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Rat Ovarian Interleukin-1α

Interleukin-1-Dependent In Vitro Expression

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Evidence exists supporting the possibility that intraovarian interleukin-1 (IL-1) may play an intermediary role in the periovulatory cascade. Although the existence of a mammalian intraovarian IL-1 system has been convincingly demonstrated, most efforts have focused on the possibility that the mammalian ovary is a site of IL-1b production, reception, and action. The objective of this study was to explore the possibility of ovarian IL-1a expression, characterize its pattern of expression by cultured ovarian cells, and study its hormonal regulation. The basal in vitro expression of IL-1a by cultured whole ovarian dispersates from immature rats increased spontaneously, reaching a peak (sixfold increase over untreated controls) at 4 h. Treatment with an IL-1 receptor antagonist (IL-1RA), human chorionic gonadorropin, or IL-1b failed to attenuate the initial 4-h burst of IL-1a expression. By contrast, treatment of whole ovarian dispersates with IL-1b for 48 h resulted in significant upregulation of IL-1a transcripts (60-fold increase). This IL-1b effect was completely blocked by cotreatment with IL-1RA, thereby suggesting mediation via a specific IL-1 receptor. The IL-1b effect proved to be protein biosynthesis and eicosanoid dependent, nitric oxide independent, and relatively specific in that it was not reproduced by a select series of other granulosa cell agonists.

Key Words: Interleukin-1α; rat; ovary; ovulation.

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Introduction

A growing body of direct and indirect evidence supports the notion that intraovarian interleukin-1β (IL-1β) may constitute an intermediary in the ovulatory process *(1)*. First, the ex vivo provision of IL-1β has been shown to bring about ovulation and to synergize with luteinizing hormone (LH) in this regard *(2,3)*. Second, the addition of an IL-1 receptor antagonist (IL-1RA) has been shown to attenuate LH-supported ovulation under both ex vivo *(4)* and in vivo *(5)* conditions. Third, some components of the intraovarian IL-1 system (e.g., IL-1β and type I IL-1 receptor [IL-1R]) appear to be expressed in vivo mainly during a narrow periovulatory window *(6–13)*. Fourth, IL-1β has been shown to induce a host of ovulation-associated phenomena (herein reviewed for the rat) such as the promotion of ovarian prostaglandin production *(14,15)*, the stimulation of ovarian hyaluronic acid biosynthesis *(16)*, the induction of ovarian collagenase activity *(17)*, the perturbation of ovarian plasminogen activator *(18,19)*, the activation of ovarian nitric oxide (NO) synthase *(20–23)* and the arrest of follicular atresia *(24)*.

Although the existence of an intraovarian IL-1 system has been convincingly demonstrated *(6–13)*, most efforts have focused on the possibility that the mammalian ovary is a site of IL-1β production *(6–9)*, reception *(11–13)*, and action *(14–24)*. In part, this preference reflects the demonstration that human ovarian IL-1β transcripts are considerably more abundant than their IL-1α counterparts *(7)*. However, the mouse *(9)* and rat *(25)* ovaries have been demonstrated to be a site of immunoreactive IL-1α production. In the mouse, the somatic cell concerned with IL-1α expression during follicular development is the theca-interstitial cell *(9)*. However, following follicular rupture, immunoreactive IL-1α at the level of the granulosa cell has also been noted *(9)*. Finally, IL-1α proved capable of enhancing LH-induced progesterone accumulation by cultured human granulosa cells *(26)*, attenuating LH-supported androgen biosynthesis by cultured rat whole ovarian dispersates *(27)*, and suppressing follicle-stimulating hormone (FSH)–stimulated progesterone accumulation by cultured rat granulosa cells *(28)*.

It was the objective of the present study to explore the possibility of ovarian IL-1α expression under in vivo conditions, characterize its basal pattern of expression by ovarian cells under in vitro conditions, and study its in vitro hormonal regulation.

Results

Reverse Transcriptase Polymerase Chain Reaction Studies of Ovarian IL-1α Transcripts

To establish ovarian IL-1α gene expression, ovaries were obtained from in vivo untreated 25-d-old rats and processed for reverse transcriptase polymerase chain reaction (RT-PCR) as described. As shown (Fig. 1), no IL-1α transcripts could be documented at a time when IL-1β transcripts were readily apparent.

