The Midcycle Increase in Ovarian Glucose Uptake is Associated with Enhanced Expression of Glucose Transporter 3

Possible Role for Interleukin-1, a Putative Intermediary in the Ovulatory Process

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Abstract

This study characterizes the rat ovary as a site of hormonally dependent glucose transporter (Glut) expression, and explores the potential role of interleukin (IL)-1, a putative intermediary in the ovulatory process, in this regard. Molecular probing throughout a simulated estrous cycle revealed a significant surge in ovarian Glut3 (but not Glut1) expression at the time of ovulation. Treatment of cultured whole ovarian dispersates from immature rats with IL-1β resulted in upregulation of the relative abundance of the Glut1 (4.5-fold) and Glut3 (3.5-fold) proteins as determined by Western blot analysis. Other members of the Glut family (i.e., Gluts 2, 4, and 5) remained undetectable. The ability of IL-1 to upregulate Glut1 and Glut3 transcripts proved time-, dose-, nitric oxide-, and protein biosynthesis-dependent but glucose independent. Other ovarian agonists (i.e., TNFα, IGF-I, interferon-γ, and insulin) were without effect. Taken together, our findings establish the mammalian ovary as a site of cyclically determined Glut1 and Glut3 expression, and disclose the ability of IL-1 to induce the ovarian expression as well as translation of Glut1 and Glut3 (but not of Gluts 2, 4, or 5). Our observations also establish IL-1 as the first known regulator of Glut3, the most efficient Glut known to date. In so doing, IL-1, a putative component of the ovulatory process, may be acting to meet the increased metabolic demands imposed on the growing follicle and the ovulated cumulus-enclosed oocyte. (*J. Clin. Invest.* 1997. 99:2274–2283.) Key words: ovary • IL-1 • glucose transporter • ovulation

Introduction

A growing body of direct and indirect evidence supports the notion that intraovarian interleukin (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 LH in this process (2, 3). Second, the addition of an IL-1 receptor antagonist has been shown to attenuate LH-supported ovulation under both ex vivo (4) and in vivo (5) circumstances. Third, some components of the intraovarian IL-1 system (e.g., IL-1β and the type I IL-1 receptor) appear to be expressed in vivo, mainly during a narrow periovulatory window (6–12). Fourth, IL-1β has been shown to induce a host of ovulation-associated phenomena in vitro, such as the promotion of prostaglandin production (13–16), the stimulation of hyaluronic acid biosynthesis (17), the induction of collagenase activity (18), and the activation of nitric oxide synthase activity (19–21).

Yet another corollary of ovulation is the so-called metabolic shift, a phenomenon first described by Armstrong and Greep (22). This periovulatory gonadotropin-driven event is characterized, in part, by the enhancement of ovarian glucose uptake. Although the precise teleologic rationale for this metabolic transformation remains unknown, it may be that the resultant high throughput of glucose substrate (or its metabolites) is designed to meet the increased energy needs of the growing follicle and the meiotically active oocyte. It is tempting to speculate that glucose transporters (Gluts)[1] may play a role in this process. This study characterizes the rat ovary as a site of hormonally-dependent Glut expression, and the potential role of IL-1, a putative intermediary in the ovulatory process, in this mechanism. Special note was made of the periovulatory increase in ovarian Glut3, the most efficient Glut known to date.

Methods

Animals. Immature female rats (Sprague-Dawley; Zivic-Miller Laboratories, Inc., Zelienople, PA) were killed by CO_2 asphyxiation. 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 generously provided by Dr. Errol B. De Souza, and Dr. C.E. Newton, (DuPont-Merck Pharmaceutical Co., Wilmington, DE). A recombinant preparation of the naturally occurring human IL-1 re-

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Received for publication 18 October 1996 and accepted in revised form 20 February 1997.

J. Clin. Invest.
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0021-9738/97/05/2274/10 $2.00
Volume 99, Number 9, May 1997, 2274–2283

1. *Abbreviations used in this paper:* AG, aminoguanidine hemisulfate salt; CHX, cycloheximide; Glut, glucose transporter; IL-1RA, IL-1 receptor antagonist; nt, nucleotide; PMSG, pregnant mare serum gonadotropins; SNAP, *S*-nitroso-*N*-acetyl-penicilamine; TS, Tris/0.9% sodium chloride buffer.

ceptor antagonist (IL-1RA) was generously provided by Dr. Daniel E. Tracey (The Upjohn Co., Kalamazoo, MI). Highly purified human chorionic gonadotropin (hCG; CR-127, 14,900 IU/mg) was generously provided by Dr. R.E. Canfield (Center for Population Research, NICHD, NIH, Bethesda, MD). Tumor necrosis factor α (TNFα) was a generous gift from Dr. Dawson (National Biological Standard Board, Hertfordshire, UK). Insulin-like growth factor-I (IGF-I) was from Bachem California (Torrance, CA). Interferon-γ was a generous gift from Dr. H. Michael Shepard (Genentech Inc., South San Francisco, CA). Rabbit polyclonal antiserum to the rat Glut1-5 was a gift from Hoffmann-La Roche (Nutley, NJ).

McCoy's 5a modified (regular and glucose-free) medium (serum-free), penicillin–streptomycin solution, L-glutamine, trypan blue stain, and bovine serum albumin (BSA) were from Life Technologies Inc., (Grand Island, NY). Collagenase (Clostridium Histolyticum, CLS type I, 144 LU/mg) was from Worthington Biochemical Corp. (Freehold, NJ). DNAse (bovine pancreas), aminoguanidine hemisulfate salt (AG), cycloheximide (CHX), insulin, RNase A, *S*-nitroso-*n*-acetyl-penicilamine (SNAP), and PMSG (pregnant mare serum gonadotropins) were from Sigma Chemical Co. (St. Louis, MO). Aprotinin, pepstatin, leupeptin, PMSF, and dithiothreitol were from ICN Biomedical (Aurora, OH). Sodium dodecyl sulfate (SDS) was from Bio-Rad (Hercules, CA).

RNase T1 was from Pharmacia LKB Biotechnology Inc., (Piscataway, NJ). T7 and SP6 RNA polymerases, pGEM4Z vector, pGEM3 vector, and other molecular biology grade reagents were from Promega Corp., (Madison, WI). [^{32}P]UTP was from DuPont New England Nuclear Research Products (Boston, MA). [^{125}I]-labeled protein A was from Amersham Corp. (Arlington Heights, IL).

Tissue culture procedures. Whole ovarian dispersates were prepared and cultured as previously described (23).

Glucose assay. Methodology conformed to that previously described (24).

