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# Interleukin-1β Stimulates Progesterone Production by *in Vitro* Human Luteal Cells: Evidence of a Mediatory Role of Prostaglandins

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**We have investigated whether IL-1β, a cytokine with an important role in ovarian physiology, is also involved in progesterone (P) synthesis in human luteal cells, and whether this effect is mediated via the cyclooxygenase (COX) pathway. Human luteal cells were cultured for 24 h in the presence of IL-1β (0.01–10 ng/ml), given alone or in combination with human chorionic gonadotropin (100 ng/ml), indomethacin (1 μg/ml), or P (100 ng/ml). We observed a significant increase in prostaglandin (PG)release after IL-1β treatment; the cytokine was more effective on $PGE_2$ than $PGF_{2\alpha}$ release. The effect of IL-1β was abolished by human chorionic gonadotropin, which had no action on basal PG levels when given alone; in contrast, P reduced basal, but not IL-1β-stimulated, PG production. Treatment with the human IL-1 receptor antagonist was associated with a decrease in both basal and IL-1β-stimulated PG production. Moreover, IL-1β induced a concentration-dependent increase in P production and release, an effect counteracted by the COX inhibitor indomethacin.**

**In conclusion, our data show the ability of IL-1β to influence P secretion via the COX pathway, thereby suggesting a possible luteotropic role in human ovary based on an autocrine-paracrine mechanism. (*J Clin Endocrinol Metab* 88: 2690–2694, 2003)**

EVIDENCE ACCUMULATED DURING the last decade has clearly shown that the cytokine IL-1β plays a pivotal role in the control of ovarian function. After the original observation that ovarian IL-1β and other proinflammatory cytokines are produced by resident ovarian macrophages (1), it was also found that IL-1β is produced by granulosa cells (2), and mRNA encoding the IL-1 receptor type I has been detected in these cells (3). In the latter, IL-1β gene expression is under the predominant control of LH (4). In addition, IL-1 has been documented in human follicular fluid (2, 5), where the concentrations of cytokine were found to correlate with those of prostaglandins $E_2$ ($PGE_2$) and $F_{2\alpha}$ ($PGF_{2\alpha}$) (6).

IL-1β stimulates cyclooxygenase (COX) activity and prostaglandin (PG) production in virtually all tissues of the body, and the COX pathway is the most extensively studied component of the signal transduction complex triggered by the cytokine (7). In this framework, several groups showed that IL-1β stimulates PG production in human and primate granulosa-luteal cells (8–10) via a mechanism involving the expression of an inducible COX isoform (11). The above-described functional interplay between IL-1β and the COX pathway has been postulated to participate in the control of several ovarian functions, including ovulation (12). One other ovarian function thoroughly investigated for IL-1β involvement is the regulation of granulosa and luteal cell secretion, in particular the biosynthesis and secretion of progesterone (P). Fukuoka *et al*. (13) reported that IL-1β has no effect on human chorionic gonadotropin (hCG)-stimulated luteinized human granulosa cells. Barak *et al.* (14) and Young *et al.* (10) found that IL-1β has no effect on basal, but inhibits gonadotropin-stimulated, P synthesis in human and primate cells. Best and Hill (15) and Kohen *et al.* (16) also found IL-1β-induced inhibition of basal and/or stimulated P production, but the presence of white blood cells in the system was needed for this effect to occur. The response to IL-1β may also vary from inhibition to stimulation of P synthesis depending on the degree of differentiation of granulosa-luteal cells (17). More recently, Chen *et al.* (4) reported that IL-1β is able to increase basal, but not gonadotropin-stimulated, P production in human granulosa-luteal cells. Thus, inhibition of, no effect on, and stimulation of P synthesis by IL-1β were reported depending on the differences in experimental models and conditions adopted; therefore, despite many efforts, the issue of the effect of IL-1β on P synthesis remains unresolved at this time.

In the present study relatively pure primary cultures of human luteal cells and assays for the measurement of $PGE_2$, $PGF_{2\alpha}$, and P were used to investigate the effects of intrinsic and exogenously added IL-1β on P biosynthesis, and whether these effects are mediated via the COX pathway. The interplay between IL-1β and hCG in regulating P synthesis was also investigated.

## Materials and Methods

### *Chemicals*

IL-1β and hCG were purchased from Roche Molecular Biochemicals (Indianapolis, IN) and from Serono (Rome, Italy), respectively. Human IL-1β receptor antagonist (IL-1ra) was obtained from R&D Systems (Minneapolis, MN). The following chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO): P, collagenase type IV, antibiotics,

Abbreviations: CL, Corpora lutea; COX, cyclooxygenase; hCG, human chorionic gonadotropin; IL-1ra, IL-1 receptor antagonist; P, progesterone; PG, prostaglandin.

glutamine, and HEPES. Ham's F-12 medium was obtained from Flow Laboratories (Milan, Italy), and fetal calf serum was obtained from Biological Industries (Kibbutz Beit HaEmek, Israel).