Time-, Human Chorionic Gonadotropin- and IL-1 Dependence of IL-1α Gene Expression by Cultured Whole Ovarian Dispersates

Although not shown, previous efforts to document rat ovarian IL-1α gene expression (in vivo) in untreated whole ovarian material using an RNase protection assay proved unsuccessful (Kol et al. unpublished observations). These findings and the preceeding RT-PCR observations suggested that, in relative terms, untreated whole ovarian tissue is not a major site of IL-1α gene expression under in vivo conditions. To determine whether ovarian IL-1α gene expression occurs in vitro, whole ovarian dispersates from immature rats were cultured for the duration indicated (up to 48 h) in the absence or presence of the indicated treatment. At the conclusion of the culture period, total cellular RNA was extracted and subjected to an RNase protection assay using antisense riboprobes corresponding to rat IL-1α and RPL19. As shown in Fig. 2 (upper left), no IL-1α signal was noted at the time of plating (time 0). Thereafter, transcripts corresponding to IL-1α increased spontaneously and rapidly to reach a 4-h peak (sixfold increase over time 0, $p < 0.05$) followed by a gradual decline to a 48-h nadir.

Given the apparent ability of human chorionic gonadotropin (hCG) to increase human ovarian IL-1α gene expression under in vivo circumstances *(7)*, the possibility of a comparable in vitro effect in the rat was evaluated. As shown in Fig. 2 (upper right), treatment with hCG was without significant effect on IL-1α expression inclusive of the early (4-h) peak (3.7-fold increase over time 0, $p < 0.05$), suggesting hCG independence. However, note that there was an increase at the 2-h time point for which statistical significance could not be documented.

Given the apparent concordance in the temporal expression patterns of human intraovarian IL-1α and IL-1β *(7)*, we also assessed whether there was a possible effect of IL-1β on IL-1α gene expression. As shown in Fig. 2 (lower left), treatment with IL-1β was without significant effect on the spontaneous early (4-h) IL-1α peak. Modest suppression of IL-1α

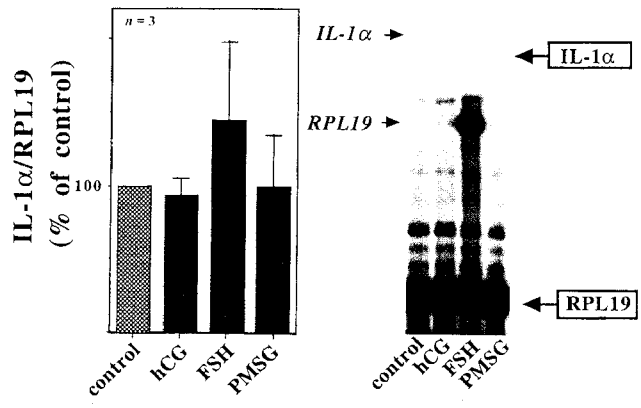

Fig. 1. RT-PCR analysis of IL-1α and IL-1β mRNA expression in the rat ovary. Ovaries were obtained from unstimulated 25-d-old immature female rats (control lane), PMSG (15 IU) injected (PMSG 48-h lane), or PMSG (15 IU) followed by hCG (15 IU) 48 h later (2-h and 48-h, post hCG lanes). RNA was extracted and reverse transcribed into cDNA. The different cDNA populations were amplified for 35 cycles with the appropriate primer pairs. Ten microliters of the PCR reactions were electrophoresed in 1.8% agarose gels. Gels were visualized with ethidium bromide, and the image was captured with a gel documentation system. The amplified products for IL-1α and IL-1β yielded 623- and 375-bp fragments lipopolysaccharide, respectively. Positive controls included speenic tissue obtained from rats treated with (+ control lane) and ovarian dispersate cell cultures treated with IL-1α (IL-1 TX lane). A negative control (H_2O lane) was also included.

transcripts at the early time points did not reach statistical significance. However, treatment with IL-1β reversed the subsequent decline of IL-1α transcripts, resulting in a secondary IL-1α peak at 48 h (20-fold increase over time 0; $p < 0.05$). These data suggest that subject to the limitations of the RNase protection assay, the transcriptional effects of exogenously provided IL-1β become apparent ≤20 h into the experiment.

