twice daily for 2.5 days by sc injection (10 μg oFSH per rat) starting in the afternoon of the fourth day after hypophysectomy. Ovaries were collected 7 days after Hx from all treatment groups (16–18 hours after the final FSH injection). Ovaries from age-matched pituitary intact rats (I) served as controls. There were at least five animals in each group for each experiment, and each experiment was performed in triplicate.

To stimulate follicular maturation, ovulation, and corpora lutea formation, pituitary intact rats (26 days of age) were injected in the morning with 15 IU PMSG sc followed 48 hours later with 15 IU hCG sc. At least three animals were collected at each of the indicated times. Each experiment was performed in triplicate.

RNA Extraction

Frozen tissue samples were homogenized in RNAzol B (Tel test, Friendswood, TX), and RNA was extracted per the vendor's protocol. The resultant RNA was resuspended in diethylpyrocarbonate (DEPC)-treated distilled, deionized water and stored at −70C. Ribonucleic acid was quantified by absorbance at 260 nm and its integrity assessed by fluorescent visualization of rRNA bands after electrophoresis of denatured RNA in a 1% agarose, 2.2 mol/L formaldehyde gel, and ethidium bromide staining.

Cloning and Characterization of Rat Probes

Partial cDNA clones were prepared by reverse transcription polymerase chain reaction (RT-PCR) using trans-species (IL-1Rs) or species-specific (RPL19) primers (Table 1) as described previously.[25] Polymerase chain reaction was performed with AmpliTaq polymerase (Perkin Elmer, Norwalk, CT) in a DNA Thermal Cycler (Perkin Elmer) using 40 (IL-1R(1)), 30 (IL-1R(2)), or 20 (RPL19) cycles at 95C for 1.5 minutes, 55C (50C for IL1R(1)) for 1.5 minutes, and 72C for 3 minutes. Polymerase chain reaction products were inserted into the pCR1000 vector (Invitrogen, San Diego, CA) according to manufacturer's protocol.

The identity of each insert was verified by sequencing of plasmid DNA with fluorescent dideoxynucleotide terminators and analysis on an Applied Biosystems (Foster City, CA) Model 373A DNA Sequencer. Both DNA strands from two clones, obtained from independent PCR amplifications, were sequenced for each gene product. Tests for homology to data base sequences at the National Center for Biotechnology Information were performed with BLASTN.[26]

The sequence of the RPL19 clone was identical to the published sequence.[27] After completion of this work, the com-

Table 1. Reverse Transcription Polymerase Chain Reaction Primers

cDNA	Primer (sense/antisense)	Length	Homologous region* (primer/cDNA)
IL-1R(1)	AAAGGATCCGATGGAAAGACATACGATGCCTA	32	10/1374–32/1396
	AGCGAATTCATTTTCTCATAGTCTTGGATTTTCTC	35	9/1730–35/1704
IL-1R(2)	CGATGCAGGCTATTACCGGTGTGT	24	1/728–24/751
	ATCCTCCCTTGTGACTGGATCAAA	24	1/1085–24/1062
RPL19	CTGAAGGTCAAAGGGAATGTG	21	1/401–21/421
	GGACAGAGTCTTGATGATCTC	21	1/595–21/575

IL = interleukin.

* The nucleotide positions of the homologous region between each primer and the published cDNA sequence are listed. IL-1R(1) primers contain nonhomologous extensions on the 5′ end. Primers were chosen using mouse IL-1R(1),[17] mouse IL-1R(2),[19] or rat RPL19[27] cDNA sequences.

plete cDNA sequences of rat IL-1R(1)[28] and IL-1R(2)[29] were published. Both IL-1R(2) clones (pWJS-II-2 and pWJS-II-7) contained an A residue instead of the G residue at position 930 of the published sequence.[29] One IL-1R(1) clone (pWJS-I-9) was identical to the published sequence[28] over the length of the probe, whereas the other (pWJS-I-3) contained a G residue instead of the A residue at position 1420. Despite these polymorphisms, no evidence of internal cleavage was observed in the RNase protection assay under conditions described later (data not shown).

RNase Protection Assay

Linearized template DNA (pWJS-I-3 for IL-1R(1) or pWJS-II-2 for IL-1R(2)) was transcribed with T7 RNA polymerase (Promega, Madison, WI) to specific activities of 400–800 Ci/mmol uridine triphosphate (UTP) (IL-1R) or 80 Ci/mmol UTP (RPL19). The DNA template was digested with 10 μg DNAse I (Gibco BRL) and the riboprobe was purified by phenol/chloroform extraction followed by ethanol precipitation in the presence of 15 μg yeast tRNA (Gibco BRL) as carrier. The expected lengths for each of the probes and subsequent protected fragments are, respectively, 374 and 307 nt (IL-1R(1)), 444 and 355 nt (IL-1R(2)), and 283 and 194 nt (RPL19).

