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Interleukin-1β Stimulates Ovarian Phospholipase A_2 (PLA_2) Expression and Activity: Up-Regulation of Both Secretory and Cytosolic PLA_2*

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ABSTRACT

Interleukin (IL)-1β has been shown to stimulate ovarian prostaglandin biosynthesis. We hypothesized that this effect entails the induction of phospholipase A_2 (PLA_2). Treatment of cultured whole ovarian dispersates of immature rat origin with IL-1β produced significant increases in [^{3}H]arachidonic acid (AA) release and [^{3}H]prostanoid accumulation as well as increases in cellular PLA_2 activity and in secretory PLA_2 and cytosolic PLA_2 transcripts. Cotreatment with IL-1 receptor antagonist reversed IL-mediated (and basal) release of [^{3}H]labeled AA and prostaglandin products, as well as cellular PLA_2 activity. Treatment with IL-1β also promoted a significant decrease in the cellular content of [^{3}H]phospholipids (apparently phosphatidylethanolamine but not phosphatidylcholine). These observations establish the ovary as a site of IL-1-dependent $sPLA_2$ and $cPLA_2$ gene expression, document the presence of a possible phosphatidylethanolamine-dependent PLA_2 activity in cultured whole ovarian dispersates, reveal the up-regulatory, receptor-mediated action of IL-1β in this regard and suggest the existence of endogenous PLA_2-stimulating/IL-1-like bioactivity. (*Endocrinology* **138:** 314–321, 1997)

THE ENZYME PHOSPHOLIPASE A_2 (PLA_2) catalyses the hydrolysis of the *sn-2* fatty acyl chain of phospholipids. Cleavage of the *sn-2* arachidonic acid (AA) residue of phospholipids provides the substrate for the biosynthesis of eicosanoids thereby giving rise to potent proinflammatory mediators [*i.e.* prostaglandins (PG), leukotrienes]. Consequently, it is the release of AA that is considered to be the rate-limiting event in the eicosanoid production cascade (1). Evidence to date (reviewed in Refs. 2–4) suggests that mammalian PLA_2 is a heterogeneous family of enzymes including two classes of proteins: secretory, low mol mass (14 kDa, $sPLA_2$), and cytosolic, high mol wt (85–110 kDa, $cPLA_2$). The former is further subclassified into a digestive group I (synthesized and secreted mainly by the pancreas), and a nondigestive group II (synthesized and secreted by many cell types).

It has been hypothesized that ovulation may constitute a cyclic inflammatory-like process (5) and that gonadotropin-inducible intraovarian interleukin-1 (IL-1; 6), an established mediator of inflammation (7), may play a central role in this regard (8–11). The observation (12–14) that IL-1 is capable of stimulating ovarian PG biosynthesis ($PGE_2 > PGF_{2\alpha}$), an established mediator of the ovulatory cascade (15), lends additional support to the notion that ovulation is an inflammatory-like process and that IL-1 may participate in this process.

It was the objective of this communication to determine whether the ability of IL-1β to stimulate PG biosynthesis involved and/or required the intervention of PLA_2. Our findings establish IL-1β as an up-regulatory agent for $sPLA_2$ and $cPLA_2$ gene expression, document the presence of a possible phosphatidylethanolamine (PE)-dependent PLA_2 activity in cultured whole ovarian dispersates from immature rats, reveal the up-regulatory, receptor-mediated, and time-dependent action of IL-1β in this regard, and suggest the existence of endogenous PLA_2-stimulating/IL-1-like activity. These observations support the proposition that PLA_2 is a key component in the IL-1-stimulated biosynthesis of ovarian prostaglandins.

Materials and Methods

Reagents and hormones

Phosphatidylcholine Lα-1-stearoyl-2-arachidonyl [arachidonyl-5,6,8,9,11,12,14,15-^{3}H(N), PCSA, 88 Ci/mmo]; $PGF_{2\alpha}$ [5,6,8,9,11,12,14,15-^{3}H(N), 200 Ci/mmol], PGE_2 [5,6,8,9,11,12,14,15-^{3}H(N), 154 Ci/mmol]; AA [5,6,8,9,11,12,14,15-^{3}H(N), 100 Ci/mmol] and [α-^{32}P] UTP (800 Ci/mmol) were purchased from DuPont, New England Nuclear (Boston, MA). The corresponding unlabeled compounds, RNase A, indomethacin, phosphatidylinositol (PI) and phosphatidylethanolamine (PE) were purchased from Sigma Chemical Co. (St. Louis, MO). McCoy's 5a (serum-free) medium, penicillin-streptomycin solution, BSA, DNase, Moloney Murine Leukemia Virus reverse transcriptase, and trypan blue stain were obtained from Life Technologies, Inc. (Grand Island, NY). Collagenase (Clostridium Histolyti-

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cum; CLS type I; 144 U/mg) was from Worthington Biochemical Corp. (Freehold, NJ). Hexane, methanol, and 2-propanol were from J. T. Baker, Inc. (Phillipsburg, NJ). Chloroform was from Fisher Scientific (Fair Lawn, NJ). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). RNase T1 was from Pharmacia (Piscataway, NJ). Ecl136II was from New England Biolabs, Inc. (Beverly, MA). T7 RNA polymerase, pGEM7Zf+ and other molecular grade reagents were from Promega (Madison, WI).

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). A recombinantly expressed preparation of the naturally occurring human IL-1 receptor antagonist (IL-1RA) was generously provided by Dr. Daniel E. Tracey (The Upjohn Co., Kalamazoo, MI).