Nucleic acid probes. The rat Glut1 and Glut3 cDNAs were generously provided by Dr. G.I. Bell (Howard Hughes Medical Institute Research Laboratories, University of Chicago, Chicago, IL). A 436 bp Glut1 cDNA (25) was cloned into a pGEM3 vector at the BamHI site. When linearized with HindIII and transcribed with T7 RNA polymerase, the construct yielded a 492-nucleotide (nt) antisense RNA probe, which upon hybridization was projected to generate a 436-nt protected fragment. A 626-bp Glut3 cDNA (26) was cloned into a pGEM4Z vector at HindII-EcoRI sites. When linearized with StyI and transcribed with SP6 RNA polymerase, the construct produced a 389-nt antisense RNA probe, which upon hybridization was projected to generate a 360-nt protected fragment. The RPL19 probe was generated and used as previously described (12).

Western blot analysis. Membranes were prepared from cultured ovarian cells by resuspending the pelleted cells in PBS with protease inhibitors (aprotinin, pepstatin, leupeptin, and PMSF [all at 10 μg/ml], pH 7.4 at 4°C] and by homogenizing by sonication using a sonifier (model 250; Branson Ultrasonics Corp., Danbury, CT), No. 2 setting, three 20-s bursts. The lysate was centrifuged at 200,000 *g* for 10 min at 4°C to pellet the membranes. Membranes were solubilized and denatured in 15 mM Tris/1.5% SDS/2.3 M urea/100 mM dithiothreitol, pH 6.8, fractionated on 10% SDS-polyacrylamide gels and electrophoretically transferred to nitrocellulose (Schleicher and Schuell, Inc., Keene, NH). Nitrocellulose filters were then incubated in 10 mM Tris/0.9% sodium chloride buffer (TS) containing 3% BSA at 37°C for 1 h. Antisera were diluted in TS/3% BSA at room temperature for 1 h, followed by a series of washes containing TS/0.1% nonidet P-40 (NP-40) and TS/0.2 M sodium chloride. [^{125}I]-labeled protein A (0.2 μCi/ml in TS/BSA) was added for 1 h at room temperature followed by the same series of washes. The antiserum used was rabbit polyclonal antiserum to the rat Glut1-5 (27). Antiserum specificity was determined by preadsorption of serum overnight at 4°C with 2.5 μg/ml of the respective homologous or nonhomologous COOH-terminal peptides. Filters were exposed to autoradiographic film (XAR5; Eastman Kodak Co., Rochester, NY). Quantification of Western blots were performed by phosphor imager analysis (Molecular Dynamics, Sunnyvale, CA).

RNA extraction. Total RNA of cultured cells was extracted (RNAZOL-B; TelTest, Friendswood, TX) according to the manufacturer's protocol.

RNAse protection assay. Linearized DNA templates were transcribed with T7 RNA polymerase (Glut1) and with SP6 RNA polymerase (Glut3) to specific activities of 800 Ci/mmol [α-^{32}P]UTP or 160 Ci/mmol [α-^{32}P]UTP (RPL19). The riboprobes were gel-purified as described (28) in an effort to eliminate transcribed products that are shorter than full-length. The assay was performed as previously described (29). Gels were exposed to film (XAR; Eastman Kodak Co.) for varying lengths of time with intensifying screens. To generate quantitative data, gels were also exposed to a phosphor screen (Molecular Dynamics). The resultant digitized data were analyzed (ImageQuant Software; Molecular Dynamics). The hormonally independent RPL19 mRNA signal was used to normalize the Glut1 and Glut3 mRNA data for possible variation in RNA loading (12). Specifically, the net protected signal (respective background subtracted) to net RPL19 signal ratio was calculated for each sample and gene of interest.

In situ hybridization. Technology conformed to that previously described (12).

Data analysis. Except when noted, each experiment was replicated a minimum of three times. Data points are presented as mean±SE, and statistical significance (Fisher's protected least significance difference) determined by ANOVA, and paired Student's *t* test. Statistical values were calculated (Statview 512+ for MacIntosh; Brain Power, Inc., Calabasas, CA).

Results

Glucose transporter proteins in the rat ovary: immune Western blot analysis of untreated IL-1β–treated and IL-1RA–treated cultured whole ovarian dispersates. To determine which glu-

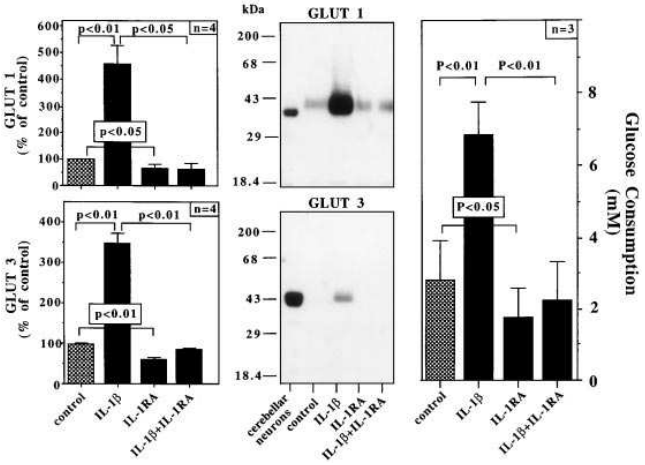


Figure 1. Glucose transporter proteins in the rat ovary: Western blot analysis of untreated, IL-1β–treated and IL-1RA–treated cultured whole ovarian dispersates. Whole ovarian dispersates (1.5×10^6 cells/60×12-mm dish) were cultured for 48 h in 3 ml of media in the absence or presence of IL-1β (50 ng/ml), with or without IL-1RA (5 μg/ml). The cells were homogenized, and total cell membranes were subjected to Western blot analysis for Glut1 and Glut3. The left panel depicts in bar graph form the mean±SE of four experiments. In each individual experiment, data were normalized relative to control. The middle panel depicts two representative autoradiographs. Cultured cerebellar neurons of rat origin were used as positive control for Glut1 and Glut3. The right panel depicts corresponding glucose consumption data reflecting the mean±SE of three experiments.

