0013-7227/97/$03.00/0
Endocrinology
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Vol. 138, No. 7
Printed in U.S.A.

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*

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ABSTRACT

This communication explores the possibility that interleukin (IL)-1β, a putative intermediary in the ovulatory process, may take part in the gonadotropin-driven midcycle diversion of ovarian carbohydrate metabolism toward glycolysis. We examined the effect of treatment with IL-1β on glucose metabolism in aerobically cultured whole ovarian dispersates from immature rats. Treatment with IL-1β increased cellular glucose consumption/uptake, stimulated extracellular lactate accumulation and media acidification, and decreased extracellular pyruvate accumulation in a receptor-mediated, time-, dose- and cell density-dependent manner. Endogenous IL-1β-like bioactivity was shown to mediate the ability of gonadotropins to exert these same metabolic effects. The IL-1β effect was also (1) apparent over a broad range of glucose concentrations, inclusive of the putative physiological window; (2) relatively specific, because tumor necrosis factor-α and insulin were inactive; (3) contingent upon cell-cell cooperation (4) and reliant on *de novo* protein synthesis. Comparison of the molar ratios of lactate accumulation to glucose consumption in IL-1β-replete *vs.* IL-1β-deplete cultures suggests that IL-β promotes the conversion of all available glucose to lactate but that other substrates for lactate production may also exist. However, no lactate was generated by cells grown under glucose-free conditions. Taken together, our data suggest that IL-1β may act as a metabolic hormone in the ovary. Subject to the limitations of the *in vitro* paradigm, our data also suggest that IL-1β may mediate the gonadotropin-associated midcycle shift in ovarian carbohydrate metabolism. By converting the somatic ovarian cells into a glucose-consuming glycolytic machinery, IL-1β may establish glycolysis as the main energy source of the relatively hypoxic preovulatory follicle and the resultant cumulus-oocyte complex. The consequent oxygen sparing may conserve the limited supply of oxygen needed for vital biosynthetic processes such as steroidogenesis. This adaptational response may also provide the glycolytically incompetent oocyte with the obligatory tricarboxylic cycle precursors it depends on to meet the increased energy demands imposed upon it by the resumption of meiosis. (*Endocrinology* **138:** 2680–2688, 1997)

A GROWING BODY of direct and indirect evidence supports the notion that intraovarian interleukin (IL)-1β[1] may be an intermediary in the ovulatory process (1). First, the provision of IL-1β has been shown to bring about ovulation and to synergize with 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 (4, 5). Third, some components of the intraovarian IL-1 system (*e.g.* IL-1β and the type I IL-1 receptor) appear to be expressed only during a narrow periovulatory window (6–9). Fourth, IL-1β has been shown to induce a host of ovulation-associated phenomena (10–13).

Yet another corollary of ovulation is the so called metabolic shift, a phenomenon first described more than 30 yr ago by Armstrong and Greep (14). This effect is characterized by the enhancement of glucose uptake as well as by the diversion of ovarian carbohydrate metabolism away from oxidative phosphorylation towards glycolysis (14–24). Although the precise teleologic rationale for the metabolic shift remains unknown, it is possible that it constitutes an adaptational response. Such an adaptational response may be designed to combat the anticipated relative hypoxia experienced by the follicle- or cumulus-enclosed oocyte (24, 25). Moreover, the resultant high throughput of monocarboxylate substrates may be designed to meet the increased energy needs of the meiotically active oocyte that cannot process glucose by itself and therefore relies on alternative energy substrates such as pyruvate and lactate (26–30).

Because intraovarian IL-1β appears to be gonadotropin dependent (6) and IL-1β has been shown to produce an aerobic glycolytic state in several extraovarian cell types

Received November 4, 1996.

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* This work was supported in part by NIH Grants HD-19998 and HD-00697 (to E.Y.A.), HD-16596 (to L.R.), the Frank C. Bressler Research Award from the University of Maryland (to D.W.P.), as well as by the Lalor Foundation, Fulbright, and Harlea Charitable Trust Fellowship Awards (to I.B.-S.).

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(31–33), we hypothesized that IL-1β may mediate the gonadotropin-induced midcycle conversion of ovarian metabolism towards glycolysis. To test this possibility, we examined the effect of treatment with IL-1β on glucose consumption and uptake, and on lactate and pyruvate formation using aerobically cultured whole ovarian dispersates from immature rats. Our results indicate that IL-1β, of either exogenous or endogenous origin, is capable of inducing glycolysis in aerobically cultured ovarian cells, an effect accompanied by up-regulation of glucose uptake. These findings support the proposition that ovarian IL-1β-like activity mediates the periovulatory, gonadotropin-induced shift in ovarian glucose metabolism.

Materials and Methods

Animals

Immature (25–28 days old) Sprague-Dawley female rats, purchased from Zivic-Miller Laboratories (Zelienople, PA), were killed by CO_2 asphyxiation. This project was approved by the Institutional Animal Care and Use Committee.

Reagents and hormones

Regular and glucose-free McCoy's 5a media (modified, without serum), penicillin-streptomycin solution, L-glutamine, trypan blue stain (0.4%), BSA, and HEPES buffer were from Life Technologies Life Sciences (Grand Island, NY). Collagenase (Clostridium Histolyticum; CLS type I; 183 U/mg) was from Worthington Biochemical Corp. (Freehold, NJ). DNase (bovine pancreas; 2100 U/mg), aminoguanidine hemisulfate salt, and sodium nitroprusside were obtained from Sigma Chemical Co. (St. Louis, MO). [^{3}H]L-glucose (20 Ci/mmol), [1,2-^{3}H]2-deoxy-D-glucose (30.8 Ci/mmol) and [^{3}H]3–0-methyl-D-glucose (60–90 Ci/mmol) were obtained from DuPont New England Nuclear Research Products (Boston, MA).