Ribonucleic acid samples (20 μg) were hybridized with excess antisense ^{32}P-RNA probes in 20 mmol/L TRIS pH 7.6, 1 mmol/L ethylenediaminetetra-acetic acid (EDTA), 0.4 mol/L NaCl, 0.1% sodium dodecyl sulfate (SDS), and 75% formamide. Positive and negative controls consisting of 20 μg yeast tRNA were included in each experiment. Reactions were denatured at 95C before overnight hybridization at 45C. After hybridization, nine volumes of 10 mmol/L TRIS-HCl, pH 7.6, 5 mmol/L EDTA, 0.3 mol/L NaCl containing 11 μg RNase A (Sigma, St. Louis, MO) and 27 U RNase T1 (Pharmacia, Piscataway, NJ) was added to each sample for a 1-hour incubation at 30C. The negative control was incubated in the same buffer without RNases. RNases were eliminated by digestion with 50 μg Proteinase K in 1.5% SDS at 37C for 15 minutes followed by phenol/chloroform extraction and ethanol precipitation in the presence of 20 μg yeast tRNA carrier. Ribonucleic acid pellets were reconstituted in 97% formamide, 1 mmol/L EDTA, 0.1% xylene cyanol/bromophenol blue and denatured at 95C before electrophoresis on 5% acrylamide gels containing 8.3 mol/L urea in 0.1 mol/L TRIS/0.1 mol/L borate (pH 8.3), and 2 mmol/L EDTA. Gels were exposed to XAR film (Kodak, Rochester, NY) for varying lengths of time with intensifying screens.

To calibrate the RNase protection assay, sense strand standard RNA was prepared by subcloning the inserts from pWJS-I-3 and pWJS-II-2 into pGEM7Zf(+) vector (Promega) and transcription in vitro. Unincorporated nucleotides were eliminated from the transcription reaction with the Centricon 100 (Amicon, Beverly, MA) and the product quantified by measuring the absorption at 260 nm. Sense strand RNA was diluted in a solution of yeast tRNA (1 mg/mL) for use in the RNase protection assay.

In Situ Hybridization

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 aminopropyltriethoxysilane-coated glass slides, and baked overnight at 60C. Wax was removed with xylene and ethanol, and slides were rinsed before treatment with pepsin (Sigma, St. Louis, MO) (4 mg/mL in 0.2 N HCl) at room temperature for 13 minutes. Slides were rinsed again and fixed with 2.7% paraformaldehyde for 5 minutes.

Riboprobes were synthesized with digoxigenin-UTP (Boehringer Mannheim, Indianapolis, IN). Riboprobes (8 ng/μL) in hybridization buffer (50% volume/volume formamide, 5% weight/volume dextran sulfate, 2× SSC, 0.1 mol/L EDTA, 150 μg/mL *Escherichia coli* tRNA, 1 mmol/L TRIS-HCl, pH 7.3) were added to each sample. Slides were covered, heated to 95C for 15 minutes in a humidified chamber, and hybridized at 47C overnight. After hybridization, slides were rinsed in 4× SSC and 2× SSC and treated with 150 μg/mL RNase A (Sigma, UK) in 2× SSC at 37C for 30 minutes.[30]

Slides were then washed, preincubated in blocking buffer (100 mmol/L TRIS-HCl, pH 7.5, 150 mmol/L 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 hours. Unbound antibody was removed by washing in 0.1 mol/L maleic acid, pH 7.5, 150 mmol/L NaCl for 30 minutes. Bound antibody was detected by incubation in 100 mmol/L TRIS-HCl, pH 9.5, 100 mmol/L NaCl, and 50 mmol/L $MgCl_2$ containing 375 μg/mL nitroblue tetrazolium and 187.5 μg/mL 5-bromo-4-

chloro-3-inodyl-phosphate (NBT/BCIP, Boehringer Mannheim) in the dark overnight. Reactions were stopped with distilled, deionized H_2O, and the slides dried and mounted in aqueous mounting medium.

Analysis and Statistics

Autoradiographs were quantified with a laser densitometer and analyzed with Image Quant Software (Molecular Dynamics, Sunnyvale, CA). Because RPL19 mRNA expression in the rat ovary is not regulated hormonally,[31] the ratio of IL-1R to RPL19 was used to correct for variations in sample handling. To compare experiments, which were assayed at different times, the IL-1R:RPL19 ratio was expressed as the fold difference from the control group (untreated pituitary intact rats). Differences between groups were tested by analysis of variance using StatView (Brain Power, Calabasas, CA) followed by Fisher least squares difference test with a significance level of $P < .05$.

RESULTS

Cloning of the Rat Type I and Type II IL-1 Receptor Probes

Ribonucleic acid from multiple rat tissues was reverse transcribed and PCR amplified as described in Materials and Methods. Of the tested tissues, only RNA from spleen yielded a product of the predicted size with the trans-species type I IL-1 receptor primers. The DNA sequence of this product was 91 and 79% identical to the corresponding segments of the mouse and human type I IL-1 receptor cDNAs, respectively. The trans-species type II IL-1 receptor primers produced a product of the predicted size with RNA from a variety of tissues. This product was 94 and 74% identical to the mouse and human cDNAs, respectively. Subsequently, both PCR products were found to be nearly identical to the published sequences for rat IL1R cDNAs (see Materials and Methods).