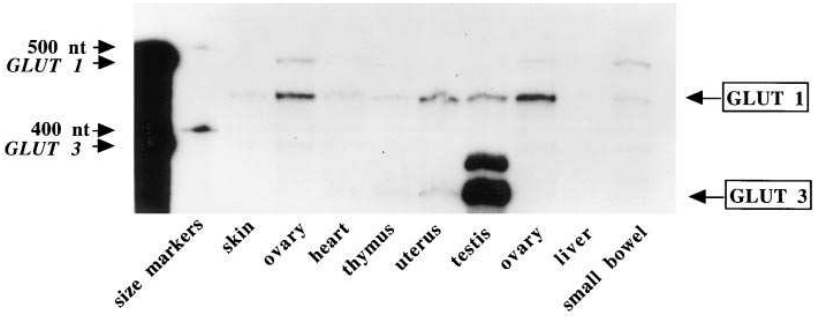


Figure 2. Glut1 and Glut3 transcripts in the rat: tissue distribution. Total RNA (20 μg) extracted from the indicated tissues of intact untreated immature rats was subjected to an RNase protection assay using antisense riboprobes corresponding to rat Glut1 and Glut3. The intensity of the signals was quantified as described. Protected fragments are depicted in boldfaced letters. The full-length riboprobes are depicted in italics.

cose transporters, if any, are prevalent in the rat ovary, and to explore the role of IL-1, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml), with or without IL-lRA (5 μg/ml). At the conclusion of the culture period, cells were subjected to Western blot analysis as described. As shown (Fig. 1; *left and middle*), the Glut1 and Glut3 proteins were barely detectable in the untreated state. Treatment with IL-1β, however, produced a marked increase in the signals corresponding to the Glut1 (4.5-fold; $P < 0.001$) and Glut3 (3.5-fold; $P < 0.01$) proteins. These IL-1 effects were completely reversed in the presence of IL-1RA, a pure competitive receptor antagonist (30). Importantly, treatment with IL-1RA by itself produced a significant ($P < 0.05$) decrease in the relative levels of the Glut1 ($P < 0.05$) and Glut3 ($P < 0.01$) proteins. Gluts 2, 4, and 5 were undetectable in both the untreated and IL-1–treated states (data not shown). The apparent molecular size differences between the positive controls (brain Gluts) and the ovarian Gluts is likely due to the tissue-specific posttranslational glycosylation patterns as previously described (25). The ability of IL-1β to upregulate the Glut1 and Glut3 proteins was closely associated with a marked increase (2.5-fold; $P < 0.05$) in glucose consumption (Fig. 1; *right*), an effect which was completely reversed by the addition of IL-1RA. These data establish the rat ovary as a site of Glut1 and Glut3 (but not of Glut2, 4, or 5) expression and translation, disclose the ability of exogenous IL-1 to induce the ovarian expression of Glut1 and Glut3, and suggest the existence of endogenous ovarian Glut-stimulating IL-1–like ovarian bioactivity.

Glut1 and Glut3 transcripts in the rat: tissue distribution. To evaluate the ovarian and extraovarian expression of Glut1 and Glut3 in the untreated immature rat, and to validate the constructs described in Methods, total RNA (20-μg) samples from ovary, small bowel, liver, skin, uterus, heart, testis, and thymus were subjected to an RNase protection assay using Glut1 and Glut3 antisense riboprobes. A protected fragment corresponding to the Glut1 transcript was noted in all tissues studied (Fig. 2), with the exception of the liver, as previously described (31). Protected fragments corresponding to Glut1 (but not Glut3) transcripts were noted in the ovary. Glut3 transcripts, in turn, were highly abundant in testis, a finding in agreement with previous observations (32). A modest Glut3 signal was also apparent in the uterus and the thymus. These findings suggest that whole ovarian material from untreated immature rats is a site of Glut1, but not Glut3, expression.

Glut1 and Glut3 gene expression: effects of follicular maturation, ovulation, and corpus luteum formation. To assess Glut1 and Glut3 gene expression in the course of a simulated estrus

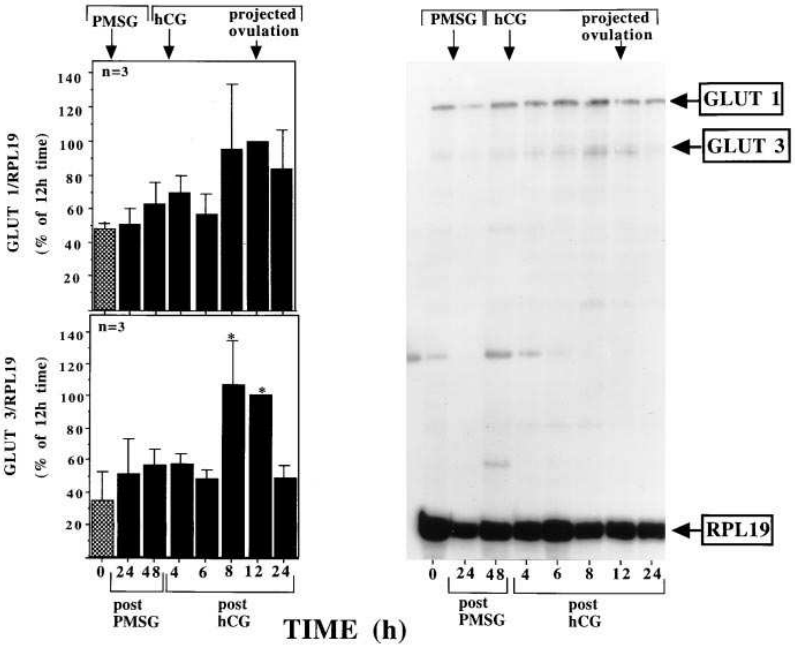
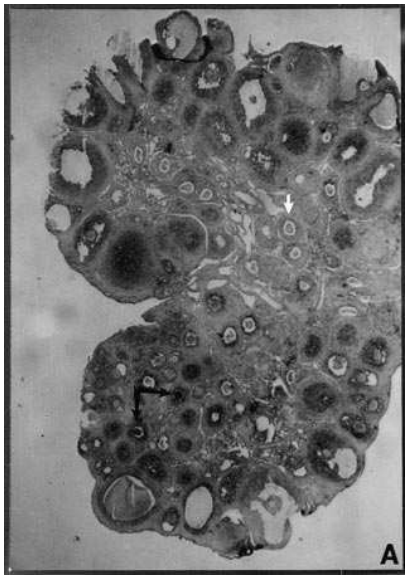
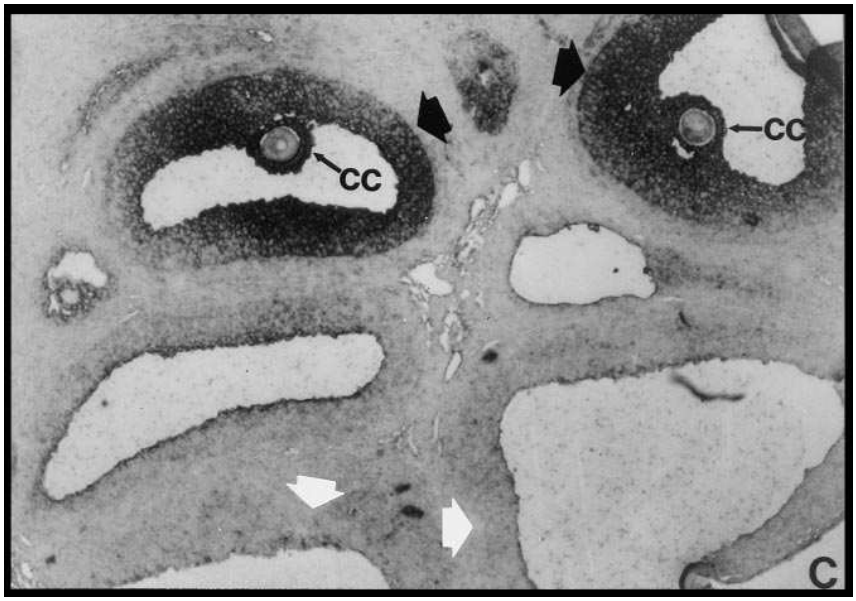