Animals and cell culture

Immature (25 to 28 day old) Sprague-Dawley female rats, purchased from Zivic-Miller Laboratories (Zelienople, PA), were killed by CO_2 asphyxiation.

Whole ovarian dispersates were prepared by collagenase digestion as previously described (16) and were cultured in serum-free McCoy's 5a medium supplemented with L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin sulfate (100 μg/ml). Cells were cultured in either 12 × 75 mm tubes (1 ml media) or in dishes (1–3 ml media) as noted and were maintained for up to 48 h at 37 C under a water-saturated atmosphere of 95% air and 5% CO_2.

Arachidonic acid release assay

Whole ovarian dispersates (5×10^5 viable cells/dish) were initially pulse-labeled with [^{3}H]AA (0.2–0.4 μCi/ml) for 24 h to allow for the incorporation of the labeled free fatty acid into esterified lipids. At the end of the incubation period, unincorporated label was removed by washing the cells three times with fresh McCoy's 5a medium. The cells were then reincubated for the duration indicated in the absence or presence of specified treatments. At the conclusion of this treatment period, conditioned media were collected, acidified, and extracted (twice) with 3 ml ethyl acetate. The latter solvent was then evaporated in a Speed-Vac centrifuge (Savant Instruments, Inc., Farmingdale, NY), and the residue resuspended in 250 μl hexane/isopropanol/acetic acid, 95/5/0.025 (vol/vol/vol). The resultant products were then fractionated by HPLC using a Waters HPLC system (Milford, MA) on a normal phase diol column (LiChrosorb Diol, 10 μm; EM Reagents, Gibbstown, NJ) with a concave gradient of hexane/isopropanol (95:5 to 60:40) at 2 ml/min. The column was calibrated with authentic [^{3}H]AA, [^{3}H]PGE_2 and [^{3}H]$PGF_{2\alpha}$. Radiolabeled AA and major eicosanoid products (PGE_2 and $PGF_{2\alpha}$) were detected and quantified by on-line scintillation counting with a Radiomatic Flow Detector (Packard Instrument Co., Meriden, CT).

TLC of [^{3}H]AA-labeled cellular phospholipids

[^{3}H]AA-labeled cells were sonicated on ice (× 2) for 5s (Vibra Cell, Sonics and Materials Inc., Danbury, CT). Thereafter, total cellular phospholipids were extracted with 3 ml chloroform/methanol, 66:33 (vol/vol). The bottom chloroform layer (containing phospholipids) was dried, and the residue resuspended in 10 μl of the same solvent. The latter phospholipid mixture was then separated by one- or two-dimensional TLC (Eastman Kodak, Rochester, NY) using a previously-described (17) solvent system, consisting of chloroform/methanol/acetic acid/water (90/8/1/0.8; vol/vol/vol/vol) in the first dimension, and a chloroform/methanol/7 M ammonium hydroxide (15/6/1; vol/vol/vol/) solvent system in the second dimension. The radioactivity in 1 cm-long segments along the TLC strips was determined using a scintillation counter. Labeled prostanoids and PCSA served as authentic controls. Unlabeled PE and PI also served as authentic controls and were visualized with iodine vapor.

Cell-free PLA_2 enzyme assay

Total cellular PLA_2 activity was determined by measuring the release of [^{3}H]AA from the *sn*-2 position of [^{3}H]PCSA substrate. Whole ovarian dispersates (5×10^5 viable cells/tube) were initially cultured as described for 48 h in the absence or presence of the specified treatments. Media were then removed, the cells washed once with 100 mM HEPES, pH 8 buffer, and suspended in 1 ml of the same. Cells were then sonicated on ice (× 2) for 5s as described above. A sonicate volume representing 1.5×10^5 cells was then incubated for 1 h at 37 C in a total assay volume of 1 ml. Assay buffer also contained 5% methanol (to dissolve substrate) and 2 mM $CaCl_2$. Total PCSA substrate concentration was 5 μM inclusive of 25×10^3 cpm of labeled substrate. Preliminary experiments disclosed that neither deoxycholic acid nor presonication of the substrate increased the reaction efficiency, and thus were not used subsequently. The enzymatic reaction was terminated with 7% formic acid (final pH 3.5), and the resultant products were extracted and detected by HPLC as described above. The ratio of labeled product to labeled substrate was multiplied by the initial substrate concentration (5 μM) to calculate the rate of enzymatic conversion to product (pmol/10^5 cells·h). Values were also corrected for substrate availability in the reaction mixture, and for product recovery.

Nucleic acid probes

A plasmid containing a 750-bp *Sma*I/*Eco*RI insert of the complementary DNA (cDNA) encoding rat type II $sPLA_2$ (18) was kindly provided by Dr. J. Ishizaki (Shionogi Research Laboratories, Osaka, Japan). For RNase protection assays, a *Bam*HI fragment was excised and subcloned into pGEM7Zf+. This latter construct was then linearized with Ecl136II and transcribed with T7 RNA polymerase in the presence of [α-^{32}P] UTP to yield a 535-nucleotide (nt) antisense riboprobe which, upon hybridization, was projected to generate a 452-nt protected fragment.