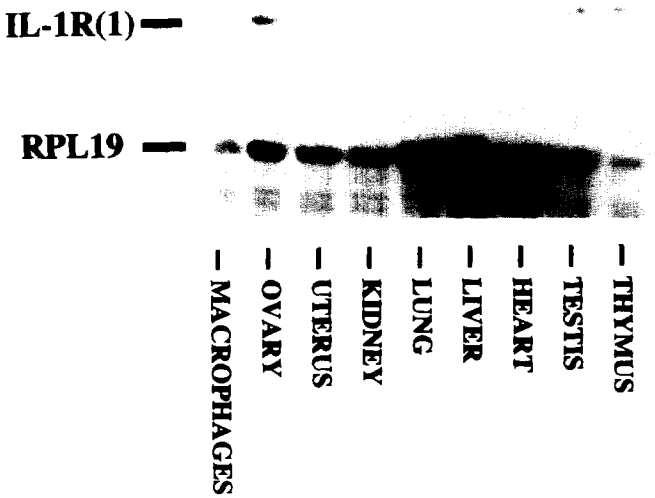

Figure 1. Type I interleukin-1 (IL-1) receptor gene expression in the immature rat: Tissue distribution. Total RNA (20 μg), extracted from the indicated tissues, was subjected to a RNase protection assay using ^{32}P-labeled rat type I IL-1 receptor (IL-1R(1)) and RPL19 antisense RNAs as described in Materials and Methods. Duration of autoradiographic exposure was 7 and 1 days(s) for the type I IL-1 receptor and RPL19, respectively. Hybridization to yeast tRNA did not produce a detectable protected fragment (not shown). The autoradiograph is from a representative experiment. Qualitatively comparable results were obtained in two additional experiments.

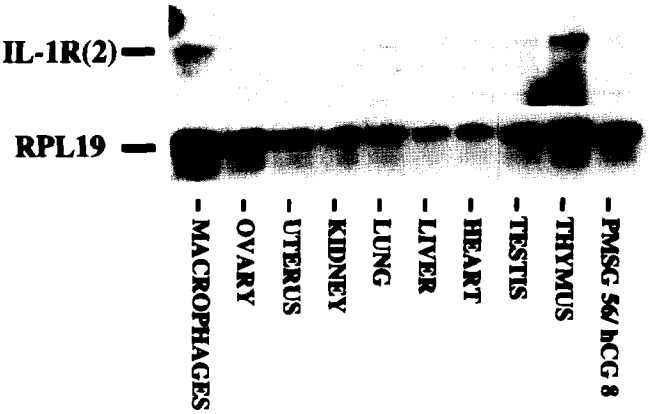

Figure 2. Type II interleukin-1 (IL-1) receptor gene expression in the immature rat: Tissue distribution. Total RNA (20 μg), extracted from the indicated tissues was subjected to a RNase protection assay using ^{32}P-labeled rat type II IL-1 receptor (IL-1R(2)) and RPL19 antisense RNAs as described in Materials and Methods. Duration of autoradiographic exposure was 21 and 1 day(s) for the type II IL-1 receptor and RPL19, respectively. Hybridization to yeast tRNA did not produce a detectable protected fragment (not shown). The autoradiograph is from a representative experiment. Qualitatively comparable results were obtained in two additional experiments.

Type I and Type II IL-1 Receptor Gene Expression in the Immature Rat: Tissue Distribution

To assess the tissue distribution of type I and type II IL-1 receptors, total RNA (20 μg), extracted from nine distinct tissues, was probed with antisense ^{32}P-labeled riboprobes generated from the cloned PCR products as described in Materials and Methods. As shown (Figure 1), a single band of approximately 307 nt, corresponding to the anticipated size of the type I IL-1 receptor protected fragment, was observed in all tissues,

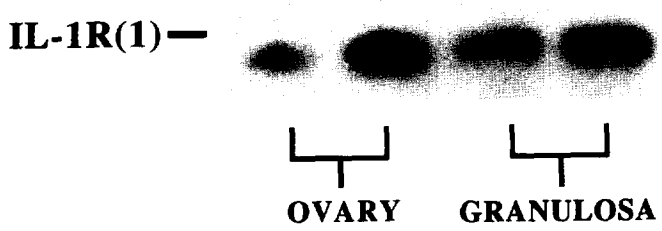

Figure 3. Ovarian type I interleukin-1 (IL-1) receptor gene expression: Granulosa cells. Total RNA (20 μg), extracted from whole ovary or from isolated granulosa cells of untreated immature rats, was subjected to a RNase protection assay using a ^{32}P-labeled antisense RNA corresponding to the rat type I IL-1 receptor (IL-1R(1)) as described in Materials and Methods. Hybridization to yeast tRNA did not produce a detectable protected fragment (not shown). Duration of autoradiographic exposure was 14 days. Individual lanes were derived from independent RNA preparations consisting of more than five animals each.

