ELSEVIER

Journal of Reproductive Immunology
45 (1999) 67–79

JOURNAL OF REPRODUCTIVE IMMUNOLOGY

www.elsevier.com/locate/jreprimm

Expression and hormonal regulation of rat ovarian interleukin-1β converting enzyme, a putative apoptotic marker: endocrine- and paracrine-dependence

Minoru Irahara [1], Motomu Ando [2], Shahar Kol [3], Eli Y. Adashi *

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

Received 24 April 1998; received in revised form 20 May 1998; accepted 3 March 1999

Abstract

It is the purpose of this paper to assess the expression, cellular localization, and hormonal regulation of rat ovarian interleukin (IL)-1β converting enzyme (ICE), a putative apoptotic marker. In agreement with previous observations ICE transcripts were noted in relatively increased abundance in the thymus, lung, spleen and small intestine. Although ICE transcripts were barely expressed in the untreated, immature rat ovary, they were apparent throughout a simulated estrous cycle. The in vivo expression of ovarian ICE rose gradually from 6 h after ovulation triggering to a peak (1.74-fold increase versus control, $P < 0.05$) 24 h after human chorionic gonadotropin administration, a marked and significant decrease to baseline being noted 24 h later. To examine the effect of in vitro culture on ovarian ICE gene expression, whole ovarian dispersates from immature rats were cultured without treatment for 72 h. ICE gene expression significantly ($P < 0.01$) increased to a maximum 24 h post

* Corresponding author. Present address: Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Utah Health Sciences Center, ARUP II, Mailbox No. 20, Suite 1100, Room No. 109, 546 Chipeta Way, Salt Lake City, UT 84108, USA. Tel.: + 1-801-585-9250; fax: + 1-801-585-9256.

E-mail address: eadashi@hsc.utah.edu (E.Y. Adashi)

[1] Present address: Department of Obstetrics and Gynecology, The University of Tokushima School of Medicine, Tokushima City, Tokushima 770, Japan.

[2] Present address: Department of Obstetrics and Gynecology, Kyorin University, School of Medicine, Tokyo 181, Japan.

[3] Present address: Department of Obstetrics and Gynecology, Rambam Medical Center, Haifa, Israel.

PII: S0165-0378(99)00019-4

plating (2.55-fold increase as compared with time zero). Treatment with IL-1β was associated with a small but statistically insignificant increase in ovarian ICE gene expression. Similarly, provision of IL-RA resulted in a modest, albeit statistically insignificant, decrease in ovarian ICE gene expression. Treatment with GnRH (but not FSH, LH or PMSG) significantly ($P < 0.05$) increased ovarian ICE gene expression (41.5% increase versus control). Treatment with dexamethasone (but not diethylstilbestrol, R5020 or R1881) produced a significant ($P < 0.05$) 42.3% decrease in ovarian ICE gene expression as compared with untreated controls. Treatment with TNFα (but not ET-1, TGFα, TGFβ, IGF-I or bFGF) produced a significant ($P < 0.01$) 2.5-fold increase in ovarian ICE gene expression as compared with untreated controls. Taken together, our present findings: (1) reaffirm the ovarian expression of the ICE gene, (2) document a periovulatory increase in ovarian ICE gene expression, (3) show the inhibitory effect of glucocorticoids in this regard, and (4) establish TNFα as an upregulator. Taken together, these findings suggest a role for ovarian ICE either in the context of apoptosis/atresia or in the context of the ovulatory process.

Keywords: Rat ovarian interleukin-1β converting enzyme; Apoptotic marker; Endocrine-dependence; Paracrine-dependence

1. Introduction

Interleukin (IL)-1β converting enzyme (ICE) is a member of a growing family of cysteine proteases (caspases) with a substrate specificity for aspartate (Cerretti et al., 1992; Thornberry et al., 1992). ICE (caspase-1) was originally discovered by virtue of its ability to cleave the inactive IL-1β precursor to the 17 kDa mature protein (Black et al., 1988). Expectedly, ICE-deficient mice proved incapable of producing mature IL-1β thereby confirming the physiological role of ICE in both the processing and export of IL-1β (Kuida et al., 1995). In addition, ICE may play additional roles in the regulation of the immune system in that ICE-deficient mice are likewise IL-1α-, TNFα-, and IL-6-poor while displaying significant resistance to endotoxin-induced septic shock (Li et al., 1995). A role for ICE in apoptosis is also the subject of intense investigation (Miura et al., 1993; Boudreau et al., 1995; Enari et al., 1995; Los et al., 1995).