A full-length cDNA of the rat 85-kDa $cPLA_2$ (19) was kindly provided by Dr. Yuji Owada (Tohoku University, Sendai, Japan) in a transcribable vector (pBluescript II SK^+). Because the transcribed region contained a poly(A) tail and several A-rich regions, the cDNA was modified to ensure the generation of a high specific activity riboprobe. Specifically, use was made of a *Bam*HI to remove some of the 3′ end of the cDNA. After self ligation, the product was linearized with *Pvu*II and transcribed with T7 RNA polymerase to yield a 328nt antisense riboprobe which upon hybridization was projected to generate a 253-nt protected fragment corresponding to the translated region of the cDNA. The rat large ribosomal protein 19 (RPL19) normalizing probe was generated as previously described (20).

RNA extraction

Total RNA from cultured cells (1.5×10^6 cells/dish) was extracted with RNAzol B (Tel Test, Friendswood, TX) according to the manufacturer's protocol.

RNAse protection assay

Linearized DNA templates were transcribed with T7 RNA polymerase to specific activities of 800 Ci/mmol [α-^{32}P]UTP ($cPLA_2$ and $sPLA_2$) or 160 Ci/mmol [α-^{32}P]UTP (RPL19). The riboprobes were gel-purified in an effort to eliminate transcribed products shorter than the full length probes. The assay was performed as previously described (21). Gels were exposed to XAR film (Kodak, Rochester, NY) for varying lengths of time with intensifying screens. To generate quantitative data, gels were also exposed to a phosphor screen (Molecular Dynamics, Sunnyvale, CA). The resultant digitized data were analyzed with Image Quant Software (Molecular Dynamics, Sunnyvale, CA). The hormonally independent RPL19 messenger RNA (mRNA) signal was used to normalize the $sPLA_2$ and $cPLA_2$ mRNA data for possible variation in RNA loading. Specifically, the net signal of the $sPLA_2$ or $cPLA_2$ protected band (respective background subtracted)/net RPL19 signal ratio was calculated for each sample.

Statistical analysis

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

Results

[^{3}H]AA incorporation into cellular phospholipids: validation of arachidonic acid release assay

To document the incorporation of labeled AA into cellular phospholipids for use in the arachidonic acid release assay, preliminary experiments were performed to establish the extent to which free [^{3}H]AA remains in the media at the conclusion of the 24 h incorporation period. This approach assumed that the fraction cleared from the media was incorporated into cellular phospholipids. Six dishes were each labeled for 24 h with [^{3}H]AA (3.9×10^5 cpm). The radioactivity remaining in the media averaged 59,805 ± 1,069 cpm, indicating an incorporation rate of 85%. Although this figure is in agreement with observations made in other experimental systems (22, 23), the actual ratio between incorporated and unincorporated AA is probably lower because nonspecific binding of the label to the substratum of the dish is a distinct possibility. Indeed, preliminary characterization of the system revealed that nonspecific association with the plasticware or with dead cells is of the same order of magnitude as incorporation into living cells. A more direct estimate of the extent of [^{3}H]AA incorporation into cellular phospholipids is shown in Fig. 1. The first radioactive peak (Rf = 0.2; *top panel*) comigrated with authentic PCSA (*bottom panel*). Authentic standards for PE and PI also comigrated in the same position (not shown). The second radioactive peak (Rf = 0.9; *top panel*) did not comigrate with any of the other authentic standards tested (AA, PGE_2 or $PGF_{2\alpha}$). This unknown compound may reflect a metabolite of AA (personal communication, Frank Hertelendy, University of St. Louis, St. Louis, MO). Importantly, pulse-labeled cells contained little, if any, free AA. The recovery ratio of labeled cellular phospholipids from AA (*i.e.* counts detected at Rf = 0.2/total counts added) was determined at the end of the incorporation period. The calculated incorporation rate of [^{3}H]AA into phospholipids was only 20%, significantly lower than the above mentioned 85%, and did not increase when BSA (0.5 mg/ml) was added to the labeling medium.

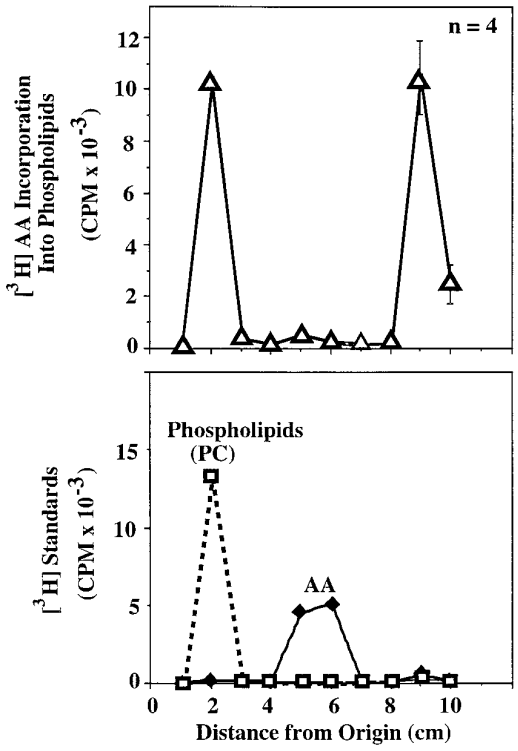

FIG. 1. [^{3}H]AA incorporation into cellular phospholipids. Whole ovarian dispersates were pulse-labeled with [^{3}H]AA for the initial 24 h of the arachidonic acid release assay as described in *Materials and Methods*. After washing, the cells were sonicated, extracted, and the total cellular phospholipids TLC-fractionated and quantified as described under *Materials and Methods*. *Top panel*, Composite data for a total of four independent experiments (mean ± SEM). *Lower panel*, Elution pattern of authentic [^{3}H]AA and a representative radiolabeled phospholipid (PC). PE and PI also comigrated in the same position as PC (Rf = 0.2).