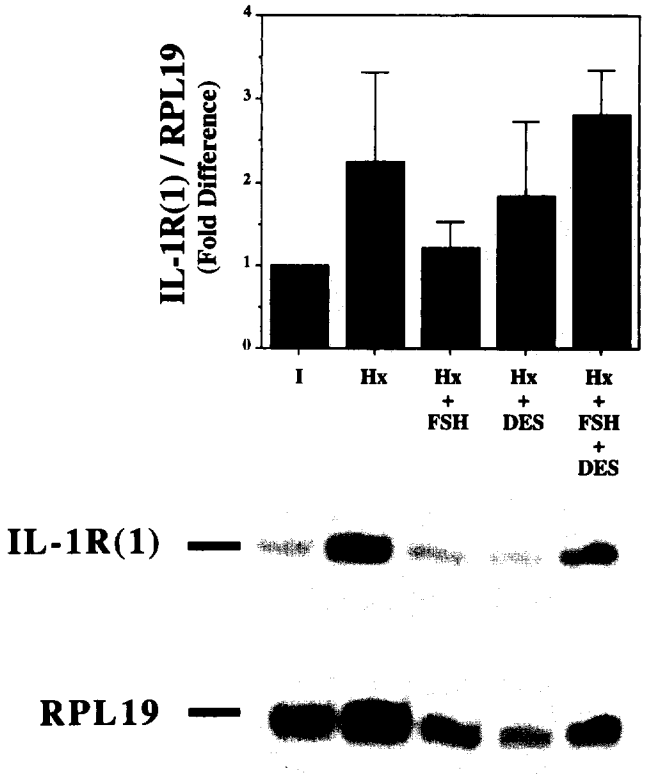

Figure 4. Ovarian type I interleukin-1 (IL-1) receptor gene expression: Effect of hypophysectomy and of hormonal replacement. Total RNA (20 μg), extracted from the ovaries of rats from the indicated treatment groups, was subjected to a RNase protection assay using ^{32}P-labeled antisense RNAs corresponding to the rat type I IL-1 receptor (IL-1R(1)) and RPL19 as described in Materials and Methods. I = intact immature rats; Hx = hypophysectomized rats; DES = diethylstilbestrol; DES and FSH designate immature Hx rats treated with these principles or combinations thereof, as outlined in Materials and Methods. Hybridization to yeast tRNA did not produce a detectable protected fragment (not shown). Duration of autoradiographic exposure was 12 and 1 day(s) for the type I IL-1 receptor and RPL19, respectively. Potential RNA loading variability was corrected by normalizing the amount of IL-1R(1) hybridization to the amount of RPL19 hybridization. The resultant data were further normalized relative to the intact immature group within each experiment. The bar graph (*top panel*) illustrates the results (mean ± standard error) of three experiments; the autoradiogram (*bottom panel*) shows the results of a representative experiment. There were at least five animals in each group for each experiment.

including the ovary. A similar tissue survey for type II IL-1 receptor transcripts revealed a protected fragment of the appropriate size (357 nt), predominantly in macrophages and thymic tissue (Figure 2). A low level of type II IL-1 receptor transcripts was also observed in lung from immature females, testis from adult males, and ovary from immature females treated with PMSG/hCG.

To ascertain if type I IL-1 receptor mRNA was expressed in granulosa cells of untreated immature animals, total RNA was extracted from freshly isolated granulosa cells and subjected to molecular probing. A single protected transcript was apparent after hybridization to RNA from whole ovaries as well as granulosa cells (Figure 3).

Ovarian Type I IL-1 Receptor Gene Expression: Hormonal Regulation and Localization

To address the possibility that rat ovarian type I IL-1 receptor gene expression is regulated by hormone treatments that alter granulosa cell proliferation and differentiation, total RNA was extracted from the ovaries of immature pituitary intact (I), hypophysectomized (Hx), or hormonally-treated Hx rats as described in Materials and Methods. Hypophysectomy resulted in a 2.2-fold increase of ovarian mRNA for type I IL-1 receptors compared with untreated animals (Figure 4), although this difference was not significant. Likewise, treatment of Hx rats with FSH and/or DES did not result in significant