### *Luteal cell culture preparation and experimental procedure*

Corpora lutea (CL) were obtained at the time of hysterectomy performed for nonendocrine gynecological disease (leiomyomatosis) in the midluteal phase of the menstrual cycle (d 5–6 from ovulation). A total of 18 patients, ranging between 30–43 yr of age, were included in the study. All of them had a history of regular menstrual cycles. Informed consent was obtained from each patient, and the study was approved by the internal review board. The age of the corpora lutea was determined as follows. All patients were monitored until ovulation by daily measurement of basal body temperature and ultrasound examination of follicular growth. When the maximal follicular diameter had reached 18 mm, daily determination of plasma P values was made. The time of ovulation (d 0) was detected by the biphasic pattern of basal body temperature, the typical ultrasound disappearance of the dominant follicle or the ultrasound detection of corpus luteum, and the rise in plasma P concentrations. At the time of surgery, plasma samples were collected immediately before anesthesia to determine plasma P concentrations. The luteal tissue removed was immediately freed from blood vessels and ovarian stroma under a dissecting microscope, dissected, and minced. Human CL cultures were performed as previously described (18), with some modification.

The luteal tissue was placed in 10 ml prewarmed Ham's F-12/HEPES medium, containing type IV collagenase (200 U/ml), then incubated at 37 C in a shaking water bath for 45 min. The medium containing the cell suspension was filtered through a 40-μm nylon mesh, and the cells obtained were centrifuged and resuspended twice in fresh medium. This procedure was repeated once with the remaining undigested tissue to obtain relatively pure luteal cells. Cells were counted in a hemocytometer, and viability was determined by the trypan blue exclusion test. The cells were diluted to a final concentration of 250,000 live cells/ml medium supplemented with 2 mM L-glutamine, 100 IU penicillin, 100 mg/ml streptomycin, and 10% fetal calf serum and cultured in 48-well plates for 24 h in 5% $CO_2$/95% air at 37 C. After this time the cells were attached to the wells, and the medium was removed and replaced with fresh serum-free medium alone (controls) or containing IL-1β (0.01–10 ng/ml) with or without 100 ng/ml hCG. In another group of experiments, we stimulated the cells with 0.1 ng/ml IL-1β alone or after a 30-min preincubation with 100 ng/ml IL-1ra or combined with hCG (100 ng/ml), P (100 ng/ml), or with indomethacin (1 μg/ml). The medium was harvested after 24 h of culture and stored at –20 C until assayed for $PGE_2$, $PGF_{2\alpha}$, or P immunoreactivity (by RIA).

### *Flow cytometric characterization of cell cultures*

Single-color fluorescence flow cytometry was performed using fluorescein isothiocyanate monoclonal antibody to CD45 obtained from BD Biosciences (Franklin Lakes, NJ). Cytometric evaluation was performed with a FACScan (BD Biosciences) equipped with LYSIS II software (BD Biosciences). The percentage of contaminating leukocytes in luteal cell preparations was calculated by the combined usage of side scatter and CD45 expression. Analysis was performed within the region defined by light scatter so as to avoid including cell debris and clumps from the analysis. The dot blot histograms in Fig. 1 show typical preparations in which the proportion of contaminating leukocytes ranged between 3–7%.

### *Analytical methods*

Commercial P RIA kits were used (Radim, Rome, Italy). The intra- and interassay coefficients of variation were 4% and 10%, respectively. The RIA sensitivity was 5 pg P/tube.

The RIAs for $PGF_{2\alpha}$ and $PGE_2$ used in this study were first characterized for measurement of prostanoids in human urine (19) and later were used successfully to measure PGs produced and released by several cell types *in vitro*, including cells from human ovaries (20). For each assay, incubation mixtures of 1.5 ml were prepared in disposable plastic tubes in which 50 μl (for $PGE_2$ or $PGF_{2\alpha}$, respectively) incubation medium were diluted to 250 μl with 0.025 M phosphate buffer (pH 7.5). Tritiated $PGE_2$ or $PGF_{2\alpha}$ (2,500–3,500 cpm) and appropriately diluted antisera were added together to a final volume of 1.5 ml. The antisera (provided by Prof. G. Ciabattoni) were used at a final dilution of 1:120,000 or 1:150,000 (for $PGE_2$ or $PGF_{2\alpha}$, respectively). A duplicate standard curve ranging from 2–400 pg/tube was run for each assay. All tubes were incubated for 24 h at 4 C. Separation of antibody-bound prostanoids was obtained with 2.5 mg charcoal (Norit-A), which absorbs 95–98% of free PGs; a charcoal suspension (2.5 mg/50 μl) in 0.025 M phosphate buffer, pH 7.5, was added to each tube after the addition of 100 μl 5% BSA. The tubes were briefly shaken and then centrifuged for 10 min at 4 C. Supernatants were decanted into 10 ml scintillation liquid. Radioactivity was measured by liquid scintillation counting. The detection limit of the assay was 2 pg/tube in all cases. The inter- and intraassay variability coefficients were 2.7% and 2.9% for $PGE_2$ and 3.2% and 2.8% for $PGF_{2\alpha}$, respectively. [$^3$H]$PGE_2$ and [$^3$H]$PGF_{2\alpha}$ were obtained from NEN Life Science Products (Milan, Italy).