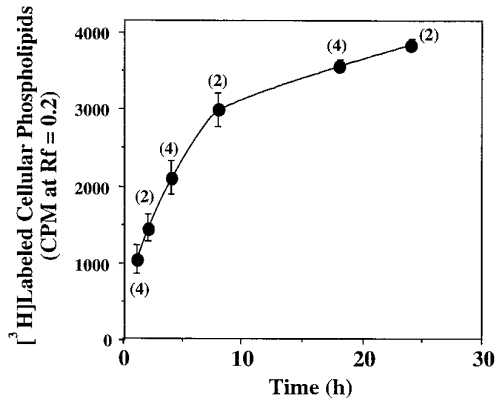

FIG. 2. [^{3}H]AA incorporation into cellular phospholipids: time course. Whole ovarian dispersates were initially pulse-labeled with [^{3}H]AA. At the indicated time points, cells were washed with fresh media, sonicated, and the total cellular phospholipids, extracted, TLC-fractionated in one dimension, and the radioactive peaks corresponding to phospholipids (Rf = 0.2) quantified. Results represent the mean ± SEM (or difference if n = 2) of two or four independent experiments (n shown *in parentheses*).

To establish the optimal duration of cellular labeling, whole ovarian dispersates were labeled with [^{3}H]AA. As shown (Fig. 2), the incorporation of [^{3}H]AA into phospholipids (Rf = 0.2) tended to plateau following 20 h of culture. These findings suggest the adequacy of 24 h of cellular labeling with [^{3}H]AA.

Effect of treatment with IL-1β on the cellular release of AA, PGE_2, and $PGF_{2\alpha}$

To assess the ability of IL-1β to stimulate rat ovarian PLA_2 activity, whole ovarian dispersates were subjected to an arachidonic acid release assay using a dose of IL-1β that maximally stimulates prostaglandin biosynthesis (12). As shown (Fig. 3), treatment with IL-1β produced progressive, time-dependent increments in the accumulation of radiolabeled products as compared with untreated control, reaching statistical significance at the 48 h time point ($P < 0.05$). Further analysis, using HPLC separation of products (Fig. 4), demonstrated that the IL-1β-induced increase in total radioactivity in conditioned media reflected time-dependent increments in [^{3}H]AA, and its downstream metabolites, [^{3}H]labeled PGE_2 and $PGF_{2\alpha}$. Replicate experiments confirmed that most of the [^{3}H]AA released from cells was further metabolized to PGE_2 and $PGF_{2\alpha}$. These observations

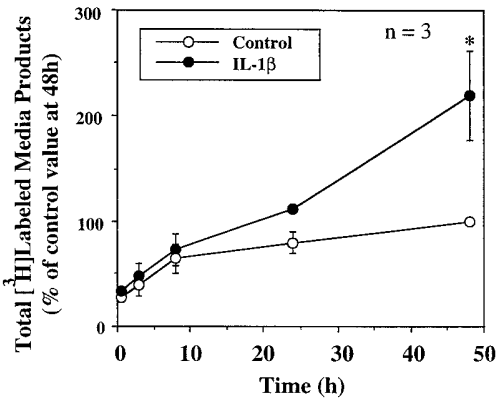

FIG. 3. Effect of treatment with IL-1β on the cellular release of total media products from [^{3}H]AA. Whole ovarian dispersates were subjected, as described in *Materials and Methods*, to an arachidonic acid release assay in the absence or presence of IL-1β. At the indicated time points, conditioned media were removed, and total cpm from ^{3}H-labeled products were determined using a scintillation counter. The results represent the mean ± SEM of three experiments. *, $P < 0.05$ *vs.* control.

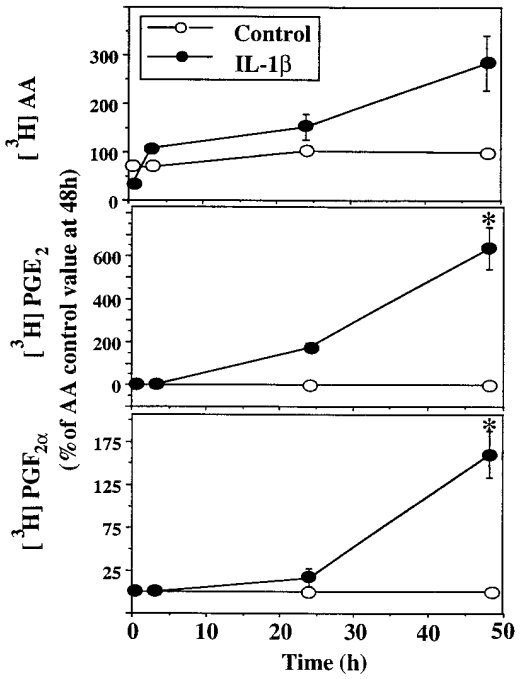

FIG. 4. Effect of treatment with IL-1β on the cellular release of [^{3}H]AA, PGE_2 and $PGF_{2\alpha}$. Whole ovarian dispersates were treated as in Fig. 3 except that, at the indicated time points, media were extracted, HPLC-chromatographed, and the relevant radioactive peaks quantified. The results represent the mean ± SEM of one to four replicates from of a representative experiment. Two additional experiments were confirmatory. *, $P < 0.05$ *vs.* value for 24 h.

suggest that IL-1β increases rat ovarian PLA_2 activity, as well as prostaglandin endoperoxide synthase activity. However, because all medium radioactivity is ultimately derived from the processing of [^{3}H]AA-labeled cellular phospholipids, the radioactivity released as prostanoid metabolites may be operationally referred to as AA released and as such may be used as a reflection of ovarian PLA_2 activity.