Recombinant human IL-1β (2×10^7 U/mg) was generously provided by Drs. Errol B. De Souza and C. E. Newton of DuPont-Merck Pharmaceutical Co. (Wilmington, DE). Recombinantly expressed, naturally occurring human IL-1 receptor antagonist (IL-1RA) was generously provided by Dr. Jerome F. Strauss III (Philadelphia, PA). Highly purified human CG (hCG; CR-127, 14, 900 U/mg) and ovine FSH (oFSH: NIH-FSH-S18, FSH potency equal to 65.6 NIH-FSH-S1 U/mg) were gifts of the National Hormone and Pituitary Program (Rockville, MD), through the NIADDK, NICHHD, and USDA. Bovine insulin was obtained from Sigma Chemical Co. (St. Louis, MO). Recombinant human tumor necrosis factor-α (TNFα; 5×10^7 U/ng) was generously provided by Dr. Jennie Mather (Genentech, Inc., San Francisco, CA).

Tissue culture

Whole ovarian dispersates were prepared as previously described (34). Purified granulosa cells were extracted by repeated follicular puncture as previously described (35). Highly purified theca-interstitial cell preparations from immature hypophysectomized rats were isolated as previously described (36). Cells were cultured in 35 × 10-mm tissue culture dishes (Falcon Plastics, Oxnard, CA) containing 1 ml of McCoy's 5a medium (modified, without serum). Media were supplemented with L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). Cell cultures were maintained for up to 96 h at 37 C under a water-saturated atmosphere of 5% CO_2 and 95% air. At the end of each experiment, collected conditioned media were stored at −20 C until assayed for their glucose or lactate content as described below. Pyruvate was assayed in freshly conditioned media.

Lactate assay

Lactate concentrations in conditioned media were determined by a modification of a previously described method (37) in which the NADH product is monitored at 340 nm after the NAD-linked conversion of lactate to pyruvate by lactate dehydrogenase with hydrazine trapping of pyruvate to ensure that the reaction goes to completion. The assay was adapted as follows to a microtiter plate system using premixed reagents from a commercially available kit (Sigma no. 826-A, Sigma Chemical Co., St. Louis, MO). Conditioned media (20 μl of 20-fold diluted) or varying volumes (20–100 μl) of L-lactic acid standard (50 μg/ml) were diluted in the microtiter plate wells to a volume of 100 μl after which were added NAD/glycine buffer solution, pH 9.2 (75 μl), and lactate dehydrogenase solution (25 μl). After a 30-min incubation period at ambient temperature, absorbance (340 nm) was read in a UVmax plate reader (Molecular Devices, Menlo Park, CA). The standard curve was linear in the range used (1–25 μg/ml), and media blanks were negligible. Lactate concentrations were calculated from the standard curve using a software package (ΔSoft, BioMetallics, Inc., Princeton, NJ) designed for the plate reader. The within-assay coefficients of variation were 14.5 and 6.9% and the between-assay coefficients of variation were 11.7 and 6.7% for the low and high standards, respectively.

Glucose assay

Glucose concentrations in conditioned media were determined by the modification of a previously described enzymatic method (38) wherein the glucose oxidase and peroxidase reactions are coupled with the chromogenic oxygen acceptor o-dianisidine. The assay was adapted as follows to a microtiter plate system using premixed reagents from a commercially available kit (Sigma no. 510-A, Sigma). Unconditioned or conditioned media (10 μl of 20-fold diluted) or varying volumes (1.5–30 μl) of standard glucose solution (50 μg/ml) were diluted in the microtiter plate wells to a volume of 30 μl to which were added the color reagent/enzyme solution (200 μl), prepared as described by the manufacturer. After a 45-min incubation period at ambient temperature, absorbance of oxidized o-dianisidine (450 nm) was read in the UVmax plate reader. The standard curve was linear in the range used (2.5–50 μg/ml). Glucose concentrations were calculated from the standard curve. The within-assay coefficients of variation were 2.7 and 1.2% and the between-assay coefficients of variation were 3.2 and 1.5% for the low and high standards, respectively. The values for glucose consumption reported herein were calculated by subtracting the value determined in conditioned media (a value containing assay variability, see below) from the glucose value in unconditioned media. This latter number is nominally 3 mg glucose/ml media (16.7 mM), but in reality varied slightly due to modest evaporation during culture. A value of 3.02 ± 0.13 mg glucose/ml (range 2.9–3.3 mg/ml) was actually measured in McCoy's media after 96 h in cell-free cultures (n = 12).

In preliminary studies, glucose was also measured by a previously described coupled reaction (39) in which glucose is first phosphorylated and the glucose-6-phosphate formed is oxidized in the presence of NAD. When we compared glucose measurement by this technique to that of the glucose oxidase method described above, both methods gave comparable results. The correlation coefficient (r^2) for the two methods, using data from five different experiments (n = 36 determinations for each method), was 0.66 ($P = 0.0001$). The slope (glucose oxidase method/NAD method) was 1.03 ($P = 0.0001$), indicating that the two methods gave essentially the same value for glucose concentration.

Pyruvate assay

Pyruvate concentrations in conditioned media were assayed by the same enzymatic reaction used to measure lactate as described above, but in the reverse direction (37). The loss of NADH is monitored at 340 nm after the NADH-linked conversion of pyruvate to lactate by lactate dehydrogenase. The assay was adapted as follows to a microtiter plate system using premixed reagents from a commercially available kit (Sigma no. 726-UV). Unconditioned or conditioned media (100 μl), or varying volumes (5–60 μl) of pyruvic acid standard (0.45 mM) were diluted in the microtiter plate wells to a volume of 100 μl after which an additional 100 μl of NADH (0.67 mM) in Tris buffer (1.5 M) was added. Initial absorbance (340 nm) was then read in a UVmax plate reader after which 25 μl lactate dehydrogenase solution (160 U/ml) was added to initiate the reaction. After a 15-min incubation period at ambient temperature, final absorbance (340 nm) was read and the change in absorbance was calculated for each sample. Pyruvate concentrations were calculated from the standard curve.