Figure 3. Ovarian Glut1 and Glut3 gene expression: effects of follicular maturation, ovulation and corpus luteum formation. Intact, 25-d–old rats were primed with 15 U PMSG. Ovulation was triggered 48 h later with 15 U hCG. The animals were killed at the indicated time points, and total ovarian RNA was extracted and subjected to a solution hybridization RNase protection assay with antisense riboprobes corresponding to rat Glut1, Glut3, and RPL19. The intensity of the signals was quantified as described. The bar graphs (*left*) depict the mean±SE of three experiments. A representative autoradiograph is shown (*right*). Data were normalized relative to the 12-h (post-hCG) value. Protected fragments are labeled in boldfaced letters. *$P < 0.05$ vs. time 0 (untreated animals).

cycle, 25-d–old rats were primed with 15 IU PMSG. Ovulation was triggered 48 h later with 15 IU hCG. The animals were killed at specific time points, and the ovaries were snap-frozen in dry ice. Total RNA was extracted, and aliquots of 20 μg were subjected to a solution hybridization RNase protection assay with antisense riboprobes corresponding to rat Glut1, Glut3, and RPL19. As shown (Fig. 3), protected fragments corresponding to Glut1 were apparent throughout the experiment. A twofold (statistically insignificant) increase was noted over untreated controls (time 0) 12 h after hCG administration (time of projected ovulation), a slight decrease being noted 12 h later. Only faint protected fragments corresponding to Glut3 were apparent in untreated animals. An increase in Glut3 transcripts, however, was noted 8 and 12 h after hCG (3- and 2.9-fold increases over untreated control [time 0] at 8 and 12 h, respectively; $P < 0.05$). The latter declined 12 h later. These data suggest that ovulation is associated with a significant increase in ovarian Glut3 (but not Glut1) expression.

Cellular localization of ovarian Glut1 and Glut3 transcripts: in situ hybridization studies. To establish the identity of the ovarian cell population responsible for Glut1 and Glut3 gene expression, ovaries were obtained from untreated, or PMSG-primed/hCG-triggered 25-d–old rats, and were processed for in situ hybridization as described. As shown, expression of Glut1 was limited to granulosa (mural, antral, and cumulus) cells, and to oocytes in both PMSG-treated (Fig. 4 *A*) and untreated (Fig. 4 *B*) rats. Follicles at different developmental stages also proved positive. A higher magnification of sections from ovaries obtained from PMSG-primed/hCG-triggered rats revealed intense staining of both antral and cumulus granulosa cells (Fig. 4 *C*). Similarly, in situ probing for Glut3 revealed a patchy positive signal in granulosa cells, and in a few oocytes from untreated (Fig. 5 *A*) and PMSG-treated rats (Fig. 5 *B*). Significant Glut3 expression appeared to be confined within the basement membrane, where an increase in intensity was noted towards the antral granulosa cell layer (Fig. 5 *C*). The theca–interstitial





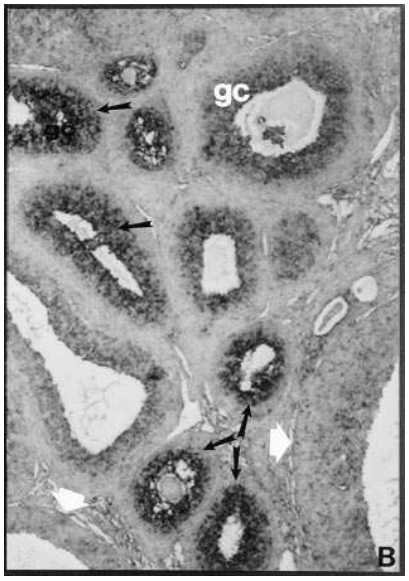


Figure 4. Cellular localization of Glut1 transcripts: in situ hybridization studies. Ovaries obtained from untreated or from PMSG-primed/hCG-triggered 25-d–old rats were processed for in situ hybridization using a digoxigenin-labeled rat Glut1 antisense riboprobe as described. (*A*) A 40-fold magnification of a section through an ovary taken 24 h after PMSG administration. The black arrows denote positive follicles with positive oocytes. The white arrow points to a negatively stained follicle. (*B*) A 100-fold magnification of a section through an untreated ovary. The black arrows denote follicles with stained granulosa cells. The positive signal appears to be confined within the basement membrane. The white arrows denote negatively stained follicles. (*C*) A 100-fold magnification of a section from an ovary obtained 6 h after hCG administration. The black arrows point to intensely stained cumulus cells (CC) surrounding modestly stained oocytes. The white arrows denote negatively stained follicles.