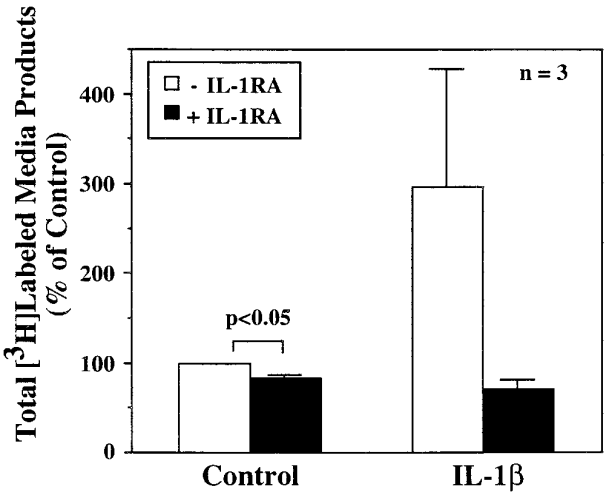

FIG. 5. IL-1β-stimulated increase in ovarian AA release: receptor mediation. Whole ovarian dispersates were treated as in Fig. 3 but were reincubated for the additional 48 h in the absence or presence of IL-1β (50 ng/ml), with or without IL-1RA (5 μg/ml). At the indicated time points, conditioned media were removed, and total cpm from ^{3}H-labeled products were determined using a scintillation counter The results represent mean ± SEM of three experiments.

IL-1β-stimulated increase in ovarian AA release: receptor-mediation

To establish whether or not the ability of IL-1β to enhance ovarian AA release is receptor-mediated, whole ovarian dispersates were subjected to an arachidonic acid release assay in the absence or presence of IL-1β, with or without IL-1RA. As shown (Fig. 5), treatment with IL-1β produced a mean 3-fold increase in [^{3}H]AA release ($P = 0.14$), which was completely blocked by the concurrent addition of IL-1RA ($P = 0.12$). Moreover, treatment with IL-1RA by itself produced a small, albeit significant ($P < 0.05$) decrease in basal [^{3}H]AA release. These findings suggest that the ability of IL-1β to enhance [^{3}H]AA release by cultured whole ovarian dispersates is IL-1 receptor-mediated. These findings also point to the existence of endogenous PLA_2-stimulating/IL-1-like activity.

Effect of treatment with IL-1β on [^{3}H]labeled cellular phospholipids

To further assess the ability of IL-1β to alter rat ovarian PLA_2 activity, the loss of cellular phospholipids during the arachidonic acid release assay was monitored. As shown (Fig. 6), treatment with IL-1β resulted in a significant ($P < 0.05$) decrease (55%) in the cellular content of [^{3}H]labeled phospholipids (Rf = 0.2, cf. Fig. 1). Treatment with IL-1β did not eliminate the second peak (Rf = 0.9, data not shown). These data confirm the finding (Figs. 3 and 4) that IL-1β stimulates AA release from ovarian cells.

To partially identify the specific phospholipids that were depleted by IL-1β (Fig. 6), cellular products were separated by TLC as shown in (Fig. 7). [^{3}H]AA was incorporated into two compounds that comigrated with authentic PC and PE. Although PC was the major labeled phospholipid noted, treatment with IL-1β depleted mainly the pool which comi-

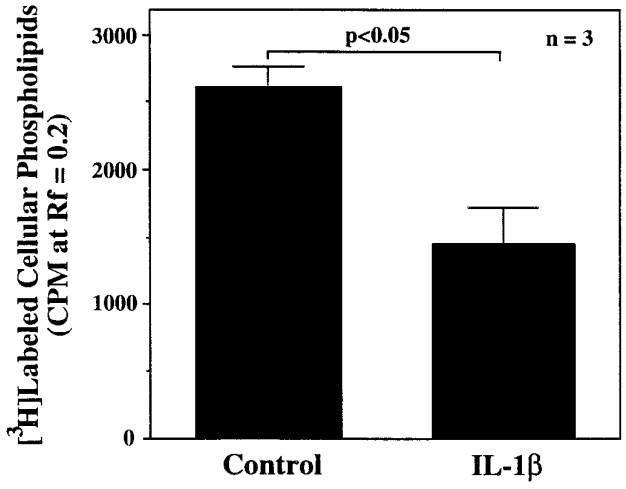

FIG. 6. Effect of treatment with IL-1β on the cellular content of [^{3}H]AA-labeled phospholipids. Whole ovarian dispersates were treated as in Fig. 3 except that, at the conclusion of the treatment period, the conditioned media were removed and the cells were washed, sonicated, extracted, TLC-fractionated in one dimension, and the sum of the radioactive peaks corresponding to phospholipids (Rf = 0.2) quantified. Results represent the mean ± SEM of three independent experiments.