Glucose uptake assay

The rate of glucose transport was measured using the glucose analogs [1,2-^{3}H]2-deoxy-D-glucose or [^{3}H]3–0-methyl glucose. Plated, pretreated cells were washed (×3) with HEPES buffer, pH 7.4, and the final wash immediately replaced with 1 ml of the reaction mix containing 1 mM of ^{3}H-labeled and unlabeled substrate. The dishes were then placed on a rotary shaker and incubated at room temperature. At the end of the reaction time (up to 15 min), the reaction mixture was aspirated off and the cells quickly washed (×4) with stopping solution [NaCl (0.9%); $HgCl_2$ (10^{-5} M); phloretin (10^{-4} M)]. The cells were allowed to dry overnight, then dissolved in NaOH (0.1 M). A portion of each sample was transferred to a plastic counting vial, scintillation fluor added, and the radioactivity determined. Nonspecific uptake (accounting for nontransporter-mediated transmembranal glucose passage) was measured for each time point by incubating the cells with a mixture of ^{3}H-labeled and unlabeled L-glucose (final concentration = 1 mM). Net specific uptake was calculated by subtracting the nonspecific from the total uptake, the results being expressed as pmol/min·mg protein.

Data analysis

Data are presented as the mean ± SE of replicate experiments (n noted on figures), each performed in duplicate. Statistical significance is denoted in the figures and was determined by ANOVA analysis for comparison of multiple groups (Fisher's protected least significant difference) or by *t* test for comparison of two groups, using Statview 512+ for MacIntosh (Brain Power, Inc., Calabasas, CA).

Results

IL-1β-stimulated lactate accumulation and glucose consumption by cultured whole ovarian dispersates: dose dependence and specificity

To determine the effect of treatment with IL-1β on glucose consumption and lactate generation, whole ovarian dispersates were cultured for 96 h under serum-free conditions in the absence or presence of increasing concentrations (0.1–50 ng/ml) of IL-1β. As shown (Fig. 1), the basal consumption of glucose and the formation of lactate over the 96-h culture period were 2.6 ± 0.5 and 5.7 ± 1.1 mM, respectively. Treatment with IL-1β produced dose-dependent increments in lactate accumulation (*open circles*) and glucose consumption (*closed circles*), the first statistically significant ($P < 0.05$) increments occurring at IL-1β concentrations of 0.5 and 1.0ng/ml, respectively. A maximal IL-1β effect (3- to 4-fold increase over untreated controls) was noted at 3–10 ng/ml, with an ED_{50} in the range of 0.3 to 0.4 ng/ml of IL-1β. In this and subsequent experiments, it is apparent that at maximal IL-1β stimulation, lactate accumulation (in molar terms) is 2- to 3-fold that of glucose consumption. This suggests that all available glucose is converted to lactate but that other substrates for lactate production may also exist.

Cellular lactate concentrations were also determined after rapid (<10 sec) washing and acidification of cells (to prevent further metabolism). Under these conditions, no lactate could be detected, suggesting that lactate is efficiently secreted, most likely via the monocarboxylate transporter (40).

The pH of media (normally pH 7.4) conditioned by IL-1β-treated cells was lowered by approximately 0.5 U as evidenced by the distinct yellowish color of the phenol red indicator dye. Media acidity apparently reflected the accumulation of lactic acid since IL-1β-treated cells that did not accumulate lactate (*e.g.* those cultured in glucose-free media, see below) did not demonstrate a pH change. The change in pH did not account for the metabolic alterations under study in that qualitatively comparable findings were documented in appropriately buffered (pH stable) media (not shown).

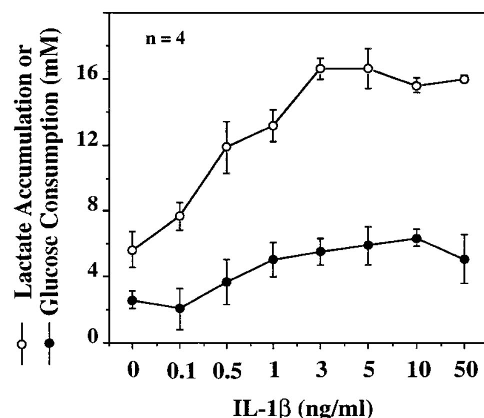

FIG. 1. IL-1β-mediated glucose consumption and lactate accumulation by cultured whole ovarian dispersates: dose response. Whole ovarian dispersates (5 × 10^5 viable cells/dish) from immature rats were aerobically cultured for 96 h in the absence or presence of increasing concentrations (0.1–50ng/ml) of IL-1β. At the conclusion of this treatment period, medium lactate accumulation (*open circles*) and glucose consumption (closed circles) were determined as described under *Materials and Methods*.

The specificity of the IL-1β metabolic effect was assessed using two other established ovarian agonists (34, 35). In contrast to IL-1β, maximally effective doses of TNFα (a cytokine possessing an overlapping range of action) or insulin (an established metabolic hormone) were unable to stimulate glucose consumption or lactate accumulation (Fig. 2). The inability of insulin to promote ovarian glucose consumption is in keeping with preliminary data (not shown) suggesting that the ovary does not express insulin-regulatable glucose transporters.

IL-1β-stimulated aerobic glycolysis: Glucose dependence and receptor mediation

To assess the possibility of lactate-generating sources other than glucose, to ascertain the role of the ambient glucose concentration, and to examine the role of the IL-1 receptor, whole ovarian dispersates were cultured for 96 h in the absence or presence of IL-1β, with and without the addition of a naturally occurring IL-1 receptor antagonist (IL-1RA). In addition, cultures were incubated in the absence or presence of various concentrations of glucose (1–3 g/liter). Under glucose-free conditions (Fig. 3), no lactate was produced, demonstrating that media glucose is required for IL-1β-induced lactate accumulation. This phenomenon could not be attributed to hypoglycemia-induced cellular dysfunction since cells grown in the absence of glucose were judged normal by the following criteria: normal cellular morphology, IL-1β-stimulated nitric oxide synthase activity, IL-1β-stimulated glucose transporter transcripts, and stable transcripts corresponding to ribosomal large protein 19, a housekeeping gene (Kol S., and Ajashi E., unpublished observations).