To explore the possibility that the early spontaneous (4-h) increase in IL-1α gene expression (Fig. 2 (upper left) may be mediated by endogenously produced IL-1, whole ovarian dispersates were cultured for the duration indicated (up to 48 h) in the presence of IL-1RA (5μg/mL), thereby creating an "vacuum." As shown in Fig. 2 (lower right), treatment with IL-1RA was without significant effect on the early (4-h) peak of IL-1α expression (2.5-fold increase over time 0, $p < 0.05$), confirming IL-1 independence.

IL-1 Receptor Mediation in IL-1α Gene Expression by Cultured Whole Ovarian Dispersates

To evaluate the possibility that the long-term IL-1 effect is receptor mediated, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (10 ng/mL), with or without human recombinant IL-1RA (5 μg/mL). As shown in Fig. 3, treatment with IL-1β produced a 100-fold increase in the relative expression of IL-1α transcripts over untreated

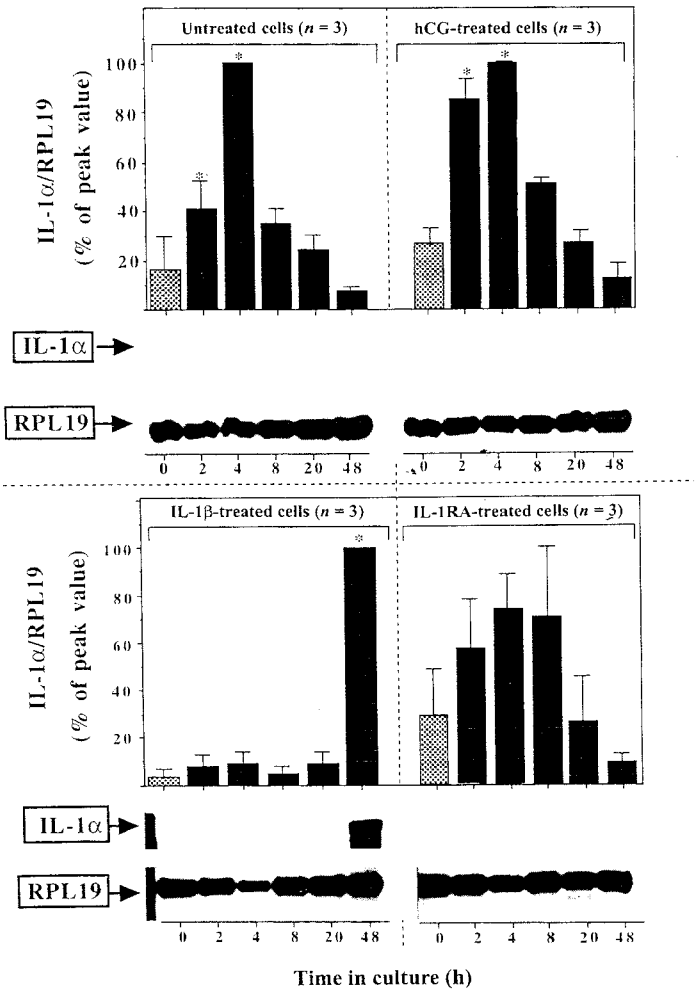

Fig. 2. Time-, hCG-, and IL-1 dependence of IL-1α gene expression by cultured whole ovarian dispersates. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for the duration indicated (up to 48 h) in the absence or presence of the indicated treatments. Total cellular RNA was extracted and subjected to an RNase protection assay using [^{32}P]-labeled antisense riboprobes corresponding to rat IL-1α and RPL19. The intensity of the signals was quantified as described. Data depicted in bar graph form the mean ± SE of three experiments carried out without treatment **(upper left)**, with IL-1β (50 ng/mL) **(lower left)**, with IL-1RA (5 μg/mg) **(lower right)**, or with hCG (10 ng/mL) **(upper right)**. Protected fragments are set in bold face letters. In each experiment, data were normalized to peak value. A representative autoradiograph is shown below each bar graph. *,=$p < 0.05$ vs time 0.

controls ($p < 0.005$). Most important, this effect was completely reversed by IL-1RA. These observations support the view that the ability of IL-1β to upregulate ovarian IL-1α transcripts is IL-1R mediated.