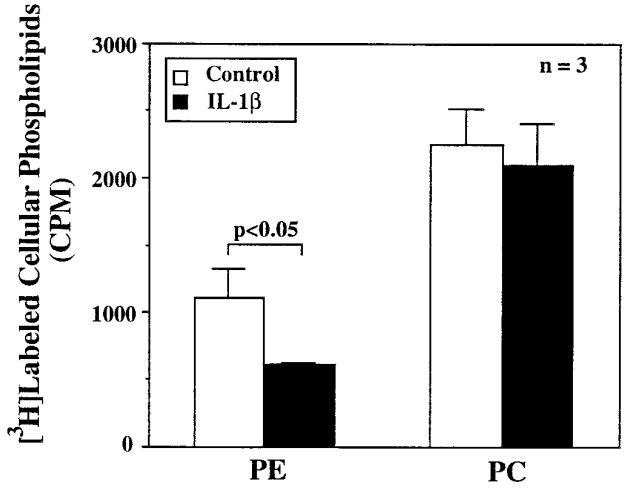

FIG. 7. Effect of treatment with IL-1β on the cellular content of specific types of [^{3}H]labeled cellular phospholipids. Whole ovarian dispersates were treated as described for Fig. 3. At the conclusion of the treatment period, the conditioned media were removed, and the cells washed, sonicated, extracted and TLC-chromatographed in two dimensions. The relevant radioactive peaks (PE, Rf = 0.3; PC, Rf = 1.0) were quantified. Results represent the mean ± SEM of three independent experiments.

grated with PE (1.8-fold decrease; $P < 0.05$). The PC pool remained unaffected.

IL-1β-induced increase in ovarian PLA_2 activity: cell-free assay

To further establish the ability of IL-1β to enhance rat ovarian PLA_2 activity, use was made of a cell-free assay system to confirm results obtained from the [^{3}H]AA-release and [^{3}H]phospholipid depletion assays (see above). The AA-containing substrate is cleaved by both $sPLA_2$ and $cPLA_2$

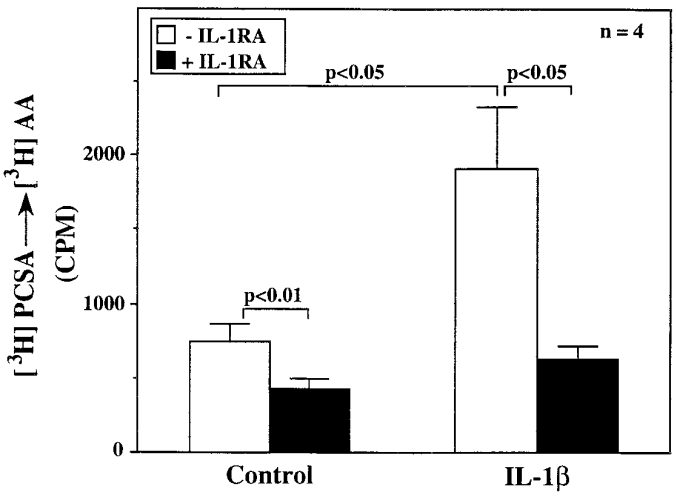

FIG. 8. IL-1β-induced increase in ovarian PLA_2 activity: cell-free enzyme assay. Whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (50 ng/ml), with or without IL-1RA (5 μg/ml). At the conclusion of this treatment interval, cellular sonicates were subjected to a cell-free PLA_2 enzyme assay. The results represent the mean ± SEM of four independent experiments.

subtypes (2–4) and thus estimates total PLA_2 activity. Preliminary studies (not shown) demonstrated that the assay was linear with respect to time and cell number in the range used. As shown (Fig. 8), treatment with IL-1β produced a 2.6 fold increase ($P < 0.05$) in total (cytosolic and secretory) cellular ovarian PLA_2 activity as compared with untreated controls. Concurrent provision of IL-1RA, produced a significant ($P < 0.01$) decrease in basal PLA_2 activity and all but abolished the IL-1β effect ($P < 0.05$). These findings also (cf. Fig. 5) suggest the existence of endogenous PLA_2-stimulating/IL-1-like activity because control levels are further reduced by the addition of IL-1RA.

Effect of IL-1β on $sPLA_2$ gene expression by whole ovarian dispersates: dose response curve

The effect of treatment with IL-1β on $sPLA_2$ gene expression *in vitro* is shown in Fig. 9. Increasing concentrations of IL-1β produced only a modest (up to 1.8-fold), albeit significant ($P < 0.05$), increase in $sPLA_2$ transcripts. A narrow response range was noted. Of note was the robust expression of $sPLA_2$ in untreated, control cells. We have shown that endogenously produced IL-1β induces this basal level of expression in control cells (Ben-Shlomo, I., S. Kol, and E. Adashi, submitted for publication). Taken together, these observations suggest that $sPLA_2$ gene expression in cultured ovarian cells is exquisitely sensitive to IL-1β as reflected by the ability of endogenously produced IL-1β to drive $sPLA_2$ expression to near maximal levels.

Effect of IL-1β on $cPLA_2$ gene expression by whole ovarian dispersates: dose response curve

The effect of treatment with IL-1β on $cPLA_2$ gene expression *in vitro* is shown in Fig. 10. Increasing concentrations of IL-1β produced significant ($P < 0.05$) increases (up to 5-fold) in $cPLA_2$ transcripts. Of note was the dramatic effect of increasing the IL-1β concentration from 0.5 to 5 ng/ml. A