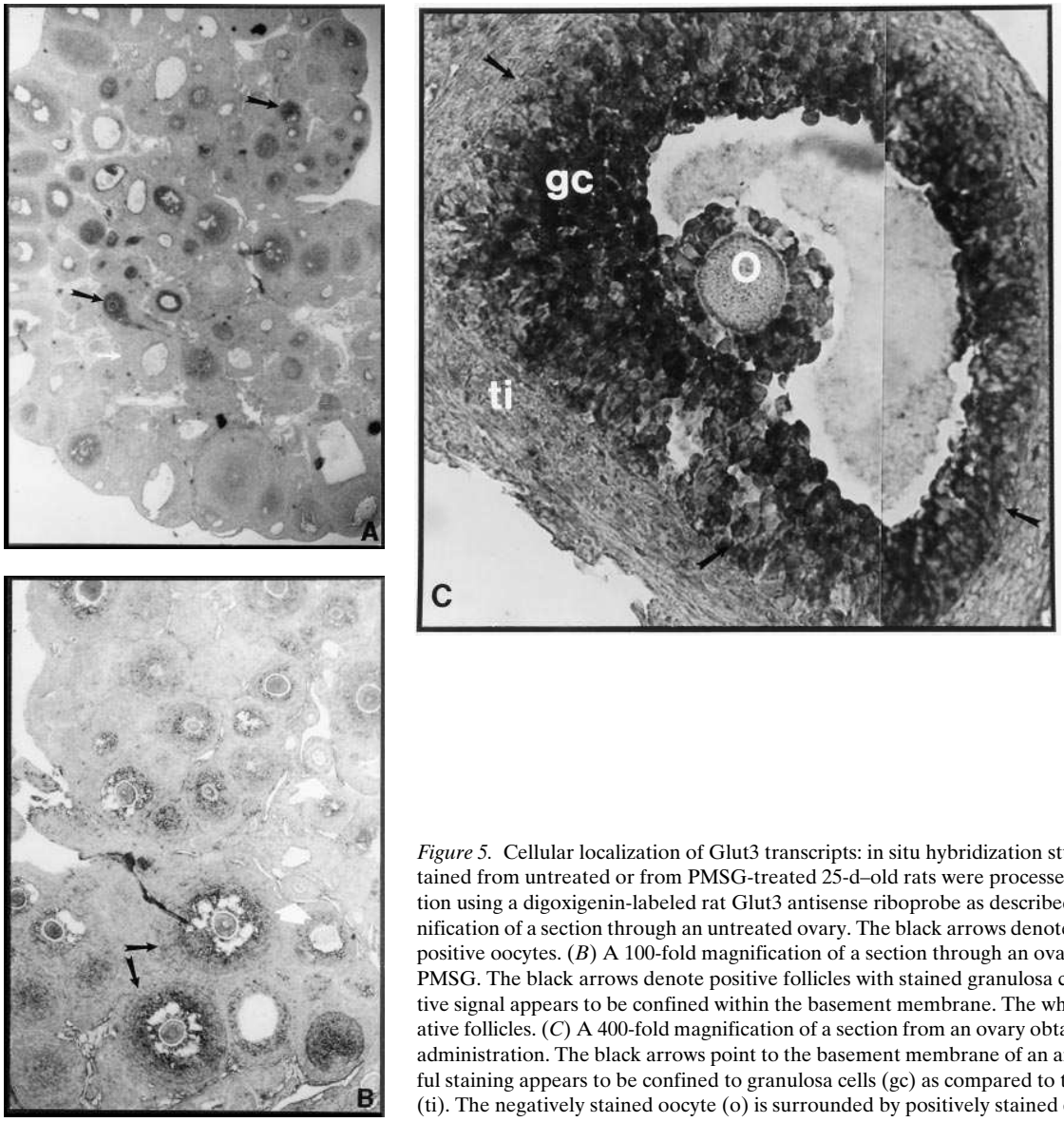


Figure 5. Cellular localization of Glut3 transcripts: in situ hybridization studies. Ovaries obtained from untreated or from PMSG-treated 25-d–old rats were processed for in situ hybridization using a digoxigenin-labeled rat Glut3 antisense riboprobe as described. (*A*) A 40-fold magnification of a section through an untreated ovary. The black arrows denote positive follicles with positive oocytes. (*B*) A 100-fold magnification of a section through an ovary obtained 48 h after PMSG. The black arrows denote positive follicles with stained granulosa cells. The patchy positive signal appears to be confined within the basement membrane. The white arrows denote negative follicles. (*C*) A 400-fold magnification of a section from an ovary obtained 48 h after PMSG administration. The black arrows point to the basement membrane of an antral follicle. Meaningful staining appears to be confined to granulosa cells (gc) as compared to theca-interstitial cells (ti). The negatively stained oocyte (o) is surrounded by positively stained cumulus cells.

compartment did not express detectable levels of either of these two transporters. Probing with sense probes proved negative (not shown). These findings document the granulosa cell as the primary site of Glut1 and Glut3 expression.

Basal and IL-1–stimulated patterns of Glut1 and Glut3 gene expression by untreated cultured whole ovarian dispersates: time dependence. To examine ovarian Glut1 and Glut3 expression under in vitro circumstances, whole ovarian dispersates were cultured for the duration indicated (up to 48 h) in the absence of any treatment. At the conclusion of the culture period, total RNA was extracted and subjected to an RNase protection assay using antisense riboprobes corresponding to rat Glut1, Glut3, and RPL19. As shown (Fig. 6), Glut1 and Glut3 transcripts were most abundant at the time of plating (time 0). Thereafter, note was made of a spontaneous decrease to a nadir at the 48-h time point (a threefold decrease over time 0 for both Glut1 and Glut3, $P < 0.05$). The declining expression of the two genes reached statistical significance (set at $P < 0.05$) after 4 and 8 h of culture for Glut1 and Glut3, respectively. To determine the effect of treatment with IL-1β on Glut1 and Glut3 transcripts in vitro, whole ovarian dispersates were cultured for the duration indicated (up to 48 h) in the presence of IL-1β (50 ng/ml). As shown (Fig. 6), treatment with IL-1β resulted in an increase in Glut1 and Glut3 transcripts (2.2- and 2.8-fold increases over time 0 for Glut1 and Glut3, respectively; $P < 0.05$ in both cases). These data suggest that IL-1β is capable of upregulating the expression of the two genes, thereby reversing the spontaneous decrease in their in vitro expression.

IL-1β–induced Glut1 and Glut3 gene expression by cultured whole ovarian dispersates: IL-1 dose dependence. To further evaluate IL-1β-induced Glut1 and Glut3 gene expression, whole ovarian dispersates were cultured for 48 h in the absence or presence of increasing concentrations of IL-1β (50 pg/ml–50 ng/ml). Dose-dependent increments were noted in the expression of Glut1 and Glut3 transcripts (Fig. 7), the corre-

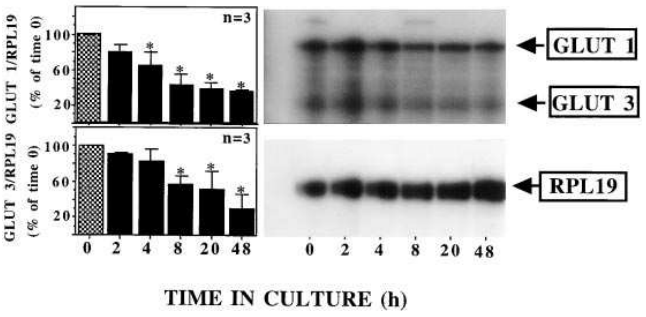


Figure 6. Glut1 and Glut3 gene expression by untreated and IL-1-treated cultured whole ovarian dispersates: time-dependence. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for the duration indicated (up to 48 h) in the absence or presence of IL-1β (50 ng/ml). Total cellular RNA was extracted and subjected to an RNase protection assay using antisense riboprobes corresponding to rat Glut1, Glut3, and RPL19. The intensity of the signals was quantified as described. The left panels depict in bar graph form the mean±SE of three experiments. In each individual experiment, data were normalized relative to the time 0 value. The right panel depicts a representative autoradiograph. The protected fragments are labeled in boldfaced letters. $*P < 0.05$ vs. time 0.

sponding ED_{50} values being 2.0 and 3.0 ng/ml. A statistically significant ($P < 0.05$) increase in Glut1 and Glut3 gene expression (over untreated controls) was reached at the IL-1β dose of 5 ng/ml. Overall 5.5- and 5.0-fold increments were noted for Glut1 and Glut3, respectively.