NO Dependence in IL-1α Gene Expression by Cultured Whole Ovarian Dispersates

To determine whether the ability of IL-1β to upregulate IL-1α gene expression is contingent on endogenously produced nitric oxide, whole ovarian dispersates were cultured for 48 h in the absence or presence IL-1β (10 ng/mL), with or without aminoguanidine hemisulfate salt (AG) (0.4 m*M*), thereby creating an NO "vacuum" *(20–23)*. As shown in Fig. 4, treatment with AG did not affect the basal or the IL-1β-induced expression of IL-1α. These observations suggest that the ability of IL-1β to upregulate IL-1α expression is nitric oxide-independent.

Eicosanoid Dependence in IL-1β-Induced IL-1α Gene Expression by Cultured Whole Ovarian Dispersates

To establish whether the effective treatment with IL-1β on IL-1α gene expression involves the intermediacy of eicosanoids, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (10 ng/mL), with or without indomethacin (10 μg/mL). As shown in Fig. 5, treatment with IL-1β produced a significant ($p < 0.005$) increase in ovarian IL-1α expression. Treatment with indomethacin by itself proved ineffective when compared with untreated controls. By contrast, concomitant treatment with both IL-1β and indomethacin resulted in significant ($p < 0.05$) inhibition of the ability of IL-1β to upregulate ovarian IL-1α expression. These observations suggest that the long-term stimulatory effect of IL-1β on ovarian IL-1α expression is eicosanoid dependent.

Protein Synthesis Dependence in IL-1β-Induced IL-1α Gene Expression by Cultured Whole Ovarian Dispersates

To determine whether the ability of IL-1β to upregulate IL-1α gene expression is contingent on intact cellular protein synthesis, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (10 ng/mL), with or without cycloheximide (CHX) (0.1 μg/mL). As shown in Fig. 6, treatment with IL-1β produced a marked (11.5-fold) increase in IL-1α transcripts as compared with control ($p < 0.001$). Cotreatment with CHX led to complete blockade of the ability of IL-1β to upregulate IL-1α transcripts. Most important, RPL19 transcripts remained unaffected, thereby arguing against an overt general toxic effect of CHX on the cellular transcription system. These observations suggest that the ability of IL-1β to induce IL-1α gene expression requires *de novo* protein biosynthesis.

Specificity Studies of IL-1α Gene Expression by Cultured Whole Ovarian Dispersates

To determine the effect of ovarian agonists other than IL-1β on IL-1α expression, whole ovarian dispersates were cultured for 48 h in the absence or presence of tumor necrosis factor-α (TNFα) (30 ng/mL), insulin-like growth factor-I(IGF-I) (50 ng/mL), activin-A (50 ng/mL), transforming growth factor-α (TGF-α) (10 ng/mL), or endothelin-1 (ET-1, 10^{-8} *M*). As shown in Fig. 7, none of these agonists affected IL-1α expression. These observations suggest that the in vitro ability of IL-1β to upregulate IL-1α transcripts is relatively

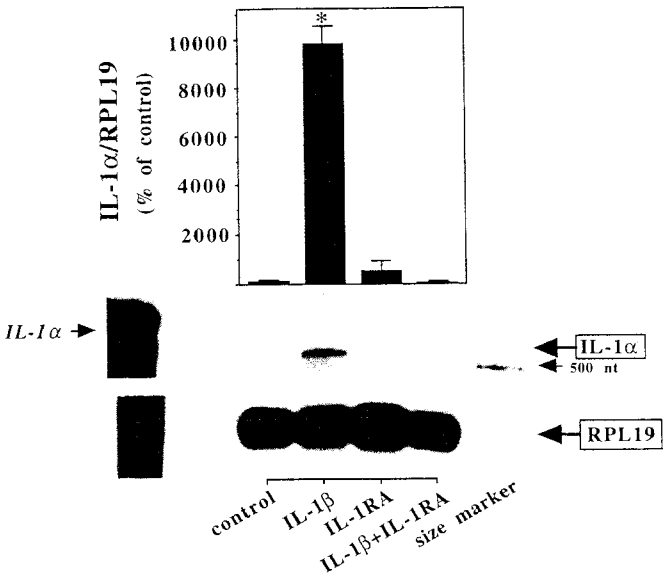

Fig. 3. IL-1 receptor mediation in IL-1α gene expression by cultured whole ovarian dispersates. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in the absence or presence of IL-1β (10 ng/mL), with or without IL-1RA (5 μg/mL). The resultant RNA samples were subjected to an RNase protection assay using [^{32}P]-labeled antisense riboprobes corresponding to rat IL-1α and RPL19. The intensity of the signals was quantified as described. **(Top)** In bar graph form the mean ± SE of three experiments. In each experiment, data were normalized to control. **(Bottom)** Representative autoradiograph. The full-length IL-1α probe is set in italics. Protected fragments corresponding to rat IL-1α and RPL19 are set in bold letters.