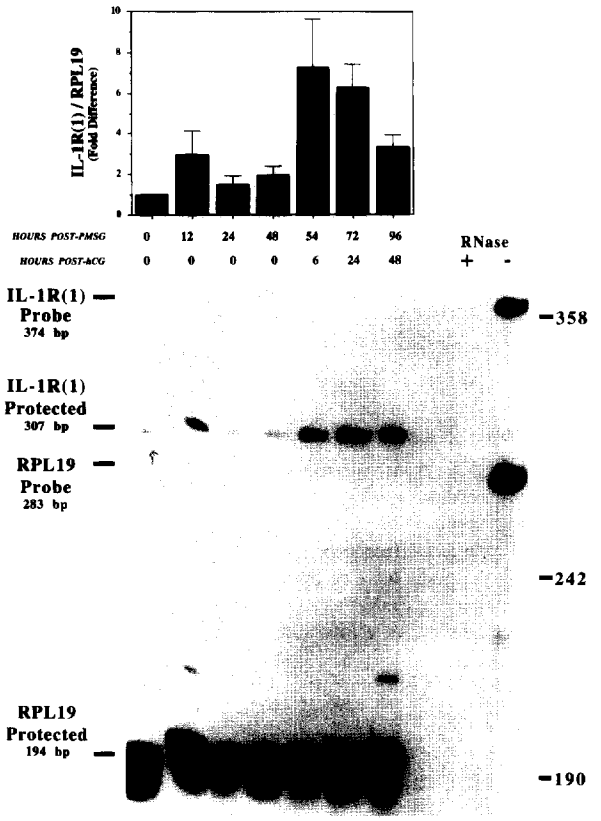

Figure 5. Ovarian type I interleukin-1 (IL-1) receptor gene expression: Effect of follicular maturation, ovulation, and luteinization. Total RNA (20 μg), extracted at the indicated times from ovaries of pregnant mare serum gonadotropin (PMSG)-primed/hCG-treated immature rats, was subjected to a RNase protection assay with ^{32}P-labeled antisense RNAs corresponding to rat type I IL-1 receptor (IL-1R(1)) and RPL19 as described in Materials and Methods. Duration of autoradiographic exposure was 1 day. At least three animals were used at each time point in each experiment. Potential RNA loading variability was corrected by normalization to RPL19. The resultant data were further normalized relative to untreated animals (0 hours post-PMSG). The bar graph (*top panel*) illustrates the results (mean ± standard error) of three experiments; the autoradiogram (*bottom panel*) shows the results of a representative experiment. The positions and predicted sizes of IL-1R(1) and RPL19 probes and protected fragments are indicated. Lanes labeled RNase + and − indicate probes hybridized to yeast tRNA and incubated with (+) or without (−) RNases as positive and negative controls.