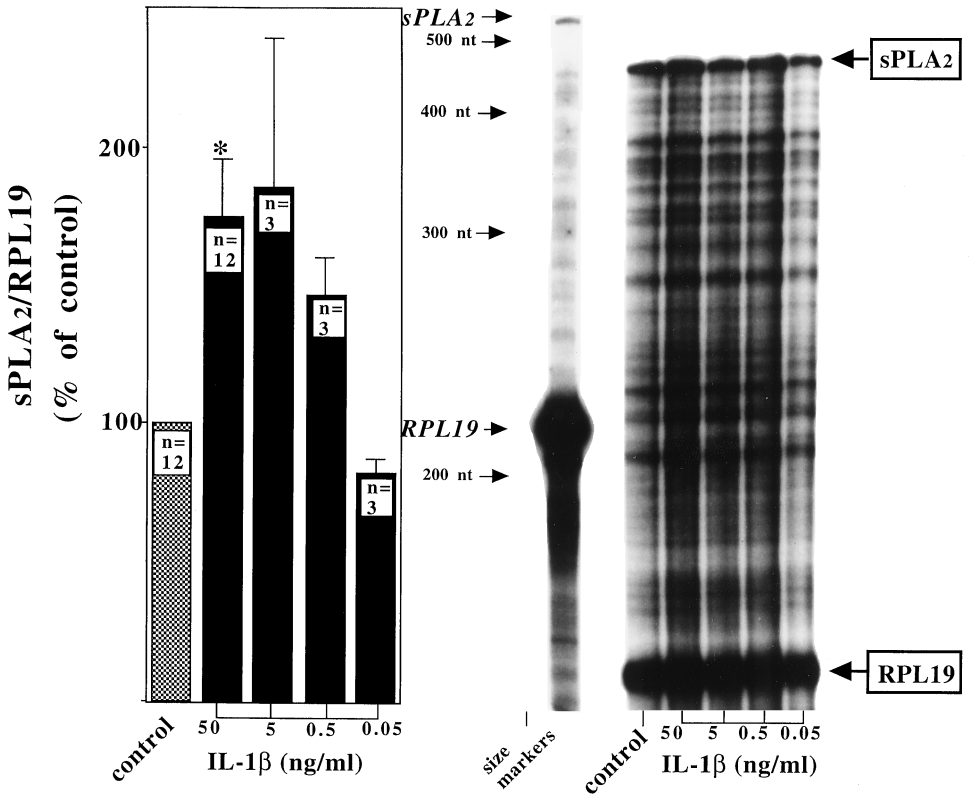

FIG. 9. $sPLA_2$ gene expression by cultured whole ovarian dispersates: effect of IL-1β. Whole ovarian dispersates were cultured for 48 h in the presence of increasing concentrations of IL-1β. Total cellular RNA was extracted and subjected to an RNase protection assay using [^{32}P]labeled rat antisense riboprobes for $sPLA_2$ and RPL19. The *left panel* depicts the normalized value for PLA_2 (mean ± SEM for the indicated number of experiments). The *right panel* depicts a representative gel. The full-length riboprobes are labeled in *italics*; the protected fragments are labeled in bold-faced letters. *, $P < 0.05$ *vs.* control. Data for untreated (control) and IL-1β (50 ng/ml)-treated cells presented in this figure were also used in another publication[2].

further increase in the IL-1β concentration resulted in only a marginal effect on $cPLA_2$ gene expression. Taken together, these observations suggest that $cPLA_2$ gene expression is induced over a relatively narrow range of IL-1β doses.

Discussion

The experiments described herein indicate that the prostaglandin-stimulatory effect of IL-1β entails a time-driven, receptor-mediated increase in ovarian PLA_2 activity. This conclusion is based on the demonstration of IL-1β-mediated AA release (Figs. 3 and 4), loss of cellular phospholipids (Figs. 6 and 7) and increased cellular PLA_2 activity (Fig. 8) that is receptor-mediated (Figs. 5 and 8). The IL-1β-stimulated PLA_2 activity is most likely dependent on PE as substrate since this compound, rather than PC, was depleted by IL-1β (Fig. 7). However, given that the TLC system used may not resolve all phospholipids, this identity remains to be established. We also noted that PC was the major labeled phospholipid (Fig. 7). The differential incorporation of AA into phospholipids (PC>PE) has also been reported for mast cells (23). Preferential depletion of the PE pool upon stimulation was also observed. Thus, it would appear that, while PC may constitute the long-term, more stable pool of arachidonate substrate, PE serves as the more available source for the biosynthesis of inflammatory mediators.

Because IL-1β up-regulates the gene expression of $sPLA_2$ and $cPLA_2$ (Figs. 9 and 10), both species may contribute to the observed increase in PLA_2 activity. The ability of IL-1β to stimulate PLA_2 activity, not hitherto reported in ovarian cells, was previously described for a number of extraovarian cell types (*e.g.* fibroblasts and mesangial cells; 24–32). The present observations (Fig. 4) also concur with our previous data (12), suggesting that IL-1β induces prostaglandin endoperoxide synthase activity, an issue beyond the scope of this communication.

The role of PLA_2 in the inflammatory processes is well established (33). Increased PLA_2 activity has been noted in a host of pathological processes (*e.g.* septic shock, connective tissue inflammatory diseases, premature labor, hypertension, and pancreatitis). However, this family of enzymes also plays many other physiological roles, including the facilitation of fat digestion, lipoprotein metabolism, lung surfactant metabolism, cell membrane homeostasis, insulin release, and sperm maturation and penetration (2, 3). Our data suggest that PLA_2 may play a role in ovarian physiology in general and in ovulation in particular (as an intermediary of IL-1β). In this connection, *in situ* hybridization and RNase protection studies identified $sPLA_2$ and $cPLA_2$ transcripts in periovulatory ovarian granulosa cells (Ben-Shlomo, I., S. Kol, and E. Adashi, submitted for publication).