The IL-1β effect on glucose consumption and lactate accumulation was apparent over a broad range of glucose

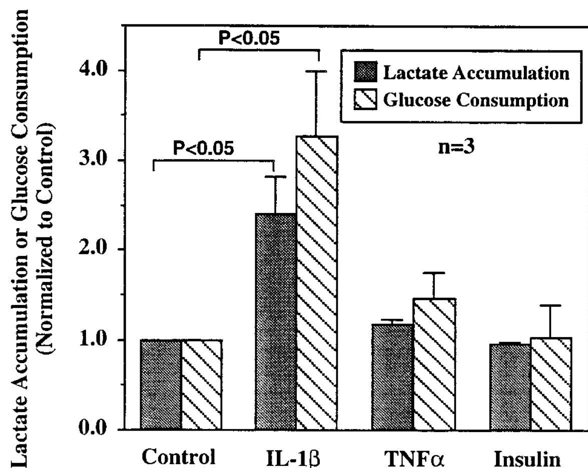

FIG. 2. IL-1β-induced aerobic glycolysis: specificity. Whole ovarian dispersates (5×10^5 viable cells/dish) were aerobically cultured for 96 h in the absence or presence of IL-1β (50 ng/ml), TNFα (10 ng/ml), or insulin (1 μg/ml) after which medium lactate concentration (*shaded bars*) and glucose consumption (hatched bars) were determined.

concentrations (Fig. 3; *solid circles*) inclusive of the putative physiological window (100–200 mg/dl; 41). The IL-1β-induced metabolic effect was glucose dose dependent up to 2 g/liter. No further increases were observed at the concentration of glucose in McCoy's 5a media (3 g/liter) that was used for most of the experiments reported herein.

The IL-1β action on glucose consumption and lactate accumulation was receptor-mediated because addition of IL-1RA (Fig. 3; *open triangles*), a reagent previously validated at the ovarian level (42), blocked the IL-1β effect thereby approximating control levels (*closed triangles*) at all glucose concentrations. Treatment with IL-1RA alone (*open squares*) was without significant effect.

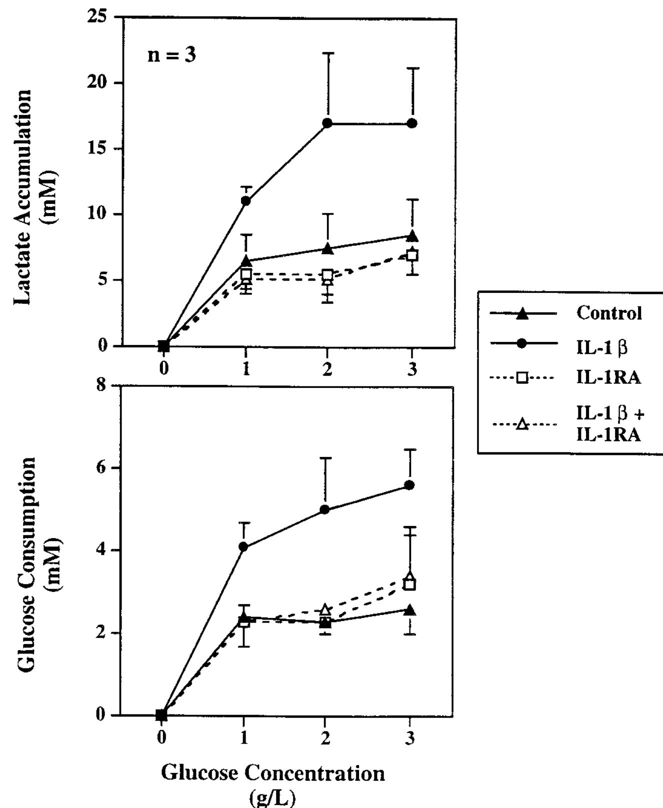

FIG. 3. IL-1β-induced aerobic glycolysis: Glucose dependence and receptor mediation. Whole ovarian dispersates (5×10^5 viable cells/dish) were aerobically cultured for 96 h using the following treatment paradigms at each of the noted glucose concentrations (0, 1, 2, 3g/liter): Control (no treatment); IL-1β (50ng/ml); IL-1RA (IL-1 receptor antagonist; 5 μg/ml); IL-1β+IL-1RA (50ng/ml and 5 μg/ml, respectively). At the conclusion of the treatment period, medium lactate concentration (*upper panel*) and glucose consumption (*lower panel*) were determined.

Comparison of lactate accumulation and glucose consumption under IL-1β-replete and IL-1β-deplete conditions

To compare the relative contribution of glucose consumption to lactate accumulation at the extremes of IL-1β exposure, whole ovarian dispersates were cultured for varying durations with either IL-1β (IL-1β-replete) or a maximally inhibitory dose of IL-1RA (IL-1β-deplete). Exposure of whole ovarian dispersates to maximally stimulatory levels of IL-1β markedly increased the molar ratio of lactate produced to glucose consumed. The resultant ratio (2.5 $\pm$ 0.2; range 2.2–2.9; n = 3) was higher than 2 (the ratio expected if glucose is completely converted to lactate), suggesting, consistent with previous reports (16, 43), that substrates other than glucose may be used for lactate production. Nevertheless, if other sources (*e.g.* amino acids) exist, they are only processed in the presence of glucose because no lactate is produced in the total absence of glucose (cf. Fig. 3). All told, these data highlight the remarkable efficiency of the IL-1β-stimulated conversion of glucose to lactate. In contrast, given IL-1β-deplete conditions, *i.e.* an IL-1β vacuum created by the addition of IL-1RA (42), the ratio of lactate accumulation to glucose consumption, (0.99 $\pm$ 0.4; range 0.5–1.8; n = 3) suggests a higher rate of oxidative phosphorylation or the possible diversion of glucose to nonglycolytic pathways. The large variability noted in this ratio for IL-1RA-treated cultures probably reflects the fact that the absolute consumption of glucose and the production of lactate in this state is very low, the values in question being at the limit of detection. These data also emphasize the stimulatory effect of both endogenous and exogenous IL-1β on ovarian glycolysis.