Flaws et al. (1995) examined the role of ICE-related proteases at the level of the ovarian follicle. These observations suggested that the expression of ICE-related proteases, but not of ICE per se, is downregulated in the context of gonadotropin-supported follicular survival. It was likewise suggested that the activity of ICE-related proteases may be involved in the activation of oligonucleosomal endonucleases, an event that could be associated with morphological indexes of apoptosis in granulosa cells during atresia. More recently, Rueda et al. (1997) observed increased ICE transcript levels in the regressed corpus luteum thereby providing the first

evidence that the ICE family of death proteases may be involved in luteolysis. It was the purpose of this communication to further assess the expression and hormonal regulation of rat ovarian ICE, a putative apoptotic marker.

2. Materials and methods

2.1. Animals

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

2.2. Hormones and reagents

Pregnant mares' serum gonadotropin (PMSG, 2100 IU/mg), phorbol myristate acetate (PMA), diethylstilbestrol (DES) and dexamethasone (DEX) were obtained from Sigma (St. Louis, MO). Highly-purified human chorionic gonadotropin (hCG; CR-127, 14 900 IU/mg) was generously provided by Dr R.E. Canfield through the Center for Population Research, NICHHD, NIH (Bethesda, MD). Ovine FSH (USDA-o-FSH-18, 65 U/mg), ovine LH (NIADDK-oLH-25, 2.3 U/mg) and ovine prolactin (NIADDK-oPrl-20, 31 IU/mg) were provided by NIADDK, NIH (Bethesda, MD). GnRH was from Peninsula Laboratories (Belmont, CA). 17,21-Dimetyl-19-nor-pregna-4,9-diene-3,20-dione (R5020), and methyltrienolone (R1881) were from Roussel-UCLAF (Romaineville, France).

Recombinant human IL-1β (2×10^7 U/mg) was generously provided by Drs Errol B. De Souza and C.E. Newton, DuPont-Merck Pharmaceutical (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, Upjohn (Kalamazoo, MI). Endothelin-1 (ET-1) was from Peninsula Laboratories (Belmont, CA). Recombinant human tumor necrosis factor-α (TNFα, 5×10^7 U/ml) was from Dr H.M. Shepard, Genentech (South San Francisco, CA). Highly-purified bovine acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) were the generous gift of Dr Denis Gospodarowicz, University of California at San Francisco (San Francisco, CA). Transforming growth factor α (TGFα) and transforming growth factor β_1 ($TGF\beta_1$) were from Oncogene Science (Uniondale, NY). Insulin-like growth factor-I (IGF-I) was from Bachem (Torrance, CA). Epidermal growth factor (EGF) was from Collaborative Biomedical Products (Bedford, MA). Leukemia in-

hibitory factor (LIF), keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF) were from PeproTech (Rocky Hill, NJ).

McCoy's 5a medium (serum-free), penicillin–streptomycin solution, L-glutamine, trypan blue stain and bovine serum albumin (BSA) were from Gibco/BRL Life Technologies (Grand Island, NY). Collagenase (*Clostridium histolyticum*; CLS type I; 144 U/mg) was from Worthington Biochemical (Freehold, NJ). DNAse (bovine pancreas), and RNase A were from Sigma. T7 and T3 RNA polymerases, and other molecular biology grade reagents were from Promega (Madison, WI). [^{32}P]UTP was from New England Nuclear (Boston, MA).

2.3. In vivo treatment paradigm

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

2.4. Tissue culture procedures

Whole ovarian dispersates were prepared and cultured as previously described (Hurwitz et al., 1991).