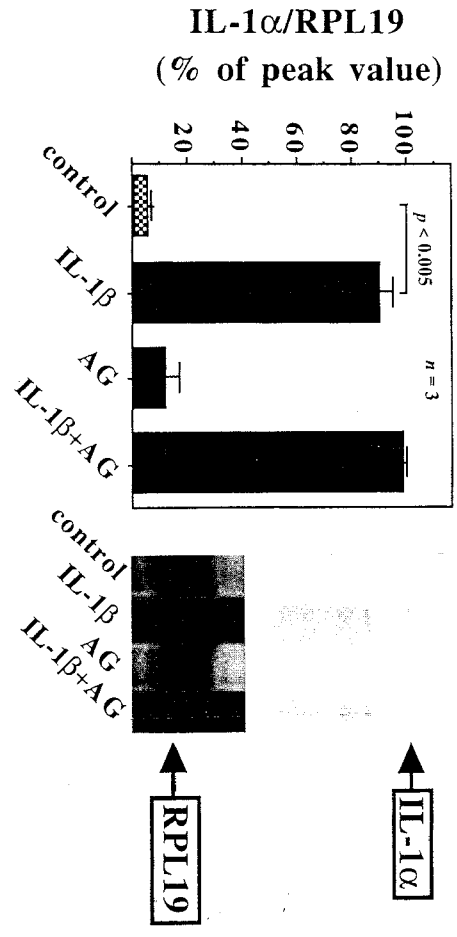

Fig. 4. NO dependence in IL-1α gene expression by cultured whole ovarian dispersates. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in the absence or presence of IL-1β (50 ng/mL), with or without AG (0.4 m*M*). The resultant RNA samples were subjected to an RNase protection assay using [^{32}P]-labeled antisense riboprobes corresponding to rat IL-1α and RPL19. The intensity of the signals was quantified as described. **(Left)** In bar graph form the mean ± SE of three experiments. In each experiment, data were normalized to the peak value. **(Right)** Representative autoradiograph. The protected fragments for IL-1α and RPL19 are set in bold letters.

specific in that it is not reproduced by a select group of granulosa cell effectors.

Discussion

Initial efforts to establish, by RNase protection assays, the ovarian expression of IL-1α revealed that, as compared with other tissues, intact whole ovarian material from untreated immature rats is not a major site of IL-1α gene expression (data not shown). It is likely that the latter observation reflects the highly compartmentalized nature of ovarian IL-1α expression. However, the use of RT-PCR technology proved equally negative (Fig. 1). Interestingly, in vitro IL-1a expression by untreated and IL-1-treated cultured whole ovarian dispersates proved quite pronounced when probed by the same RNase protection technology (Figs. 2–7).

Note, however, that there was a 4-h "burst" of IL-1α transcripts in cultured whole ovarian dispersates (Fig. 2). This reproducible IL-1/hCG-independent phenomenon has previously been documented for IL-1β, type I IL-1 receptor, and IL-1RA transcripts (Kol et al., unpublished observations). Although the precise molecular and cellular mechanism(s) underlying this phenomenon remain unknown, it is tempting to speculate that the dispersion and plating of ovarian cells may constitute a self-limited injurious process leading to an adaptational response akin to inflammation. To the extent that ovulation constitutes an inflammatory-like process *(29)*, the interim burst in the expression of IL-1α may represent a crude simulation of intraovarian events reminiscent of ovulation. Although the dependence of IL-1α on IL-1 has been demonstrated in several extraovarian sites *(30)*, the ability of IL-1β to modulate the expression of this gene in the context of the ovary has not been investigated *(1)*. Our present studies (Figs. 2–7) document the ability of IL-1β to produce marked increments in IL-1α transcripts as assessed in cultured whole ovarian dispersates from immature rats. The documentation of autocrine amplification of IL-1 expression is in keeping with the possibility that IL-1α may partake in the exponential process triggered by the gonadotropin surge.