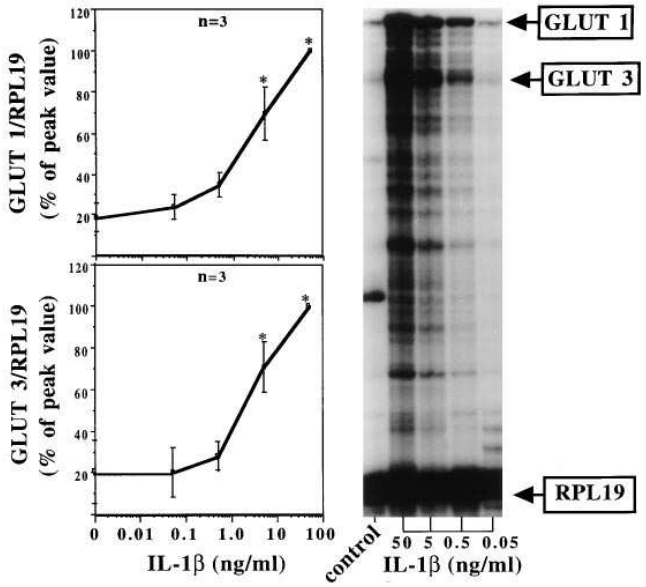


Figure 7. IL-1β–induced Glut1 and Glut3 gene expression by cultured whole ovarian dispersates: IL-1 dose-dependence. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in the absence or presence of the indicated concentrations of IL-1β. Total cellular RNA was extracted and subjected to an RNase protection assay using antisense riboprobes corresponding to rat Glut1, Glut3, and RPL19. The intensity of the signals was quantified as described. The left panels depict in bar graph form the mean±SE of three experiments. In each individual experiment, data were normalized relative to the peak value. The right panel depicts a representative autoradiograph. The protected fragments are labeled in boldfaced letters. $*P <$ 0.05 vs. control.

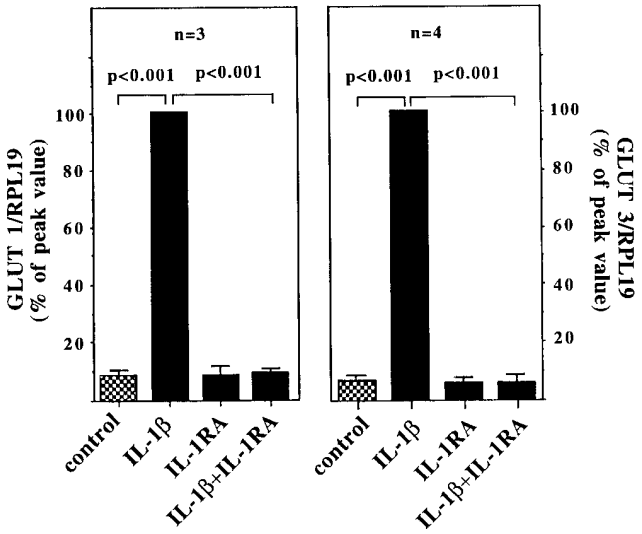


Figure 8. IL-1β–induced Glut1 and Glut3 gene expression by cultured whole ovarian dispersates: IL-1 receptor mediation. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml), with or without IL-1RA (5 μg/ml). The resultant RNA samples were subjected to an RNase protection assay using antisense riboprobes corresponding to rat Glut1, Glut3, and RPL19. The intensity of the signals was quantified as described. The bar graph depicts the mean±SE of three experiments (Glut1) or four experiments (Glut3). In each individual experiment, data were normalized relative to the peak IL-1β value.

IL-1β–induced Glut1 and Glut3 gene expression by cultured whole ovarian dispersates: IL-1 receptor mediation. To further evaluate the dependence of ovarian Glut1 and Glut3 transcripts on IL-1, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml), with or without human recombinant IL-1RA (5 μg/ml). As shown (Fig. 8), treatment with IL-1β (but not IL-1RA) produced an increase in the relative expression of Glut1 (11-fold; $P < 0.001$) and Glut3 (16-fold; $P = 0.0001$) transcripts over untreated controls. Cotreatment with IL-1RA produced complete blockade of the IL-1 effect.

IL-1β–induced Glut1 and Glut3 gene expression by cultured whole ovarian dispersates: glucose dependence. To determine if the observed IL-1β–induced Glut1 and Glut3 gene expression is driven by declining glucose concentration, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml) in regular McCoy's 5a medium (glucose concentration = 3,000 mg/liter) or in a glucose-free counterpart. As shown (Fig. 9), medium glucose concentration was without effect on the IL-1β–induced expression of Glut1 and Glut3. Importantly, RPL19 transcripts remained unaffected through all experimental circumstances, thereby arguing against an overt general toxic effect of glucose deficiency on the cellular transcription system.

IL-1β–induced Glut1 and Glut3 gene expression by cultured whole ovarian dispersates: protein synthesis dependence. To determine if the ability of IL-1β to induce Glut1 and Glut3 gene expression is contingent upon intact cellular protein synthesis, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml), with or without cyclo-

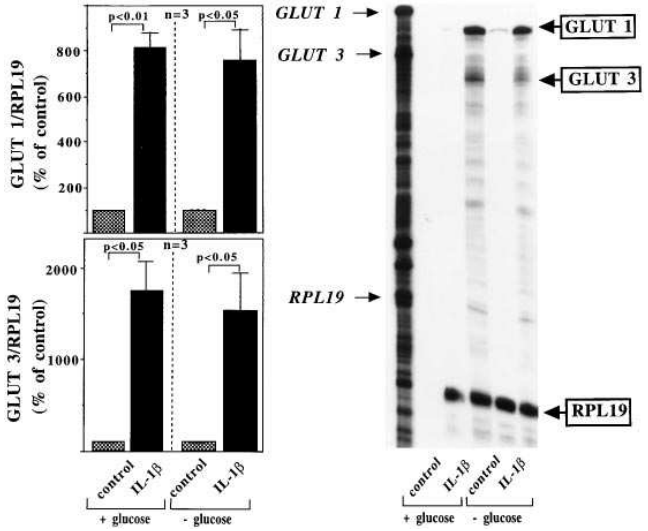


Figure 9. IL-1β–induced Glut1 and Glut3 gene expression by cultured whole ovarian dispersates: glucose dependence. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in regular ([glucose] = 3,000 mg/l) or glucose-free medium in the absence or presence of IL-1β (50 ng/ml). Total cellular RNA was extracted and subjected to an RNase protection assay using antisense riboprobes corresponding to rat Glut1, Glut3, and RPL19. The intensity of the signals was quantified as described. The left panel depicts in bar graph form the mean±SE of three experiments. In each individual experiment, data were normalized relative to control. The right panel depicts a representative autoradiograph. The full-length riboprobes are labeled in italics, and the protected fragments are labeled in boldfaced letters.