2.5. Nucleic acid probes

The rat ICE cDNA was generously provided by Dr Karen M. Keane of Parke-Davis Pharmaceutical (Ann Arbor, MI). The original rat ICE cDNA (Keane et al., 1995) was inserted into a pBluescriptIIKS+ vector. T3-driven transcription of the StyI-linearized construct yielded a 396 nt riboprobe which upon hybridization was projected to generate a 384 nt protected fragment. The RPL19 probe was generated and employed as previously described (Scherzer et al., 1996).

2.6. RNA extraction

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

2.7. RNAse protection assay

Linearized DNA templates were transcribed with the appropriate RNA polymerase to specific activities of 800 Ci/mmol [α-^{32}P]UTP (ICE) and of 160 Ci/mmol [α-^{32}P]UTP (RPL19). The riboprobes were gel-purified as described by Kol et al. (1996) in an effort to eliminate transcribed products

shorter than the full length probes. The assay was performed as previously described (Lowe et al., 1987). The gels were exposed to XAR film (Kodak, Rochester, NY) for varying lengths of time with intensifying screens. To generate quantitative data, the gels were also exposed to a phosphor screen (Molecular Dynamics, Sunnyvale, CA). The resultant digitized data were analyzed with Image Quant Software (Molecular Dynamics, Sunnyvale, CA). The hormonally-independent RPL19 mRNA signal was used to normalize the ICE data for possible variation in RNA loads. Specifically, the net protected signal (respective background subtracted) to net RPL19 ratio was calculated for each sample and gene of interest.

2.8. Data analysis

Except as noted, each experiment was replicated a minimum of three times. Data points are presented as mean $\pm$ S.E. Statistical significance (Fisher's protected least significance difference) was determined by ANOVA and Student's t-test. Statistical values were calculated using Statview 512 + for MacIntosh (Brain Power, Calabasas, CA).

3. Results

3.1. Tissue distribution of ICE transcripts in the immature rat

To further validate the ICE constructs described in Section 2, total RNA (20 mg) samples from various tissues of immature untreated rats were subjected to an RNase protection assay with an antisense riboprobe corresponding to rat ICE. As shown in Fig. 1, and in agreement with previous observations (Keane et al., 1995), protected fragments corresponding to ICE transcripts were noted in relatively increased abundance in the thymus, lung, spleen and small intestine. ICE transcripts, in turn, were noted in decreasing abundance in the heart, kidney, brain, liver, uterus and muscle. ICE transcripts were minimally expressed in the untreated immature rat ovary. These findings suggest that, in relative terms, untreated whole ovarian tissue is not a major site of ICE gene expression.

3.2. Ovarian ICE gene expression in vivo: effects of follicular maturation, ovulation and corpus luteum formation

To assess ICE gene expression in the course of a simulated estrous cycle, 25 day old rats were initially primed with PMSG (15 IU). Ovulation was triggered 48 h later with hCG (15 IU). The animals were sacrificed at the

indicated time points, the ovaries snap-frozen at −70°C, total RNA was extracted and subjected to a solution hybridization RNase protection assay with antisense riboprobes corresponding to rat ICE and RPL19. As shown in Fig. 2, protected fragments corresponding to ICE transcripts were apparent throughout the experiment. The in vivo expression of ovarian ICE rose gradually from 6 h after hCG administration to a peak (1.74-fold increase versus control, $P < 0.05$) at a point 24 h after hCG triggering. A marked significant ($P < 0.01$) decrease to baseline was noted 24 h later.

3.3. ICE gene expression by cultured whole ovarian dispersates: time-dependence

To examine the effect of in vitro culture on ovarian ICE gene expression, whole ovarian dispersates from immature rats were cultured without treatment for up to 72 h. ICE gene expression increased significantly ($P < 0.01$) to a maximum at a point 24 h post plating (2.55-fold increase as compared with time zero). No further increments were noted thereafter.