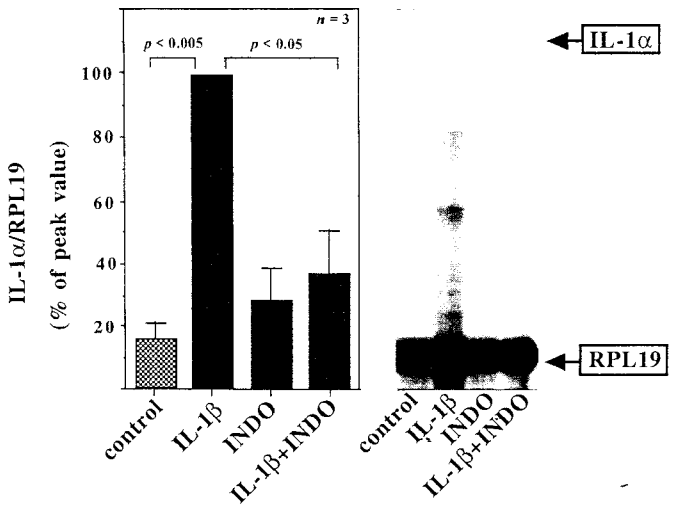

Fig. 5. Eicosanoid-dependence in IL-1β-induced IL-1α gene expression by cultured whole ovarian dispersates. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured in the absence or presence of IL-1β (10 ng/mL), with or without indomethacin (INDO) (10 μg/mL). The resultant RNA samples were subjected to an RNase protection assay using [^{32}P]labeled antisense riboprobes corresponding to rat IL-1α and RPL19. The intensity of the signals was quantified as described. **(Left)** In bar graph form the mean ± SE of three experiments. In each experiment, data were normalized to peak value. **(Right)** Representative autoradiograph. The protected fragments are depicted in bold letters.

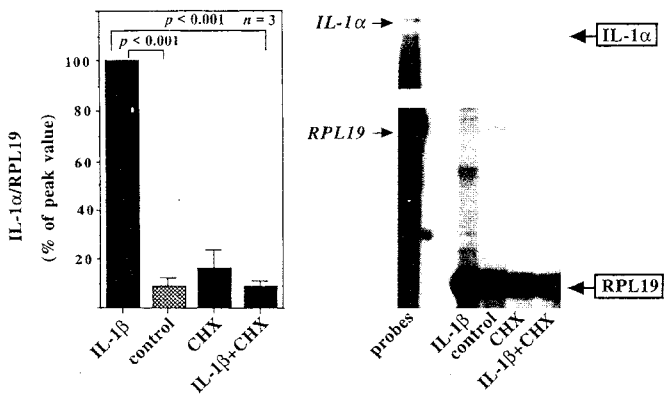

Fig. 6. Protein synthesis dependence in IL-1α gene expression by cultured whole ovarian dispersates. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in the absence or presence of IL-1β (50 ng/mL), with or without CHX (0.1 μg/mL). The resultant RNA samples were subjected to an RNase protection assay using [^{32}P]-labeled antisense riboprobes corresponding to rat IL-1α and RPL19. The intensity of the signals was quantified as described. **(Left)** In bar graph form the mean ± SE of three experiments. In each experiment, data were normalized to peak value. **(Right)** Representative autoradiograph. The protected fragments are set in bold letters. The full-length riboprobes are set in italics.

The IL-1β effect appeared to be receptor-mediated in that the concurrent presence of IL-1RA abolished the IL-1β effect (Fig. 3). Presumably, only the type I IL-1R was involved *(31)* since the type II receptor does not appear to engage in signaling but rather serves as a stationary binding protein or "decoy" receptor *(32)*. Moreover, ovarian transcripts corresponding to the type I IL-1R are more abundant than those for the type II receptor *(12)*. Taken together, these observations support the view that ovarian IL-1α transcripts are IL-1 dependent and that the IL-1 effect under study is receptor mediated. These findings establish IL-1α as yet another ovarian "end point" of IL-1 apart from the induction of prostaglandin biosynthesis *(14,15)*, the promotion of proteoglycan formation *(16)*, the induction of collagenanse activity *(17)*, the modulation of plasminogen activator activity *(18,19)*, the activation of NO synthase *(20–23)*, and the arrest of follicular atresia *(24)*.