IL-1β-stimulated aerobic glycolysis: time and cell density dependence

To evaluate the time requirements of the IL-1β effect, whole ovarian dispersates were cultured for the duration indicated in the absence or presence of a maximal stimulatory dose of IL-1β. As shown (Fig. 4), treatment with IL-1β resulted in marked enhancement (of up to 2.2-fold) of both parameters beginning with the 48-h time point. Whereas treatment with IL-1β produced a statistically significant increment in lactate accumulation by 48 h, significant alteration in glucose consumption was only documented by the 72 h time point. No IL-1β effect was noted for a series of time points during the first 48 h (not shown). This initial delay is suggestive of the induction of an intermediate factor(s) between IL-1β stimulation and the final endpoint of glucose

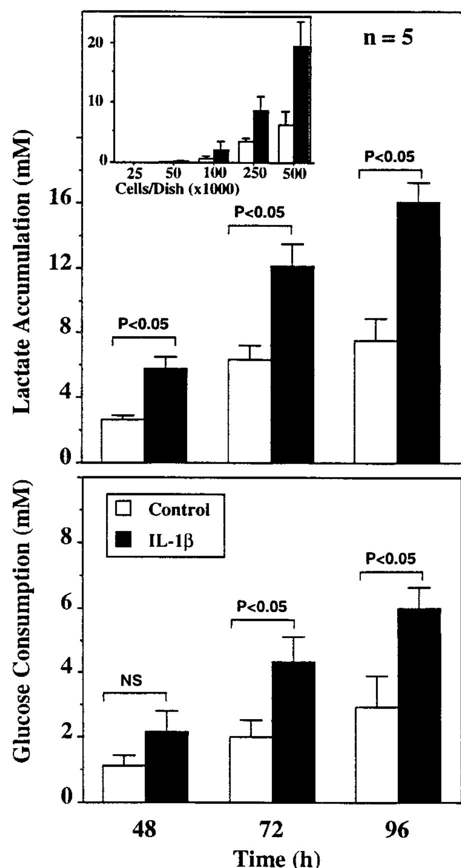

FIG. 4. IL-1β-induced aerobic glycolysis: time- and cell density dependence. Whole ovarian dispersates (5×10^5 viable cells/dish) were aerobically cultured for up to 96 h in the absence or presence of IL-1β (50ng/ml), after which medium lactate concentration (upper panel) and glucose consumption (lower panel) were determined. *Inset:* Cells were cultured in the absence or presence of IL-1β (50ng/ml) for 96 h at different cell densities (0.25–500 $\times 10^3$ cells/dish).

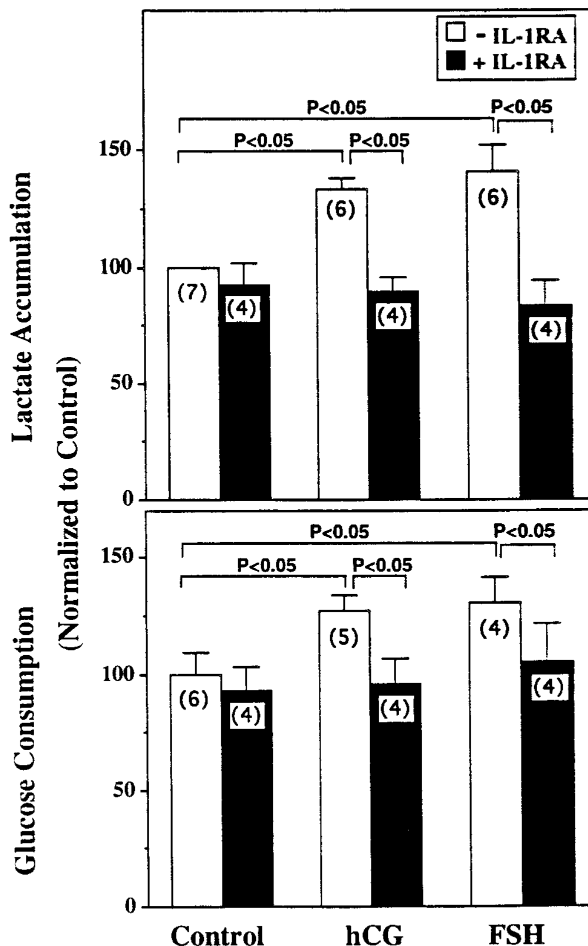

FIG. 5. Gonadotropin-stimulated glucose consumption and lactate accumulation. Whole ovarian dispersates (5×10^5 viable cells/dish) were aerobically cultured for 96 h in the absence or presence of hCG (10 ng/ml) or FSH (100 ng/ml), with or without IL-1RA (5 μg/ml). At the conclusion of the treatment period, medium lactate concentration (*upper panel*) and glucose consumption (*lower panel*) were determined (n is noted *in parentheses* for each treatment group).

consumption and lactate production. In fact, when cells were cultured in the presence of cycloheximide (0.1 μg, an inhibitor of protein synthesis), IL-1β-stimulated glucose consumption and lactate accumulation was completely inhibited (n = 3 experiments; not shown). These data suggest that protein synthesis is required for IL-1β-driven glycolysis.