3.4. ICE gene expression by cultured whole ovarian dispersates: IL-1-independence

To explore the possibility of IL-1-dependent ovarian ICE gene expression, whole ovarian dispersates were cultured for 48 h in the absence or

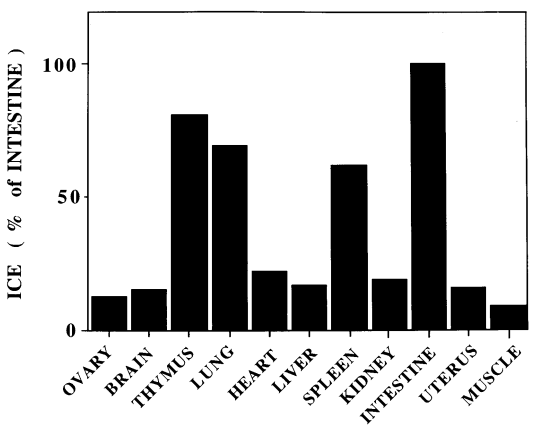

Fig. 1. Tissue distribution of ICE transcripts in the immature rat. Total RNA (20 μg), extracted from the indicated tissues of immature untreated rats, was subjected to an RNase protection assay using an antisense riboprobe corresponding to rat ICE. The intensity of the signals was quantified as described in the text and normalized relative to the signal generated by ICE transcripts in the intestine.

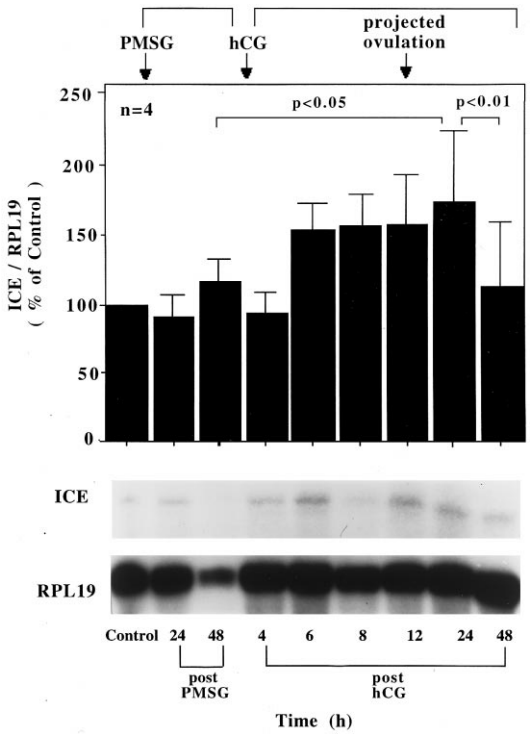

Fig. 2. Ovarian ICE gene expression in vivo: effects of follicular maturation, ovulation and corpus luteum formation. Immature rats were initially primed with PMSG (15 IU). Ovulation was triggered 48 h later with hCG (15 IU). The animals were sacrificed at the indicated time points, the ovaries snap-frozen at $-70°C$, total RNA extracted and subjected to a solution hybridization RNase protection assay with antisense riboprobes corresponding to rat ICE and RPL19. The intensity of the signals was quantified as described in the text. The top panel depicts (in bar graph form) the mean $\pm$ S.E. of four experiments. The lower panels constitute representative autoradiographs. In each individual experiment data were normalized relative to the time zero (control) value.

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 was associated with a small but statistically insignificant increase in ovarian ICE gene expression. Similarly, provision of IL-RA resulted in a modest, albeit statistically insignificant, decrease in ovarian ICE gene expression.

3.5. ICE gene expression by cultured whole ovarian dispersates: effect of ovarian agonists or steroid hormones

Given the ability of hCG to increase ovarian ICE gene expression under in vivo circumstances (Fig. 2), the possibility of a comparable in vitro effect

was evaluated. Whole ovarian dispersates were cultured for 48 h in the absence or presence of several ovarian agonists (including hCG) or steroid hormones. All ovarian agonists with the exception of GnRH were without significant effect on ovarian ICE gene expression. Treatment with GnRH (1 μg/ml) significantly ($P < 0.05$) increased ovarian ICE gene expression (1.42-fold increase versus control). In contrast, treatment with PMA, FSH, LH, PMSG, hCG, or PRL proved without effect. Similarly, all steroid hormones used with the exception of dexamethasone were without effect on ovarian ICE gene expression. Treatment with DEX (10^{-7} M) produced a significant ($P < 0.05$) 42.3% decrease in ovarian ICE gene expression as compared with untreated controls. In contrast, treatment with DES, R5020, or R1881 proved without effect.