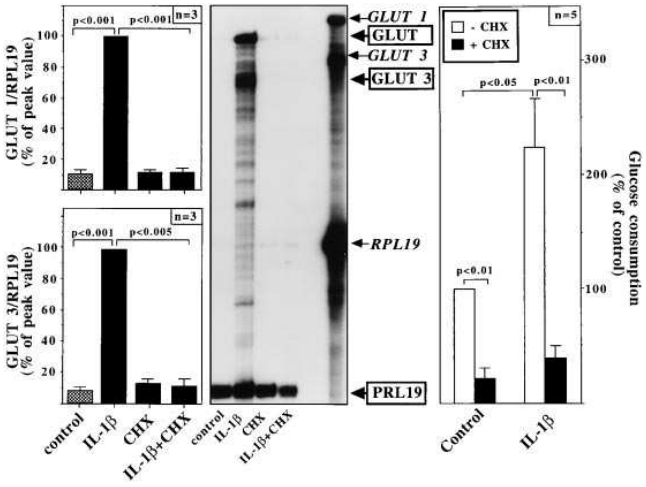


Figure 10. Glut1 and Glut3 gene expression by cultured whole ovarian dispersates: protein synthesis dependence. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml), with and without cycloheximide (CHX, 0.1 μg/ml). Total cellular RNA was extracted and subjected to an RNase protection assay using antisense riboprobes corresponding to rat Glut1, Glut3, and RPL19. The intensity of the signals was quantified as described. The left panels depict in bar graph form the mean±SE of three experiments. In each individual experiment, data were normalized relative to the peak value. The middle panel depicts a representative autoradiograph. The full-length riboprobes are labeled in italics and the protected fragments in boldfaced letters. The right panel depicts corresponding glucose consumption data reflecting the mean±SE of five experiments.

heximide (CHX; 0.1 μg/ml). Treatment with CHX completely abolished the ability of IL-1β to upregulate Glut1 and Glut3 expression (Fig. 10; *left and middle*) and to promote glucose consumption (Fig. 10; *right*). Importantly, 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 Glut1 and Glut3 gene expression as well as to promote glucose consumption requires de novo protein biosynthesis.

IL-1β–induced Glut1 and Glut3 gene expression by cultured whole ovarian dispersates: nitric oxide dependence. To determine whether the ability of IL-1β to induce Glut1 and Glut3 gene expression is contingent upon endogenously produced nitric oxide (19–21), whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml), with or without AG (0.4 mM), an established inhibitor of ovarian nitric oxide synthase activity (19, 20). As shown (Fig. 11), treatment with AG by itself was without effect on the spontaneous expression of Glut1 and Glut3. Cotreatment with AG, however, resulted in 50 ($P < 0.001$) and 60% ($P < 0.001$) decrements in IL-1β–induced Glut1 and Glut3 gene expression, respectively. Treatment with SNAP (0.1 mM), however, an established nitric oxide generator, was without effect on Glut1 or Glut3 expression (data not shown).

IL-1β–induced Glut1 and Glut3 gene expression by cultured whole ovarian dispersates: specificity studies. To determine if the observed upregulation of ovarian Glut1 and Glut3 gene expression is specific to IL-1β, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml), TNFα (30 ng/ml), interferon-γ (1,000 U/ml), IGF-I (50 ng/ml), or insulin (1 μg/ml). Treatment with IL-1α resulted in the expected upregulation of Glut1 and Glut3 gene expression. None of the other agonists employed, however, produced a significant change in Glut1 and Glut3 gene expression as compared with untreated controls (not shown).

Discussion

Glut1 and Glut3 proteins are members of a family of glucose transporters composed of six distinct proteins (for review see reference 31). Glut1 is a widely expressed protein, the liver constituting a notable exception (32). Glut1 is almost invariably the major glucose transporter isoform expressed in cultured cells, and as such is responsible for basal glucose transport. Glut3 in turn is the most prominent isoform in the brain (33–36), but has also been detected in the testis (32, 37) and placenta (38). Like Glut1, Glut3 is characterized by a high-affinity dissociation constant (K_m) for glucose (39). The maximal transport capacity (V_{max}) of Glut3, however, is highest among all the known Glut isoforms thereby rendering it uniquely suited to engage in high-volume glucose transport.

Our present observations establish the mammalian ovary, and specifically the granulosa cell, as a site of Glut1 and Glut3 gene expression and translation (Figs. 1–5). As such, this report establishes for the first time the identity of the glucose transporters prevalent in the rat ovary. Although Glut1 is often thought of as responsible for basal glucose transport, the

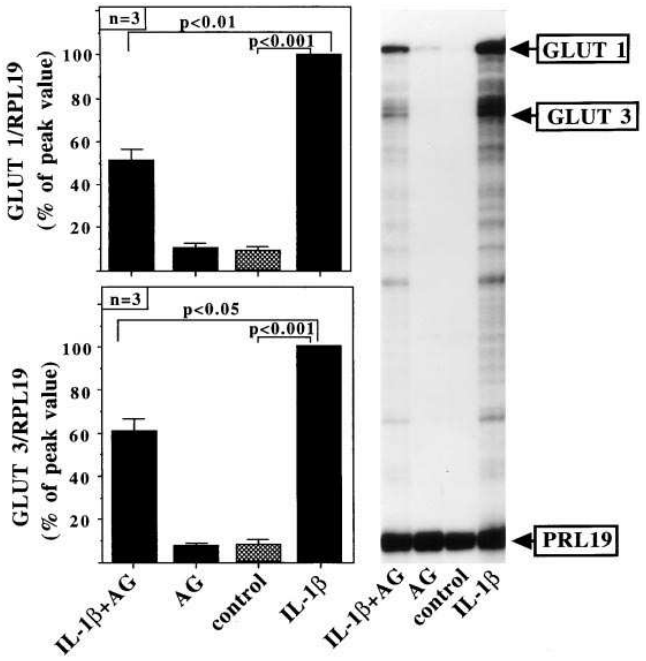


Figure 11. Glut1 and Glut3 gene expression by cultured whole ovarian dispersates: nitric oxide dependence. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml), with and without aminoguanidine (AG, 0.4 mM). Total cellular RNA was extracted and subjected to an RNase protection assay using antisense riboprobes corresponding to rat Glut1, Glut3, and RPL19. The intensity of the signals was quantified as described. The left panels depict in bar graph form the mean±SE of three experiments. In each individual experiment, data were normalized relative to the peak IL-1β value. The right panel depicts a representative autoradiograph. The protected fragments are labeled in boldfaced letters.