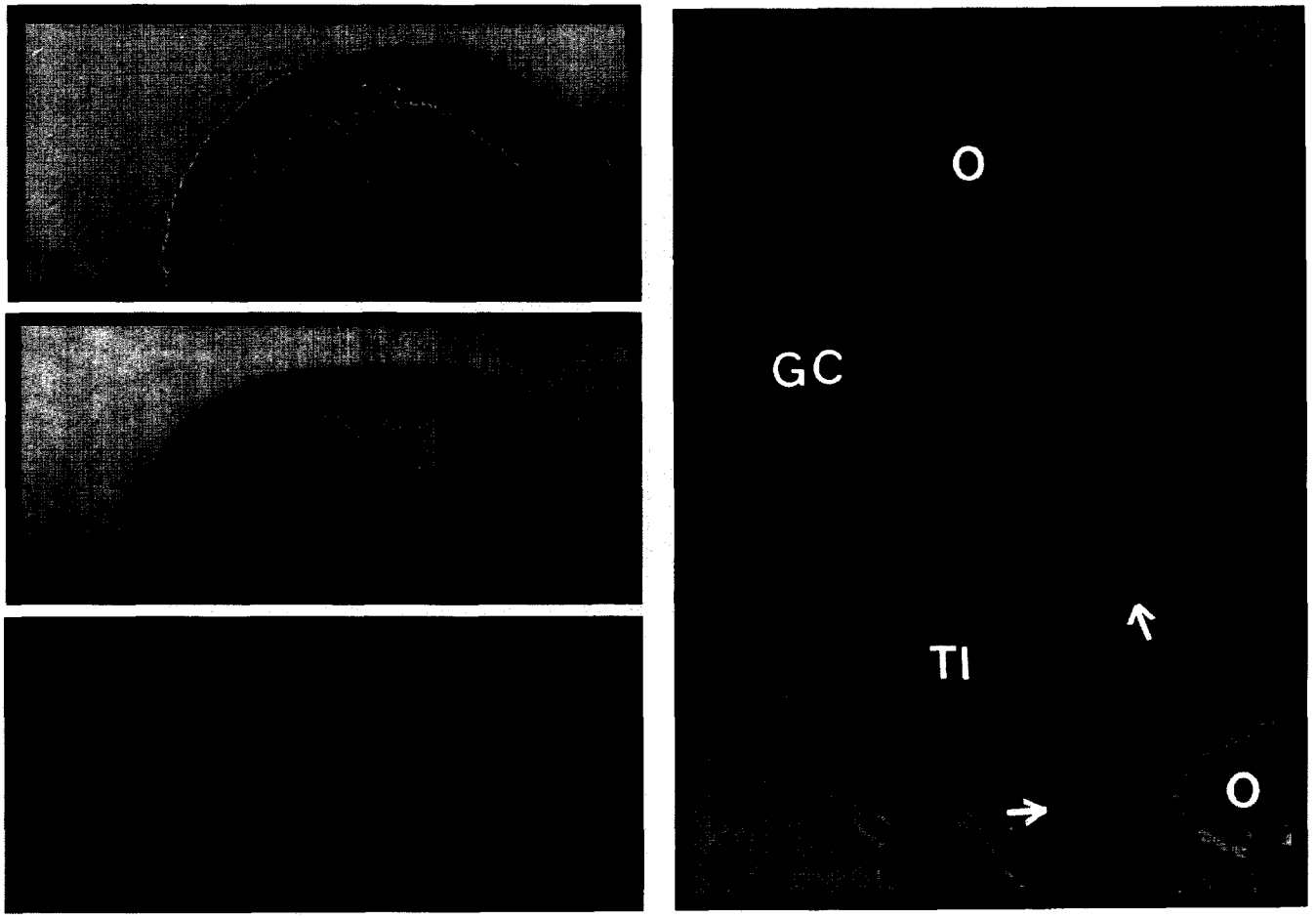

Figure 6. Ovarian type I interleukin-1 (IL-1) receptor gene expression: In situ hybridization. Ovaries collected 12 hours after hCG treatment of PMSG-primed rats were used for in situ hybridization with digoxigenin-labeled riboprobes as described in Materials and Methods. Sections were hybridized with riboprobes for antisense type I IL-1 receptor (A, B, and D) or for pCR1000 vector without insert (C). Hybridization with sense strand type I IL-1 receptor riboprobe was not different from that for vector alone. Panels A, B, and C are adjacent sections from the same ovary. Panels A and B were hybridized on separate occasions with different probe preparations demonstrating the reproducibility of the assay. Slides were photographed at a magnification of 40× (A, B, and C) or 400× (D). O = oocyte; GC = granulosa cells; TI = theca-interstitial. The basolateral follicular wall is indicated by the arrows.

changes in the whole ovarian amount of type I IL-1 receptor mRNA.

To ascertain if type I IL-1 receptor gene expression is altered during the final stages of follicle maturation and ovulation, RNA was extracted from the ovaries of immature rats that had been treated sequentially with PMSG and hCG. Basal amounts of type I IL-1 receptor transcripts did not change 12, 24, or 48 hours after the administration of 15 IU of PMSG (Figure 5). However, a marked increase (7.3-fold) in the abundance of type I IL-1 receptor mRNA was observed in ovarian RNA collected 6 hours after hCG administration ($P < .05$). Receptor mRNA content was slightly lower 24 hours after hCG but still significantly above basal levels ($P < .05$). By 48 hours after hCG, the intraovarian amount of type I IL-1 receptor mRNA had declined to baseline.

In situ hybridization was used to localize IL-1R(1) expression in the preovulatory rat ovary (Figure 6). In ovaries collected 12 hours after hCG treatment, hybridization was observed consistently in the granulosa cells (Figures 6A, B). Hybridization of adjacent sections with sense strand probe (not shown) or with vector sequences alone (Figure 6C) did not produce any signal. Granulosa cells of both antral and preantral follicles were positively stained (Figure 6D). Hybridization to oocytes (O) or theca interna (TI) was not observed in this time period.

Ovarian IL-1 Receptor Gene Expression: Relative Abundance of the Two Receptor Types

Using an in vitro-transcribed standard RNA (Figure 7), the peak levels of type I IL-1 receptor mRNA in the PMSG/hCG-treated ovary is estimated to be 2 pg per 20 μg of total RNA. Type II IL-1 receptor mRNA was reproducibly detected in three independent RNA samples from lung tissue. The amount of type II IL-1 receptor mRNA in these lung samples is estimated to be 0.07 pg per 20 μg of total RNA (Figure 8). Type II IL-1 receptor mRNA in one PMSG/hCG-treated ovary was at the detection limit of the assay (0.03 pg per 20 μg), whereas two other PMSG/hCG-treated ovary samples were below this threshold. Thus, the ovarian content