3.6. ICE gene expression by cultured whole ovarian dispersates: effects of selected cytokines and growth factors

To determine the effect of treatment with selected multifunctional peptidergic regulators on ovarian ICE gene expression, whole ovarian disper-

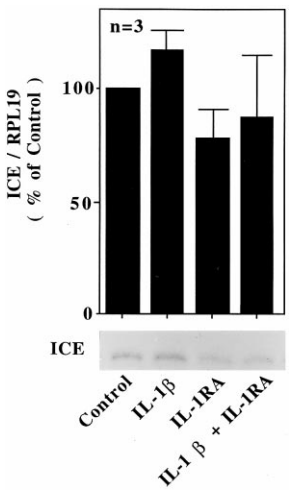

Fig. 3. ICE gene expression by cultured whole ovarian dispersates: IL-1-dependence. Whole ovarian dispersates (1.5×10^6 viable cells/dish) were cultured for 48 h in the absence or presence of IL-1β (10 ng/ml), with or without human recombinant IL-1 RA (5 μg/ml). The resultant RNA samples were subjected to an RNase protection assay using antisense riboprobes corresponding to rat ICE and RPL19. The intensity of the signals was quantified as described in the text. The top panel depicts (in bar graph form) the mean ± S.E. of three experiments. The lower panel constitutes a representative autoradiograph. In each individual experiment data were normalized relative to the control value. IL-1RA, interleukin-1 receptor antagonist.

sates were cultured for 48 h in the absence or presence the indicated agents. All agents tested with the exception of TNFα, were without effect on ovarian ICE gene expression. Treatment with TNFα (10 ng/ml) produced a significant ($P < 0.01$) 2.5-fold increase in ovarian ICE gene expression as compared with untreated controls. In contrast, treatment with IL-1β (Ben-Shlomo et al., 1997), ET-1, LPS, TGFα, TGFβ$_1$(Adashi et al., 1989), HGF, LIF, EGF, KGF, IGF-I (Adashi et al., 1990), aFGF (Hurwitz et al., 1990) or bFGF (Hurwitz et al., 1990) proved without effect.

4. Discussion

The present study explored the expression and hormonal regulation of rat ovarian ICE. This work was prompted by the proposed role of IL-1 in ovulation (Ben-Shlomo and Adashi, 1994) and atresia (Chun et al., 1995; Billig et al., 1996, Hsueh et al., 1996; Kaipia et al., 1996; Schun et al., 1996; Kaipia and Hsueh, 1997) as well as the growing appreciation of the possible involvement of ICE in apoptosis (Miura et al., 1993; Boudreau et al., 1995; Enari et al., 1995; Los et al., 1995). In this context, note was made of the fact that in relative terms, the ovary is not a major site of ICE expression (Fig. 1). Moreover, ovarian ICE expression proved relatively constituitive, modulatory influences being limited to a number of select regulators. As such, these findings suggest a role for ICE in ovarian physiology albeit with a yet-to-be-assigned function.

One central finding of this paper concerns the apparent midcycle upregulation of ovarian ICE gene expression. Indeed, note was made of a substantial increase in ICE gene expression 24 h following the administration of hCG (Fig. 2). On the one hand, these findings are compatible with the view that IL-1 may play a role in the ovulatory process (Ben-Shlomo and Adashi, 1994). Indeed, several observations support the possibility of upregulation of intraovarian IL-1 at midcycle (Hurwitz et al., 1992a,b; Brannstrom et al., 1993; Peterson et al., 1993; Brannstrom et al., 1994; Simon et al., 1994a,b). On the other hand, assuming a role for ICE in apoptosis, these findings could be interpreted to suggest a marked increase in atresia at midcycle. Alternatively, one cannot rule out possible role(s) for ICE in the early phases of corpus luteum formation (Rueda et al., 1997). Irrespective of the precise role of ICE at midcycle, these observations document the endocrine-dependence of ovarian ICE gene expression implicating by extension the ICE gene in ovarian physiology.