### *Data analysis*

Data were first analyzed by Kolmorogov-Smirnov test to assess differences in the general shapes of distribution. Normally distributed data were then analyzed by one-way ANOVA with Bonferroni correction to perform pairwise comparison group means.

## Results

### *Effect of IL-1β on prostanoid release from human luteal cells*

Luteal cells were incubated for 24 h in the presence of graded concentrations of IL-1β (0.01–10 ng/ml), and the production of prostanoids was estimated by measuring the amounts of $PGE_2$ and $PGF_{2\alpha}$ released into the incubation

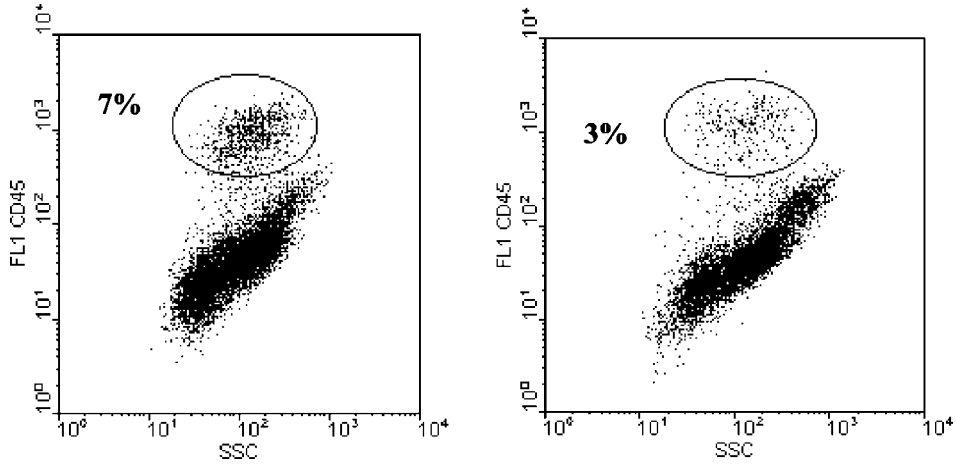


FIG. 1. Dot plot histogram generated by fluorescein isothiocyanate-conjugated monoclonal antibody raised against human CD45. The proportion of contaminating leukocytes was less than 10% in all experiments. The data shown are representative of three independent experiments.

medium. IL-1β was able to stimulate in a concentration-dependent manner the release of both PGs (Fig. 2). Statistical difference with respect to controls was achieved from 0.1 ng/ml onward in the case of $PGF_{2\alpha}$ and from 0.01 ng/ml onward in the case of $PGE_2$, indicating that the cytokine was about 10-fold more potent in stimulating $PGE_2$ than $PGF_{2\alpha}$ release in this paradigm. A difference was also observed in activity, as IL-1β produced maximal increases of +163.7% and +98.4% *vs.* controls on the release of $PGE_2$ and $PGF_{2\alpha}$, respectively (Fig. 2).

A further series of experiments was carried out with 0.1 ng/ml IL-1β, with or without a 30-min preincubation in the presence of plain medium or medium containing 100 ng/ml IL-1ra. Figure 3 shows that IL-1ra was able to abolish the IL-1β-induced increase in both $PGE_2$ and $PGF_{2\alpha}$ release. Moreover, IL-1ra also significantly decreased basal PG production, suggesting that in this experimental paradigm endogenous IL-1β plays a role in the control of baseline PG biosynthesis via a paracrine/autocrine mechanism.

### *Effects of hCG and P on basal and IL-1β-stimulated prostanoid release from human luteal cells*

Luteal cells were exposed for 24 h to 0.1 ng/ml IL-1β and 100 ng/ml hCG, given alone or in combination. As expected, IL-1β stimulated both $PGE_2$ and $PGF_{2\alpha}$ release in a significant manner, whereas hCG had no effect. However, the latter was able to completely antagonize the stimulatory effects of IL-1β on both PGs (Fig. 4).