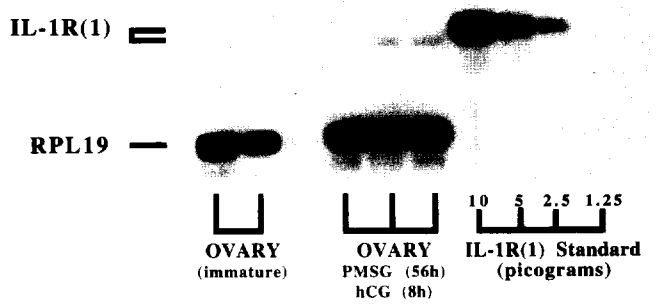

Figure 7. Ovarian type I interleukin-1 (IL-1) receptor gene expression: Determination of abundance. Sense strands of type I IL-1 receptor (IL-1R(1)) RNA were transcribed from a plasmid containing the IL-1R(1) cDNA, quantified, and diluted as described in Materials and Methods. The indicated amounts of sense strand standard (in 10 μL of 1 mg/mL yeast tRNA) were used in a RNase protection assay with ^{32}P-labeled IL-1R(1) antisense RNA. A representative titration of IL-1R(1) sense RNA (n = 3) is shown along with RNA (20 μg each) obtained from ovaries of immature rats and ovaries of treated rats (pregnant mare serum gonadotropin [PMSG]/hCG). Each lane is derived from an independent RNA sample consisting of at least three animals. We estimated that 20 μg of RNA obtained from PMSG (56 hours)/hCG (8 hours) contained approximately 2 pg of IL-1R(1) mRNA protected fragment. The protected fragment with IL-1R(1) standard was five nucleotides larger than the protected fragment with tissue mRNAs because of retention of polymerase chain reaction primer sequences during the subcloning process. Duration of autoradiographic exposure was 1 day.

of IL-1R(1) transcripts is estimated to be 70-fold higher than its IL-1R(2) counterpart.

DISCUSSION

The present studies describe the isolation of riboprobes that are suitable for detection of the two types of rat IL-1 receptors with highly sensitive RNase protection assays. Using these probes it was found that, in the rat, the type I IL-1 receptor was expressed in a variety of organs, including ovary (Figure 1). This ubiquitous expression in rat is consistent with in situ hybridization[20] and RNase protection studies in mice.[21] The presence of high basal transcription elements in the human type I IL-1 receptor gene promoter also supports widespread tissue distribution of this receptor.[32] In contrast, type II IL-1 receptor mRNA was less abundant in most rat tissues. In the rat ovary, type II mRNA was approximately 70-fold less abundant than its type I counterpart (Figures 7 and 8). Although type II IL-1 receptor expression was not detected in many tissues with the RNase protection assay, RT-PCR did indicate a widespread tissue distribution of this receptor as well (data not shown). Our inability to detect type I IL-1R transcripts by RT-PCR may be due to mismatches in the trans-species sense strand primer that were ultimately uncovered by publication of the rat cDNA sequence.[28] This exemplifies the potential uncertainties using the trans-species primers in RT-PCR and underscores the importance of verifying negative results with an alternative technique.

Within the immature rat ovary, type I IL-1 receptor transcripts were detected in preparations of granulosa cells (Figure 3). This observation contrasts with in situ observations of ovaries from mice, in which type I IL-1 receptor transcripts[20] and protein[7] were localized to oocytes but not to the granulosa cells of secondary or tertiary follicles. However, the presence of IL-1 receptors on immature rat granulosa cells can be inferred by the ability of IL-1 to inhibit hormone-induced differentiation of these cells in culture.[33–37]

Despite detection of type I IL-1 receptor transcripts in immature granulosa cells, ovarian expression of type I IL-1 receptors was not altered by hormonal treatments that influence the number and differentiation of the granulosa cells. Specifically, hypophysectomy (which reduces the ovarian content of preantral granulosa cells), DES (which increases the number of preantral granulosa cells), and FSH (which induces granulosa cell differentiation) all failed to produce significant changes in the amount of IL-1 receptor mRNA.

However, ovarian type I IL-1 receptor expression was significantly stimulated by hormonal treatments (PMSG/hCG) that induced ovulation and promoted luteinization of the granulosa cells. The relative abundance of ovarian type I IL-1 receptor transcripts increased 7.3-fold 6 hours after hCG, 6 hours before anticipated ovulation. During this time of heightened ovarian expression, type I IL-1R transcripts were clearly localized within the granulosa cells (Figure 6). Unlike the prior