current demonstration of IL-1 inducibility might argue otherwise. Still, Glut1 expression proved stable across a simulated estrus cycle as assessed by molecular probing of whole ovarian material (Fig. 3). The testicular presence of Glut3 has previously been documented (32, 37). Possible ovarian expression however, has not been assessed. The current observations support the view that Glut3 expression is not limited to the brain, and that reproductive tissues may well be a site of Glut3 gene expression. Given that the ovarian expression of Glut3 in vivo is particularly pronounced during a relatively narrow periovulatory window (Fig. 3; 8–12 h after hCG administration), it is tempting to speculate that Glut3 may play a meaningful role in ovarian physiology, possibly in the context of the ovulatory cascade. Clearly, the ability of Glut3 to engage in high-volume glucose transport renders it especially suitable for the hypothesized increase in the metabolic demands imposed by terminal follicular maturation and the resumption of meiosis. The above conclusions must be tempered by the realization that caution must be exercised when extrapolating from the immature to adult state, not to mention from the superovulated to the naturally cycling state.

Studies at the transcript level suggest that the IL-1 effect is characterized by approximate ED_{50s} of 2.0 and 3.0 ng/ml for Glut1 and Glut3, respectively. These ED_{50s} are higher than those required for the induction of secretory PLA_2 transcripts (0.3 ng/ml) or nitric oxide synthase activity (0.7 ng/ml), comparable to those required for the induction of cytosolic PLA_2 (2 ng/ml) and prostaglandin endoperoxide synthase-2 (2 ng/ml) transcripts, but lower than those required for the induction IL-1β (6 ng/ml) or type I IL-1 receptor (10 ng/ml) transcripts (20). To the extent that IL-1 may play a role in the ovulatory cascade (1–5), these observations suggest that the induction of glucose transporters may constitute a relatively early event in the sequence leading to follicular rupture.

The apparent dependence of Glut1 and Glut3 on IL-1 is not limited to the exogenously provided hormone. The use of IL-1RA exposed endogenous IL-1-like bioactivity (Fig. 1) at the protein level. Other evidence supporting the existence of endogenous intraovarian IL-1 bioactivity consists of the demonstration of IL-1β transcripts in cultured whole ovarian dispersates (6), and the detection of immunoreactive IL-1β in media conditioned by the same (Kol et al., unpublished observations). Evidence for endogenous ovarian IL-1–like bioactivity was also previously noted in the context of other ovarian IL-1 endpoints such as nitric oxide generation (20), or $sPLA_2$ and $cPLA_2$ induction (Kol et al., unpublished observations). Evidence against the involvement of endogenous IL-1 is represented by the fact that treatment with IL-1RA failed to decrease Glut1 or Glut3 transcripts (Fig. 8). Whether of endogenous or exogenous origin, the IL-1 effect proved receptor-mediated (Figs. 1 and 8), and relatively specific in that it was not reproduced by select growth factors and cytokines.

Given the reported upregulation of Glut1 in response to declining glucose concentrations (40), consideration was given to the possibility that the ability of IL-1β to upregulate Glut1 and Glut3 expression/translation may reflect, if only in part, the declining glucose content of the medium. Our findings, however, reveal the ambient glucose concentrations to be without significant effect on the basal or IL-1β–induced content of Glut1 and Glut3 transcripts (Fig. 9). Consequently, the ability of IL-1β to induce Glut1 and Glut3 transcription/translation must be attributed to mechanisms other than the induction of hypoglycemia. Conceivably, the activation of nitric oxide synthase (19–21) (Fig. 11) or of prostaglandin biosynthesis (13–16) may be at play. Certainly, IL-1-induced gene expression for both Glut1 and Glut3 requires de novo protein biosynthesis (Fig. 10). The nature of the proteins required for the realization of IL-1 action remains a matter of speculation.

While IL-1 was shown to stimulate ovarian nitric oxide synthase (20), the involvement of which in the ovulatory process was suggested (19), our data failed to document a direct effect of nitric oxide on Glut1 or Glut3 expression. Blocking nitric oxide production with AG (Fig. 11) however, resulted in significant decrements in IL-1–induced Glut1 and Glut3 expression, suggesting that the stimulatory effect of IL-1β on the expression of these genes is mediated, at least in part, by endogenously produced nitric oxide. Current views favor the notion that Glut3 expression is constitutive in nature. Indeed, we remain unaware of regulatory agents capable of altering Glut3 expression. In this respect, this report is unique in that it establishes IL-1 as the first known regulator of Glut3 gene expression. Moreover, our current observations offer a novel outlook on the interaction between a proinflammatory mediator (41) and a membrane protein concerned with the transport of glucose. Given the obvious energy needs of the inflammatory process, it appears intuitive that facilitation of glucose transport might constitute part of such a process. Given that

ovulation has also been likened to an inflammatory process (42), the IL-1–mediated induction of Glut1 and Glut3 may represent a specific adaptational response of the mammalian ovary.

Our findings point to the intraovarian IL-1 system as a major regulator of follicular carbohydrate economy. This new role for IL-1 in ovarian physiology further suggests physiologic relevance. Specifically, IL-1 may be of import in the context of meeting the metabolic demands imposed on the developing follicle. The endowment of somatic ovarian cells with glucose transporters may thus be viewed as a crucial step in follicular development. Indeed, it is the somatic component of the follicle, most notably, the granulosa cell layer and the cumulus complex which are responsible for sustaining the oocyte during the periovulatory period. Conceivably then, endogenously elaborated IL-1–like bioactivity may mediate the periovulatory, gonadotropin-induced shift in follicular metabolism (22).

Acknowledgments

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

This work was supported in part by NIH Research Grants HD-19998 and HD-30288 (E.Y. Adashi) and by German Israeli Fund 200-202.07194 (I. Ben-Shlomo). The first two authors have contributed equally to the conception and execution of this work.

Shahar Kol is a recipient of a 1994 Merck Senior Fellow Award of the Endocrine Society, and of an American Physician Fellowship Award. Izhar Ben-Shlomo is the recipient of a Lalor Foundation Fellowship, an International Fellowship Award from the Israeli Medical Association, a Fulbright Fellowship, and a Harlea Charitable Trust Award. Kristina Ruutiainen is the recipient of a Fogarty International Fellowship Award, a Lalor Foundation Award, and a Finnish Culture Foundation Award.

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