Increases in glucose consumption and lactate accumulation were also cell density dependent (Fig. 4; *inset* for lactate only), over a cell density range of 0.25 to 5×10^5 cells/ml. However, a statistically significant ($P < 0.01$, n = 4) difference between control and IL-1β-treated cells was observed only at a cell density of 5×10^5 cells/ml.

Effect of treatment with gonadotropins on glucose consumption and lactate accumulation: intermediary role of endogenous IL-1β

Because gonadotropins have previously been implicated in the induction of the midcycle metabolic shift (14–24), we examined the impact of treatment with gonadotropins on ovarian glycolysis as well as on the intermediary paracrine role of endogenous IL-1β in this regard. Whole ovarian dispersates were cultured for 96 h in the absence or presence of maximally stimulating doses of hCG or FSH, with or without IL-1RA. As shown (Fig. 5), treatment with either FSH or hCG produced significant ($P < 0.05$), albeit modest (30–40%), increments in glucose consumption and lactate accumulation. Furthermore, the concurrent provision of IL-1RA, significantly ($P < 0.05$) reduced the FSH and hCG effects to below control levels thereby implicating endogenous IL-1-like activity as an intermediary in this process.

The role of cell-cell interaction in IL-1β-induced aerobic glycolysis

To assess the relative contribution of the different populations of ovarian somatic cells to the IL-1β-induced metabolic effect, whole ovarian dispersates, isolated granulosa cells, purified theca-interstitial cells, or contact-dependent cocultures thereof were cultured for 96 h in the absence or presence of IL-1β. As shown (Fig. 6), treatment of whole ovarian dispersates with IL-1β, resulted in 1.5- and 2.2-fold increments ($P < 0.05$) in glucose consumption and lactate accumulation, respectively (cf Fig. 1). In contrast, treatment

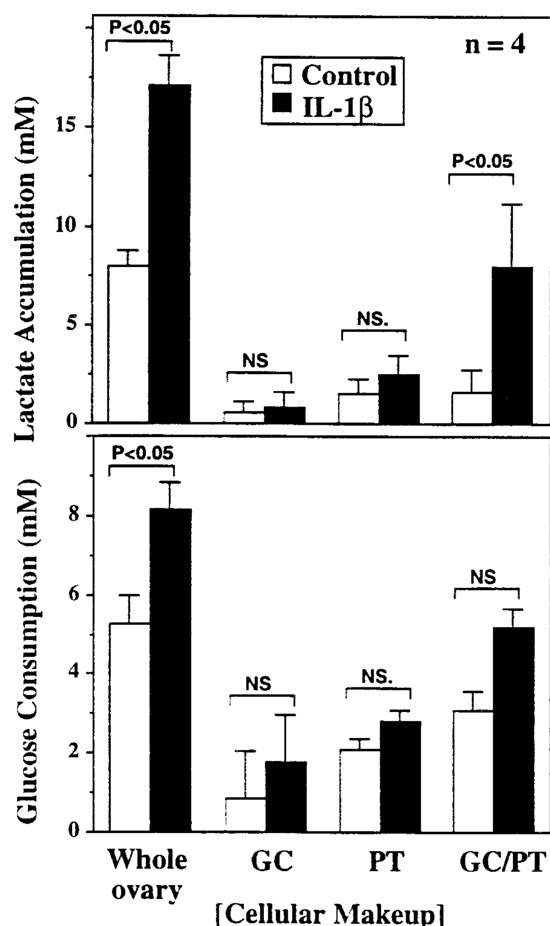

FIG. 6. Cell-cell interaction in IL-1β-induced aerobic glycolysis. Whole ovarian dispersates (5×10^5 cells), purified granulosa (GC; 4×10^5 cells) or purified theca-interstitial (PT; 1×10^5 cells/ml) cells were prepared as described in *Materials and Methods* and aerobically cultured separately or in mixture (GC/PT) for 96 h in the absence or presence of IL-1β (50 ng/ml) after which media were analyzed for lactate concentration (*upper panel*) or glucose consumption (*lower panel*).

of isolated cellular populations of either granulosa cells or purified theca-interstitial cells produced negligible increments in glucose consumption and lactate accumulation. However, contact-dependent cocultures of these two cell types (granulosa cells/purified theca-interstitial cells), reconstituted at a putative physiological cellular ratio (4:1), allowed IL-1β to produce significant ($P < 0.05$) marked increments in lactate accumulation (4.9-fold), an increase greater than expected from simple additivity. Alterations in glucose consumption followed a similar trend but did not reach statistical significance.

Media pyruvate accumulation

The conversion of pyruvate to lactate by lactate dehydrogenase is the final metabolic step in glycolysis. Because pyruvate is the putative obligatory energy source for the developing denuded oocyte (26–29), we examined the effect of treatment with IL-1β on pyruvate accumulation in media conditioned by whole ovarian dispersates (Fig. 7). Media pyruvate content was considerably lower (two orders of

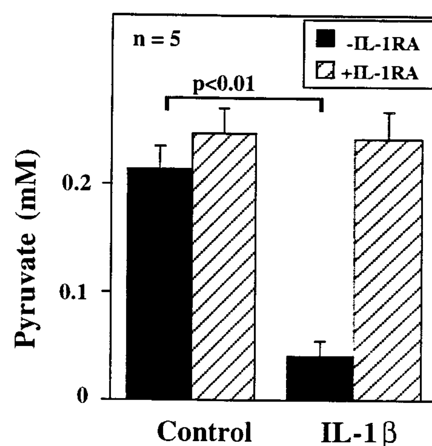

FIG. 7. IL-1β-mediated pyruvate depletion. Whole ovarian dispersates (5×10^5 viable cells/dish) from immature rats were aerobically cultured for 96 h in the absence or presence of a maximally stimulating dose of IL-1β (50 ng/ml), with and without IL-1RA (5 μg/ml) after which media pyruvate concentrations were determined.

magnitude) than lactate content in control cultures. Treatment with IL-1β resulted in an 81% reduction in media pyruvate accumulation, an effect reversed by the concurrent application of IL-1RA. The ability of IL-1β to inhibit pyruvate accumulation was time, dose, and cell density dependent (not shown). These data suggest that IL-1β is capable of modulating the pyruvate content of conditioned media in a direction opposite to that observed for lactate and that this effect is also receptor-mediated. The decline in media pyruvate levels could not be ascribed to pyruvate instability because the content of pyruvate added to unconditioned (no cell) media was unchanged after a 96-h incubation period.