In an effort to characterize better the IL-1 effect, we set out to examine the possible intermediary role of NO. Although the role of NO in ovarian physiology remains to be identified, relevance to the ovulatory process has been proposed *(33)*. Given the proposed role of IL-1 in the ovulatory process *(1–5,10)* and its established ability to activate the inducible variety of ovarian NO synthesis*(20–23)*, we set out to examine the role of NO in IL-1 hormonal action. To this end use was made of amino guanidine (AG), an established inhibitor of the inducible variety of ovarian NO synthase *(20)*. As shown in Fig. 4, the generation of a NO "vacuum" was without significant effect on the ability of IL-1β to upregulate IL-1α transcripts. In fact, the steady-state levels of IL-1α transcripts increased with AG treatment, suggesting a negative feedback effect of NO. However, the latter effect was not statistically significant. Consequently, the ability of IL-1 to upregulate IL-1α transcripts appears to be NO independent. Conceivably, then, the IL-1 effect may be mediated through the activation of the sphingomeyelin-ceramide *(34,35)* pathway, whose potential role in the transduction of the IL-1 signal is currently under active investigation. By contrast (Fig. 5), the IL-1 effect entailed activation of the eicosanoid pathway *(14,15)*. Irrespective of the transduction pathway, the IL-1 effect proved specific (Fig. 7) and contingent on intact cellular protein synthesis (Fig. 7). The nature of the intermediary obligatory protein(s) remains unknown.

Materials and Methods

Animals

Immature Sprague-Dawley female rats from Zivic-Miller (Zelienople, PA) were sacrificed by CO_2 asphyxiation on d 25 of life. The project was approved by the Institutional Animal Care and Use Committee.

Hormones and Reagents

Recombinant human IL-1β (2×10^7 U/mg) was provided by Drs. Errol B. De Souza and C. E. Newton, DuPont-Merck Pharmaceutical (Wilmington, DE). A recombinantly expressed

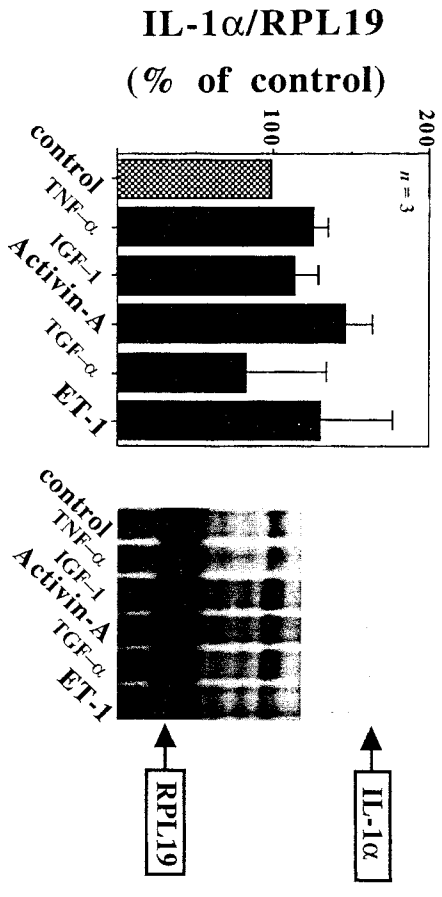

Fig. 7. Specificity studies of IL-1α gene expression by cultured whole ovarian dispersates. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in the presence of TNF-α (30 ng/mL), IGF-1 (50 ng/mL), activin-A (50 ng/mL), TGF-α (10 ng/mL), or ET-1 ($10^{-8}M$). The resultant RNA samples were subjected to an RNase protection assay using [^{32}P]-labeled antisense riboprobes corresponding to rat IL-1α and RPL19. The intensity of the signals was quantified as described. **(Left)** In bar graph form the mean ± SE of three experiments. In each experiment, data were normalized to control. **(Right)** Representative autoradiograph. The protected fragments are set in bold letters.

preparation of the naturally occurring human IL-1RA was provided by Dr. Daniel E. Tracey, Upjohn (Kalamazoo, MI). Highly purified hCG (CR-127, 14,900 IU/mg) was provided by Dr. R. E. Canfield through the Center for Population Research, NICHHD, National Institues of Health (Bethesda, MD). Activin-A was a gift from Dr. Jennie Mather, Genentech (South San Francisco, CA). ET-1 was from Peninsula (Belmont, CA), and TGFα was from Oncogene Science (Uniondale, NY). TNFα was a generous gift from Dr. Dawson, National Biological Standard Board (Hertfordshire, UK). IGF-1 was from Bachem (Torrance, CA).