In examining the in vitro regulation of ovarian ICE, note was made of an apparent constituitive increase in ICE expression 24 h after plating. It is quite possible that the latter phenomenon is attributable to the collagenase-

mediated dispersion and the process of plating, the temporary impact of which on cellular function is generally appreciated. However, once upregulated, ovarian ICE gene expression remained unchanged for the duration of the 72 h culture period. As such, these findings suggest a relatively steady level of ovarian ICE expression in vitro following the stabilization of the culture conditions.

To examine the possibility that ovarian ICE-expression may be IL-1-dependent, the effects of treatment with IL-1β or its antagonist were explored. Use of the latter was dictated by the desire to induce an IL-1 vacuum as previously shown in a similar paradigm (Kokia et al., 1994). The data suggest independence of either exogenous or endogenous IL-1 in that treatment with either IL-1β or IL-1RA was without effect on basal ovarian ICE gene expression. These findings argue against the possibility of a positive upregulatory IL-1-dependent loop designed to further enhance IL-1 generation by way of amplification of precursor conversion.

The in vitro examination of the effect of treatment with established ovarian agonists revealed the regulation of ovarian ICE gene expression to be highly selective. Indeed, a substantial array of peptidergic agonists, A- and C-kinase activators, and key steroid hormones proved without effect. Special mention is made of LH and hCG which in vitro appeared incapable of modulating the ovarian ICE gene in contradistinction to the in vivo paradigm (Fig. 2). It is highly likely that the inability of LH and hCG to effect in vitro upregulation of ovarian ICE gene expression may be attributable to the fundamental difference between the in vivo and in vitro paradigm. In addition, the whole ovarian dispersates under study were not FSH/PMSG-primed as was true of the in vivo paradigm.

The above notwithstanding, treatment of whole ovarian dispersates with DEX resulted in marked attenuation of ovarian ICE gene expression. DEX, an anti-inflammatory principle, has been shown in both ovarian (Irahara et al., unpublished observations) and extraovarian (Pfeilschifter and Schwarzenbach, 1990) settings to inhibit IL-1 action. By extending this inhibitory effect to the IL-1-generating ICE, DEX may accomplish a more complete blockade of the IL-1 effect and by extension of the inflammatory reaction. In contrast, treatment with TNFα, a pro-inflammatory cytokine, resulted in marked increments in ovarian ICE gene expression. It does appear likely that TNFα may engage in the upregulation of ovarian ICE expression in the interest of enhanced IL-1 generation and in the context of an inflammatory response. It is also possible that TNFα, by virtue of its apoptosis-promoting potential (Nagata, 1997) may be enhancing ovarian ICE gene expression in the context of its role as a putative apoptotic principle. The present data do not permit a meaningful distinction between the above possibilities.

Taken together, our present findings reaffirm the ovarian expression of the ICE gene, document a periovulatory increase in ovarian ICE gene expression, establish its IL-1-independence, show the inhibitory effect of glucocorticoids in this regard and establish GnRH and TNFα as upregulators. Taken together, these findings suggest a role for ovarian ICE either in the context of apoptosis/atresia or in the context of the ovulatory process.

Acknowledgements

The authors wish to thank Carol W. Resnick for her kind advice and assistance. We also wish to thank Linda Elder for dedicated assistance in the preparation of this manuscript. This work was supported in part by NIH grant HD-30288(EYA).

References

Adashi, E.Y., Hernandez, E.R., Resnick, C.E., Purchio, A.F., Twardzik, D.R., May, J.V., 1989. Ovarian transforming growth factor-β (TGFβ): sites(s) and mechanism(s) of action. Mol. Cell. Endocrinol. 61, 247–256.

Adashi, E.Y., Resnick, C.E., Rosenfeld, R.D., 1990. Insulin-like growth factor-I (IGF-I) and IGF-II hormonal action in cultured rat granulosa cells: mediation via type I but not type II IGF receptors. Endocrinology 126, 216–222.