The effect of treatment with IL-1β on glucose uptake by cultured whole ovarian dispersates

Glucose transport capacity constitutes a major regulatory step in the ability of tissues to increase their glucose utilization. To assess whether the IL-1β-mediated increase in glucose consumption reflects an increase in glucose uptake, whole ovarian dispersates were incubated for 48 h in the absence or presence of a maximal stimulatory dose of IL-1β. At the conclusion of this incubation period, cellular glucose uptake capacity was determined over a 15-min period. As shown (Fig. 8, *left panel*), the rate of specific [^{3}H]2-deoxy-D-glucose uptake by control cells was linear throughout. Treatment with IL-1β markedly increased the rate of glucose uptake, demonstrating an initial linear response for the first few min, and a progressive increase in [^{3}H]2-deoxy-D-glucose uptake towards an apparent plateau. These findings indicate that the IL-1β-induced increase in glucose consumption was attributable, in part, to an increase in glucose uptake.

The rate of cellular glucose uptake was determined with varying concentrations (0.5–5.0 mM) of the [^{3}H]2-deoxy-D-glucose substrate. Eadie-Hofstee transformation of the data (Fig. 8, *right panel*) disclosed a modest elevation in K_m (1.7-fold) and an increase in V_{max} of 3.6-fold in IL-1β-treated as compared with control cells. Although the variance for V_{max} was high for both groups (mean ± SEM for controls = 6.5 ± 2.0 nmol/mg protein/min and for IL-1β-treated cells =

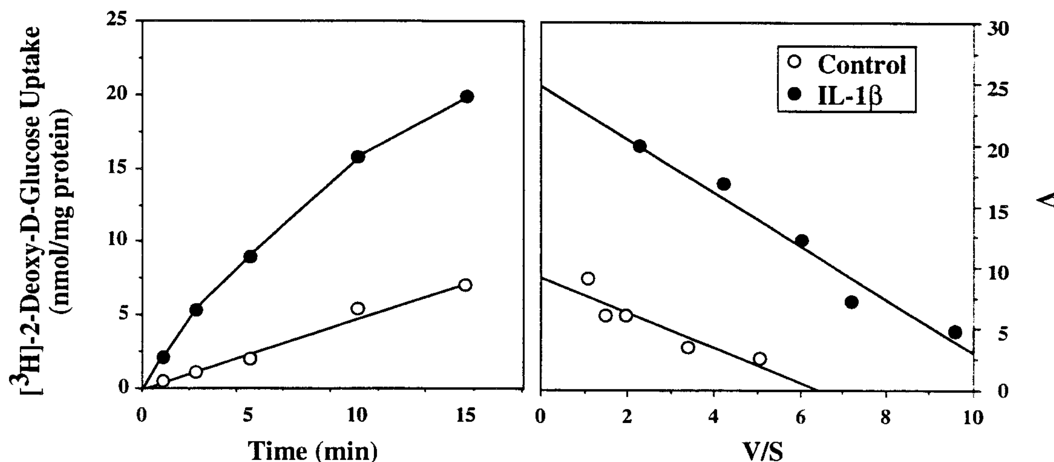

FIG. 8. IL-1β-stimulated glucose uptake. Whole ovarian dispersates (5×10^5 viable cells/dish) were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml). At the conclusion of this incubation period, cellular glucose transport was determined as described under *Materials and Methods*. *Left panel*, Glucose uptake as a function of time (a representative experiment from two independent experiments is shown); *right panel*, the rate (V) of cellular glucose transport was determined using different concentrations (0.5–5.0 mM) of the [1,2-^{3}H]2-deoxy-D-glucose substrate (S). The Eadie-Hofstee transformation of the data (*right panel*) represents one experiment from three similar, independent experiments.

22.6 ± 6.7 nmol/mg protein/min), the V_{max} was elevated in the IL-1β-treated group in each experiment. The mean ratio of IL-1β/control was significantly higher than zero ($P < 0.05$) for both the K_m and the V_{max}. The increase in V_{max} suggests an increase in the number of transporter proteins in IL-1β-treated cells. The change in the apparent Km calculated in these experiments should be viewed with caution since this result may reflect, in part, the dissociation constant of 2-deoxy-D-glucose for hexokinase, rather than for the glucose transporters (44). Preliminary experiments using [^{3}H]3–0-methyl glucose, a nonphosphorylatable substrate, also disclosed elevated V_{max} values for IL-1β-treated cells as compared with controls (not shown).

Discussion

Treatment with IL-1β induces a significant increase in ovarian glucose consumption (and uptake) as well as in media lactate accumulation. Because the relative accumulation of lactate can be taken as a crude reflection of the intensity of the glycolytic process, the above constellation indicates a shift in cellular metabolism toward the glycolytic processing of glucose despite ample oxygen supply. The ovarian shift toward aerobic glycolysis mimics a similar shift that has long been noted in proliferating and tumor cells (45, 46).

Characterization of the IL-1β metabolic effect revealed it to be receptor mediated (Fig. 3) and dose (Fig. 1), time (Fig. 4)- and cell density (Fig. 4; *inset*) dependent but glucose concentration-independent (Fig. 3). The IL-1β action was relatively specific in that other ovarian effectors, TNFα (35), and insulin (34) were incapable of producing a similar effect (Fig. 2). Of particular note is the lack of effect by insulin on glucose consumption in the ovary. These data suggest that ovarian glucose transporters do not include the insulin-coupled variety (glucose transporter 4).