Although the role of PLA_2 in ovulation and/or luteinization has not been fully elucidated, an increase in ovarian PLA_2 activity in response to the LH surge (34) was recently reported. Because preovulatory ovarian IL-1β gene expression is probably gonadotropin dependent (6, 35), we speculate that the LH-induced stimulation of PLA_2 activity is IL-1β mediated. This robust enzyme may participate in the rapid cellular and tissue remodeling that characterizes the ovary during the periovulatory period. Moreover, by promoting PG biosynthesis, PLA_2 may act in concert with other factors [*e.g.* plasminogen activator, collagenase (36, 37)] to promote follicular rupture and the release of the cumulus/

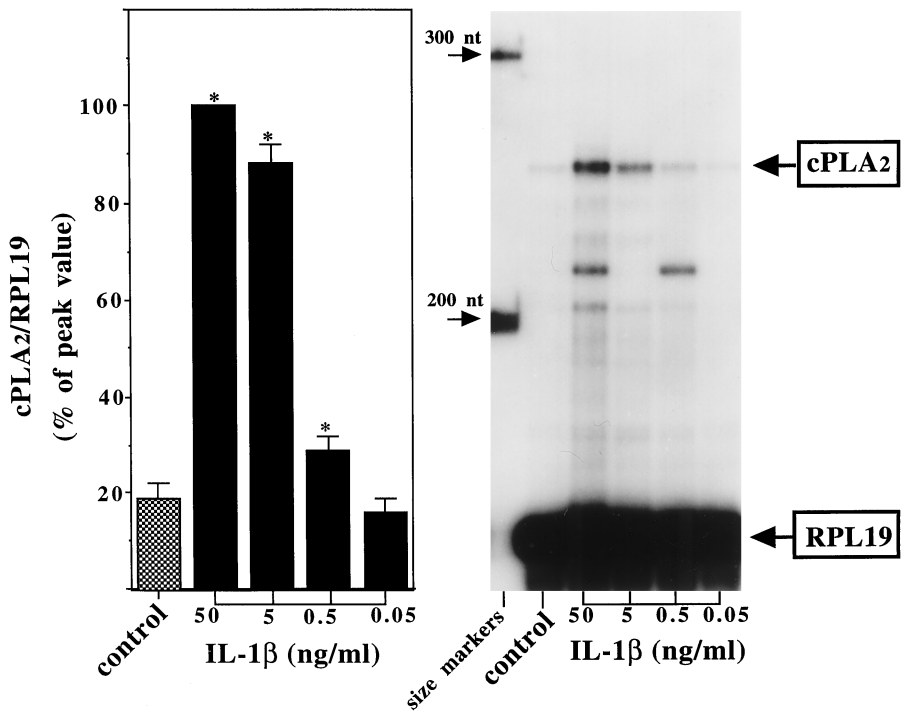

FIG. 10. $cPLA_2$ gene expression by cultured whole ovarian dispersates: effect of IL-1β. Whole ovarian dispersates were cultured and analyzed as described in Fig. 9 except that transcripts for $cPLA_2$ were detected.

oocyte complex (38, 39). Reported alterations in PLA_2 activity during luteal regression in pseudopregnant and pregnant rats (40) support the notion that PLA_2 may also be involved in corpus luteum formation and regression.

Apart from documenting the ability of exogenously provided IL-1β to stimulate ovarian PLA_2 activity, our present findings also suggest the existence of endogenous PLA_2-stimulating/IL-1-like activity. Indeed, the ability of IL-1RA to inhibit basal PLA_2 activity (Figs. 5 and 8) underscores the possibility that cultured whole ovarian dispersates release IL-1-like bioactivity. Although the presence of IL-1β (or IL-1α) in media conditioned by whole ovarian dispersates is yet to be demonstrated, prior studies have clearly established whole ovarian dispersates as a site of IL-1β gene expression (6). Preliminary observations (Kol, S., M. Ando, and E. Adashi, unpublished data) further suggest that the ovary is also the site of modest IL-1α gene expression. Taken together, these observations support the concept that both exogenous and endogenous IL-1 exert an up-regulatory effect on ovarian PLA_2 activity.

At the level of gene expression, our observations suggest that $sPLA_2$ transcripts are substantially stimulated by what are probably low levels of endogenously elaborated IL-1β. This conclusion is supported by the finding that a further increase in IL-1β concentrations (by adding exogenous IL-1β) results in only a modest further increase in $sPLA_2$ expression (Fig. 9). Presumably then, $sPLA_2$ is highly sensitive to stimulation by even low concentrations of IL-1β. In contrast, the overall increase in $cPLA_2$ transcripts following treatment with IL-1β was far more substantial compared with $sPLA_2$ (5 *vs.* 1.8-fold). It seems likely that $cPLA_2$ is less sensitive to stimulation by IL-1β because higher, exogenously provided doses of IL-1β are required to effect a meaningful increase (Fig. 10). Perhaps the bulk of steady-state cellular PLA_2 activity in untreated cells is attributable to $sPLA_2$.

In summary, our current observations document the presence of possible PE-dependent PLA_2 activity in cultured whole ovarian dispersates from immature rats, reveal the up-regulatory, receptor-mediated, and time-dependent action of IL-1β in this regard, support the existence of endogenous PLA_2-stimulating/IL-1-like activity, and establish IL-1β as an up-regulatory agent for $sPLA_2$ and $cPLA_2$ gene expression. As such, these observations support the proposition that PLA_2 is a key component in the IL-1β-stimulated biosynthesis of ovarian prostaglandins.

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