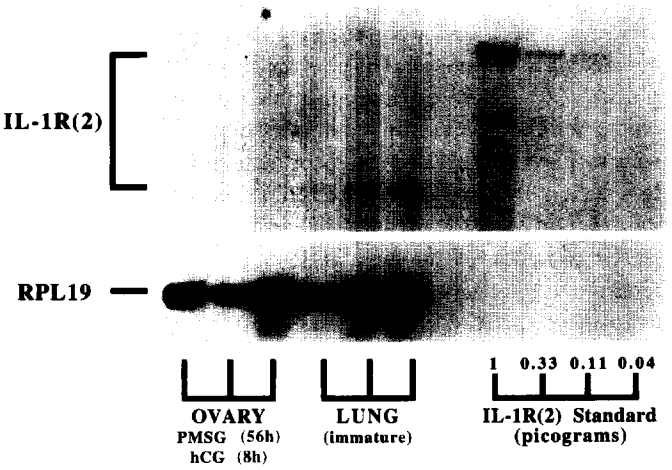

Figure 8. Ovarian type II interleukin-1 (IL-1) receptor gene expression: Determination of abundance. Sense strands of type II IL-1 receptor (IL-1R(2)) RNA were transcribed from a plasmid containing the IL-1R(2) cDNA, quantified, and diluted as described in Materials and Methods. The indicated amounts of sense strand standard (in 10 μg of 1 mg/mL yeast tRNA) were used in a RNase protection assay with ^{32}P-labeled IL-1R(2) antisense RNA. A representative titration of IL-1R(2) sense RNA (n = 3) is shown along with RNA obtained from ovaries and lungs of PMSG/hCG-treated or untreated immature rats. Each lane is derived from an independent RNA sample. We estimated that 20 μg of RNA from PMSG/hCG-treated rat ovary contains less than 0.03 pg of IL-1R(2) mRNA protected fragment and that 20 μg of RNA obtained from rat lung contains approximately 0.07 pg of IL-1R(2) RNA protected fragment. The protected fragment with IL-1R(2) standard is 53 nucleotides larger than the protected fragment with tissue mRNAs because of the retention of vector sequences during the subcloning process. Duration of autoradiographic exposure was 24 days. PMSG = pregnant mare serum gonadotropin.

finding of a preovulatory increase in expression of IL-1β transcripts in the same experimental model,[15] the relative abundance of ovarian type I IL-1 receptor transcript was still elevated 24 hours after hCG. This raises the possibility that increased type I IL-1R expression in the rat ovary is maintained in the early corpora lutea. Indeed, type I IL-1R protein increases in the mouse granulosa cells before ovulation and remains elevated in the resultant corpora lutea.[7] Type I IL-1R transcripts have also been detected in human granulosa-luteal cells isolated during oocyte retrieval.[5,6]

The mechanism of this periovulatory increase in type I IL-1 receptor expression is unknown but could involve either the cAMP or protein kinase C signal transduction pathways as it does in other cells.[29,38–40] However, activation of the cAMP transduction system is unlikely to be the sole causative agent of the periovulatory ovarian increase because PMSG (Figure 5) and FSH (Figure 4), by themselves, did not alter the amount of ovarian type I IL-1 receptor transcripts.

The previously demonstrated increase in ovarian IL-1β gene expression[15] may result in increased periovulatory receptor expression because ligand-induced enhancement of type I receptor expression has been demonstrated in fibroblasts,[39,40] cultured secretory endometrial cells,[41] and macrophage-replete preparations of granulosa-luteal cells.[6] However, increased IL-1β expression does not always result in increased receptor expression.[6,41–43] Although treatment with prostaglandins also increases type I IL-1 receptor expression in fibroblasts, the intermediacy of prostaglandins in the ligand-induced increase is unclear.[39,40] Because prostaglandins are a recognized component of the preovulatory cascade, they may be the agents responsible for increased receptor expression in the ovary. Resolution of these two, nonexclusive mechanisms awaits further in vivo and in vitro experiments.

The presence of the type II IL-1 receptor has not been reported in the ovary of any species. Because the abundance of ovarian type II IL-1 receptor transcripts was at the detection limit of the RNase protection assay (less than 0.03 pg per 20 μg total RNA), it cannot be concluded whether hormone treatments affect type II IL-1 receptor gene expression in the ovary. However, the relevance of type II IL-1 receptor to ovarian physiology is questionable given the preponderance (more than 70-fold) of type I IL-1 receptor mRNA. If the increase in ovarian type I IL-1 receptor mRNA leads to increased expression of ovarian receptors, as it does in most other cell types,[38,44–46] and if the primary purpose of type II IL-1 receptor is to serve as a binding protein,[23,24] the present results suggest that the preovulatory ovary has an increased sensitivity to IL-1 action that is retained after ovulation. We should note, however, that ovarian IL-1 activity may still be modulated by the naturally occurring receptor antagonist (IL-1ra), the expression of which has not been addressed in this model system.

It has been postulated that intraovarian IL-1β (of theca-interstitial or resident macrophage origin) interacts with intraovarian receptors (on granulosa cells) to coordinate and amplify key components of the ovulatory cascade. Support for this hypothesis has already been demonstrated by 1) the preovulatory induction of IL-1β transcripts in the rat[15] as well as human[5,6] ovary; 2) the in vitro ability of IL-1β to activate several established components of the preovulatory cascade including collagenase activity,[8] proteoglycan/hyaluronic acid production,[9] and prostaglandin biosynthesis[10]; and 3) the ex vivo capacity of IL-1β to induce ovulation in its own right[11,12] or of IL-1 receptor antagonist to inhibit LH/hCG action in this regard.[13,14] However, the observation of overtly normal fertility in mice that lack active IL-1β because of genetic ablation[47,48] argues against an obligatory role for this cytokine in the ovulatory cascade. The present demonstration of up-regulation of the type I IL-1β receptor mRNA in the granulosa cells of the periovulatory ovary designates these cells as potential sites of IL-1 action and supports the growing body of evidence that IL-1 does indeed play some in vivo role in ovulation. The insights derived from the investigation of intraovarian IL-1 receptor regulation will contribute to the understanding of the molecular events underlying the ovulatory process.

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