Ben-Shlomo, I., Adashi, E.Y., 1994. Interleukin-1 as a mediator in the ovulatory sequence: evidence for a meaningful role of cytokines in ovarian physiology. Curr. Sci. Ser. 1, 187–192.

Ben-Shlomo, I., Kol, S., Roeder, L., et al., 1997. Interleukin (IL-1β) increases glucose uptake and induces glycolysis in aerobically-cultured rat ovarian cells: evidence that IL-1β may mediate the gonadotropin-induced midcycle 'metabolic shift'. Endocrinology 138, 2680–2688.

Billig, H., Chun, S.-Y., Eisenhauer, K., Hsueh, A.J., 1996. Gonadal cell apoptosis: hormone-regulated cell demise. Hum. Reprod. Update 2, 103–117.

Black, R.A., Kronheim, S.R., Cantrell, M., et al., 1988. Generation of biologically active interleukin-1β by proteolytic cleavage of the inactive precursor. J. Biol. Chem. 263, 9437–9442.

Boudreau, N., Sympson, C.J., Werb, Z., Bissel, M.J., 1995. Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. Science 267, 891–893.

Brannstrom, M., Wang, L., Norman, R.J., 1993. Ovulatory effect of interleukin-1 on the perfused rat ovary. Endocrinology 132, 399–404.

Brannstrom, M., Norman, R.J., Seamark, R.F., Robertson, S.A., 1994. Rat ovary produces cytokines during ovulation. Biol. Reprod. 50, 88–94.

Cerretti, D.P., Kozlosky, C.J.C., Nelson, B., et al., 1992. Molecular cloning of the interleukin-1β converting enzyme. Science 256, 97–99.

Chun, S.-Y., Eisenhauer, K.M., Kubo, M., Hsueh, A.J.W., 1995. Interleukin-1β suppresses apoptosis in rat ovarian follicles by increasing nitric oxide production. Endocrinology 136, 3120–3127.

Enari, M., Hug, H., Nagata, S., 1995. Involvement of an ICE-like protease in Fas-mediated apoptosis. Nature 375, 78–80.

Flaws, J.A., Kugu, K., Trbovich, A.M., et al., 1995. Interleukin-1β-converting enzyme-related proteases (IRPs) and mammalian cell death: dissociation of IRP-induced oligonucleosomal endonuclease activity from morphological apoptosis in granulosa cells of the ovarian follicle. Endocrinology 136, 5042–5053.

Hsueh, A.J.W., Eisenhauer, K., Chun, S.-Y., Hsu, S.-Y., Billig, H., 1996. Gonadal cell apoptosis. Recent Prog. Horm. Res. 51, 433–456.

Hurwitz, A., Hernandez, E.R., Resnick, C.E., Packman, J.N., Payne, D.W., Adashi, E.Y., 1990. Basic fibroblast growth factor (bFGF) inhibits gonadotropin-supported ovarian androgen biosynthesis: mechanism(s) and site(s) of action. Endocrinology 126, 3089–3096.

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

Hurwitz, A., Loukides, J., Ricciarelli, E., et al., 1992a. 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. 89, 1746–1754.

Hurwitz, A., Ricciarelli, E., Botero, L., Rohan, R.M., Hernandez, E.R., Adashi, E.Y., 1992b. Endocrine- and autocrine-mediated regulation of rat ovarian (theca-interstitial) interleukin-1β gene expression: gonadotropin-dependent preovulatory acquisition. Endocrinology 129, 3427–3429.

Kaipia, A., Hsueh, A.J.W., 1997. Regulation of ovarian follicle atresia. Annu. Rev. Physiol. 59, 349–363.

Kaipia, A., Chun, S.-Y., Eisenhauer, K., Hsueh, A.J.W., 1996. Tumor necrosis factor-α and its second messenger, ceramide, stimulate apoptosis in cultured ovarian follicles. Endocrinology 137, 4864–4870.