Importantly, the IL-1β-induced increase in glucose consumption was associated with an increase in glucose uptake (Fig. 8). This phenomenon could be attributable to an increase in the number of glucose transporter proteins in IL-1β-treated ovarian cells. Such a possibility is supported by the observation that IL-1β up-regulates the steady-state levels of transcripts and of proteins corresponding to several glucose transporters in a number of extraovarian cell types (47, 48).

The ability of IL-1β to bring about a metabolic shift in ovarian cells was time dependent but not immediate (Fig. 4). Neither glucose consumption nor lactate generation could be detected up to 24 h in culture even in the presence of IL-1β. The promotion of glucose uptake and the induction of the glycolytic state may require an as-yet-to-be defined set of transcriptional/translational events without that the cellular reprogramming may not be realized. For example, the modulation of distinct species of glucose transporter(s), monocarboxylate transporters, glutamate transporter(s), and/or of select glycolytic and oxidative enzymes may take place. Studies are currently underway to examine these possibilities.

Our observation that the IL-1β-induced metabolic shift in ovarian glucose utilization is reversed by IL-1RA (Fig. 3) is an indication that this effect is receptor-mediated. In addition, treatment with IL-1RA attenuated the metabolic actions of FSH and hCG (Fig. 5), suggesting the existence of endogenously produced, IL-1-like bioactivity. Given the ability of LH/hCG to increase the steady state levels of intraovarian IL-1β transcripts in the preovulatory rat ovary (6), it is conceivable that the *in vivo* ability of the midcycle gonadotropin surge to effect a metabolic shift may be due, in part, to the preovulatory induction of intraovarian IL-1β.

Our findings (Fig. 6) suggest that the full magnitude of the metabolic shift requires both cellular components of the follicle (*i.e.* the granulosa and theca-interstitial cells). These observations are in keeping with previous reports that the ovarian action of IL-1β is contingent on contact-independent cell-cell cooperation (11–13).

A metabolic shift of the nature we describe in this study may be the result of multiple regulatory effects on several

metabolic pathways. Indeed, significant differences were noted in the molar ratio of lactate produced to glucose consumed when comparing IL-1β-replete and IL-1β-deplete circumstances. In the presence of maximally stimulating levels of IL-1β, 2.5 ± 0.2 lactate molecules were produced per glucose molecule consumed. In an IL-1RA-induced IL-1β vacuum, this ratio was only 0.9 ± 0.4. These observations suggest that in the presence of IL-1β, every available glucose molecule is converted to lactate (expected ratio of 2). That the ratio is even higher (2.5 ± 0.2) suggests, consistent with previous reports (16, 43), that lactate-generating sources other than glucose may have been used in IL-1β-treated cultures. The exact nature and relative contribution of such accessory precursors (*e.g.* glutamine, alanine, glycogen, or other substrates) is of interest and is currently under active investigation in our laboratory.

Our data further demonstrate an IL-1β-mediated decrease in media pyruvate accumulation (Fig. 7), concordant with the IL-1β-mediated increase in lactate accumulation. This decline in pyruvate most likely reflects a change in the redox state of the cell, resulting from IL-1β-stimulated glycolysis. Indeed, the glycolytically induced depletion of NAD would be expected to drive the conversion of pyruvate to lactate to generate additional NAD to maintain the glycolytic process. However, pyruvate depletion could also be related to the activation and/or utilization of other metabolic pathways. For example, IL-1β-treated cells may increase the uptake of pyruvate into the tricarboxylic acid cycle via pyruvate carboxylase, its conversion to alanine, or its utilization as a metabolic scavenger of oxygen-free radicals. *In vivo*, the requisite utilization of pyruvate by the glycolytically incompetent oocyte may drive the depletion of pyruvate from the glycolytically competent somatic cells.

The mechanism by which IL-1β provokes the transition to glycolysis remains to be determined. It may be that the IL-1β-stimulated increase in glucose uptake (Fig. 8) triggers the glucose-mediated enhancement of aerobic glycolysis as described by Crabtree (49). Whether IL-1β also exerts direct stimulatory effects on glycolytic enzymes is a subject for future investigation.

In summary, these observations lend support to the notion that IL-1β serves as a mediator of the gonadotropin-associated midcycle shift in ovarian carbohydrate metabolism. The rationale for such a shift remains a mystery. It could be argued that the conversion of the somatic ovarian cell into a glycolytic machinery is necessary to meet the energy needs of the maturing but glycolytically inactive (26–29) oocyte, which depends on extracellular (cumulus cell-derived) sustenance (50–54) in general and on monocarboxylate glycolytic products in particular (26–29). Conceivably, the relatively hypoxic oocyte environment (25) may require a sparing of the utilization of oxygen by the tricarboxylic acid cycle thereby conserving oxygen for other vital tasks such as steroidogenesis. Leese *et al.* (43) have suggested that the early shift toward aerobic glycolysis may prepare embryos for the relatively anoxic environment of implantation. Alternatively, metabolic antioxidants, such as pyruvate (55), may protect the oocyte from oxygen-free radicals, which may be generated during the dynamic periovulatory period. Undoubtedly, other possibilities exist. In any case, these studies, for the first time, implicate IL-1β as a key player in modulating the energy needs of the cycling ovary, a topic first addressed in 1962 (14).

Acknowledgment

The authors wish to thank Ms. Cornelia T. Szmajda for her assistance in the preparation of this manuscript and Ms. Susan Lautar for able technical assistance. We also thank Dr. Ian Simpson (NIH, MD), Dr. Steven Downs (Marquette University, Milwaukee, WI), Dr. Jerome F. Strauss (University of Pennsylvania, Philadelphia, PA), and Dr. Karl Brand (University of Erlangen-Nurenberg, Erlangen, Germany) for valuable advice and discussions.

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