McCoy's 5a medium (serum-free), penicillin-streptomycin solution, L-glutamine, trypan blue stain, and bovine serum albumin were from Life Technologies (Grand Island, NY). Collagenase (*Clostridium histolyticum*; CLS type I; 144 U/mg) was from Worthington (Freehold, NJ). DNase (bovine pancreas), AG, RNase A, indomethacin, and CHX were from Sigma (St. Louis, MO). RNase T1 was from Pharmacia (Piscataway, NJ). pGEM4Z and other molecular grade reagents were from Promega (Madison, WI).

Tissue Culture Procedures

Whole ovarian dispersates were prepared and cultured as previously described *(36)*.

Nucleic Acid Probes

A rat IL-1α cDNA was generously provided by Dr. A. Shaw of Glaxo (Geneva, Switzerland). To generate a suitable riboprobe, an *XbaI* to *Pst*I fragment of the cDNA was subcloned into pGEM4Z. The latter plasmid was linearized with *Eco*RI and transcribed with T7 RNA polymerase to yield a 565-nt antisense riboprobe, which on hybridization was projected to generate a 522-nt protected fragment.

The ribosomal protein L19 (RPL19) probe was generated as previously described *(12)*. The construct was transcribed with T7 RNA polymerase to generate a 214-nt probe capable of protecting a 153-nt mRNA segment.

RNA Extraction

RNA of cultured cells was extracted with RNAZOL-B (Tel Test, Friendswood, TX) according to the manufacturer's protocol.

RNase Protection Assay

Linearized DNA templates were transcribed with T7 RNA polymerase to specific activities of 800 Ci/mmol [α-^{32}P]UTP (IL-1α) or 160 Ci/mmol [α-^{32}P]UTP (RPL19). The riboprobes were gel purified as previously described *(37)* in an effort to eliminate transcribed products shorter than the full-length probes. The assay was performed as previously described *(38)*. Gels were exposed to XAR film (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 mRNA signal was used to normalize the IL-1α mRNA data for possible variation in RNA loads. Specifically, the net protected IL-1α signal (respective background subtracted) to net RPL19 signal ratio was calculated for each sample.

Reverse Transcriptase Polymerase Chain Reaction

Total cellular RNA was extracted by the SV Total RNA Isolation System (Promega). First-strand complementary DNA was synthesized from 1 μg total RNA using 200 U of Moloney murine leukemia virus RT (Life Technologies, Gaithersburg, MD) for 1 h at 37°C after oligo (deoxythymidine) priming. Aliquots of the first-strand reaction (1 μL) were used as templates for subsequent PCR reactions. For detection of IL-1α transcripts, the following primers were used:

1. Forward: 5'-CTAAGAACTACTTCACACCGCA-3'

2. Reverse: 5'-CTGGAATAAAACCCACTGAGGTAGG-3'

The IL-1β primer sequences were as follows:

1. Forward: 5'-CTTTCGACAGTGAGGAGAATGACC-3'
2. Reverse: 5'-CAGACAGCACGAGGCATTTTTG-3'

Each PCR reaction mixture (25 µL) contained 0.2 µ*M* of each primer, 0.75 U of *Taq* DNA Polymerase (Perkin-Elmer, Norwalk, CT), 1.5 m*M* $MgCl_2$, 0.2 m*M* dNTP, and 10X reaction buffer. Amplification was performed under the following conditions: 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min. After the final amplification, the reaction was elongated for 7 min at 72°C. PCR products (10 µL) were electrophoresed in 1.8% agarose gels containing ethidium bromide, visualized with ultraviolet light, and digitally photographed with a gel documentation system (Bio-Rad, Hercules, CA). The identities of positive bands were confirmed by direct sequencing of the PCR products.

The PCR products were 623 and 375 bp for IL-1α and IL-1β, respectively. A positive control was derived from spleen tissue samples obtained from rats treated with lipopolysaccharide (Sigma) intraperitoneally.

Data Analysis

Except as noted, each experiment was replicated a minimum of three times. Data points are presented as mean ± SE. Data were expressed in terms of percentage of peak value. The reference point represents the peak value in each figure. Statistical significance (Fisher's Protected Least Significance Difference) was determined by analysis of variance and student's t-test. Statistical values were calculated using Statview 512+ for MacIntosh (Brain Power, Calabasas, CA).

Acknowledgments

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