Keane, K.M., Giegel, D.A., Lipinski, W.J., Callahan, M.J., Shivers, B.D., 1995. Cloning, tissue expression and regulation of rat interleukin 1β converting enzyme. Cytokine 7, 105–110.

Kokia, E., Iben-Shlomo, I., Adashi, E.Y., 1994. The ovarian action of IL-1 is receptor-mediated: reversal by a naturally occurring interleukin-1 receptor antagonist. Fertil. Steril. 63, 176–181.

Kol, S., Ben-Shlomo, I., Adashi, E.Y., Rohan, R.M., 1996. Simplified riboprobe purification using translucent straws as gel tubes. Gen. Anal. Biomol. Eng. 12, 129–132.

Kuida, K., Lippke, J.A., Ku, G., et al., 1995. Altered cytokine export and apoptosis in mice deficient in interleukin-1β converting enzyme. Science 267, 2000–2003.

Li, P., Allen, H., Banerjee, S., et al., 1995. Mice deficient in IL-1β-converting enzyme are defective in production of mature IL-1β and resistant to endotoxic shock. Cell 80, 401–411.

Los, M., Craen, M.V., Penning, L.C., et al., 1995. Requirement of ICE/CED-3 protease for FAS/APO-1-mediated apoptosis. Nature 375, 81–83.

Lowe, W.L., Roberts, C.T., Lasky, S.R., LeRoith, D., 1987. Differential expression of alternative 5′ untranslated regions in mRNA encoding rat insulin-like growth factor 1. Proc. Natl. Acad. Sci. USA 84, 8946–8950.

Miura, M., Zhu, H., Rotello, R., Hartwieg, E.A., Yuan, J., 1993. Induction of apoptosis in fibroblasts by IL-1β converting enzyme, a mammalian homolog of the *C. elegans* cell death gene ced-3. Cell 75, 653–660.

Nagata, S., 1997. Apoptosis by death factor. Cell 88, 355–365.

Peterson, C.M., Hales, H.A., Hatasaka, H.H., Mitchell, M.D., Rittenhouse, L., Jones, K.P., 1993. Interleukin-1β (IL-1β) modulates prostaglandin production and the natural IL-1 receptor antagonist inhibits ovulation in the optimally stimulated rat ovarian perfusion model. Endocrinology 133, 2301–2306.

Pfeilschifter, J., Schwarzenbach, H., 1990. Interleukin-1 and tumor necrosis factor stimulate cGMP formation in rat renal mesangial cells. FEBS Lett. 273, 185–187.

Rueda, B.R., Tilly, K.I., Botros, I.W., et al., 1997. Increased bax and interleukin-1B-converting enzyme messenger ribonucleic acid levels coincide with apoptosis in the bovine corpus luteum during structural regression. Biol. Reprod. 56, 186–193.

Scherzer, W.J., Ruutianen-Altman, K.S.L., Putowski, L.T., Kol, S., Adashi, E.Y., Rohan, R.M., 1996. Detection and in vivo hormonal regulation of rat ovarian type I and type II interleukin-1 receptor mRNA: Increased expression during the periovulatory period. J. Soc. Gynecol. Invest. 3, 131–140.

Schun, S.-Y., Eisenhauer, K.M., Minami, S., Billig, H., Perlas, E., Hsueh, A.J.W., 1996. Hormonal regulation of apoptosis in early antral follicles: follicle-stimulating hormone as a major survival factor. Endocrinology 137, 1447–1456.

Simon, C., Frances, A., Piquette, G., Polan, M.L., 1994a. Immunohistochemical localization of the interleukin-1 system in the mouse ovary during follicular growth, ovulation, and luteinization. Biol. Reprod. 50, 449–457.

Simon, C., Tsafriri, A., Chun, S.-Y., Piquette, G.N., Dang, W., Polan, M.L., 1994b. Interleukin-1 receptor antagonist suppresses hCG-induced ovulation in the rat. Biol. Reprod. 51, 662–667.

Thornberry, N.A., Bull, H.G., Calaycay, J.R., et al., 1992. A novel heterodimeric cysteine protease is required for interleukin-1β processing in monocytes. Nature 356, 768–774.