To ascertain whether the inhibitory effect of hCG on IL-1β was direct or mediated by an increase in P synthesis in luteal cells, the latter were incubated for 24 h with 0.1 ng/ml IL-1β, 100 ng/ml P, or their combination. P failed to counteract the stimulatory effects of IL-1β on PG release. However, when given alone, P displayed an unexpected capability to significantly reduce PG production and release (Fig. 5).

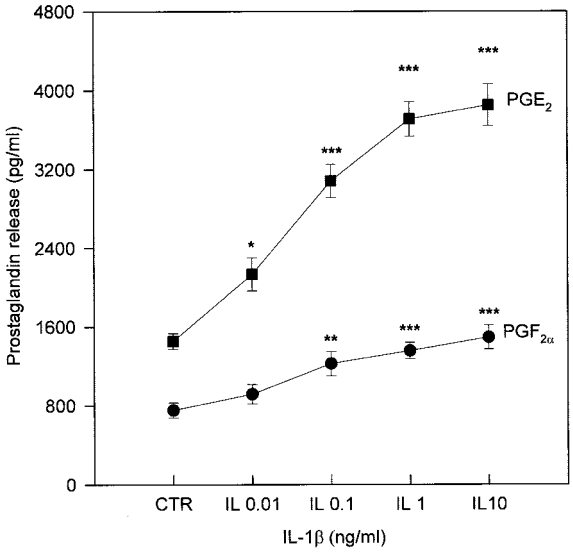


FIG. 2. Dose-dependent effects of IL-1β on $PGE_2$ and $PGF_{2\alpha}$ release by human luteal cells. Luteal cells were cultured for 24 h with medium alone (CTR) or with increasing concentrations of IL-1β (0.01–10 ng/ml). Data are expressed as the mean ± SEM of 12 experiments. Significance *vs.* CTR: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

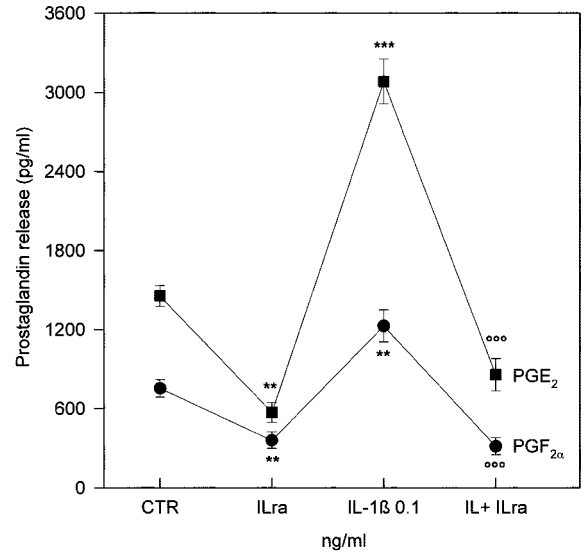


FIG. 3. ILra effect on IL-1β-induced $PGE_2$ and $PGF_{2\alpha}$ release by human luteal cells. Luteal cells were incubated for 24 h with medium alone (CTR) or containing IL-1ra (100 ng/ml), IL-1β (0.1 ng/ml), or their combination. Data are expressed as the mean ± SEM of six experiments. Significance *vs.* CTR: **, $P < 0.01$; ***, $P < 0.001$. Significance *vs.* IL-1β: °°°, $P < 0.001$.

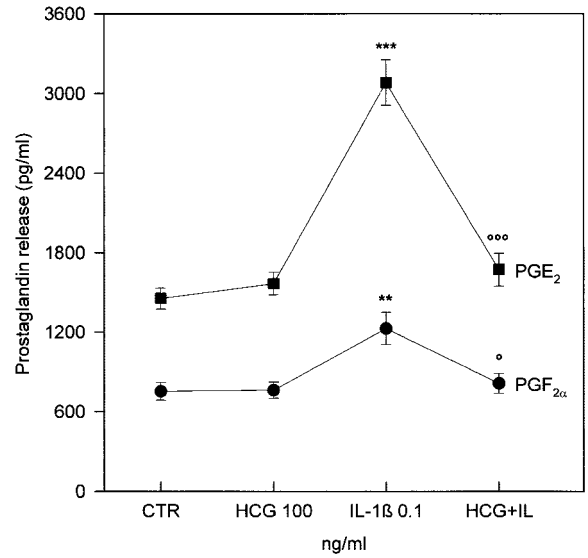


FIG. 4. Effect of hCG on IL-1β-induced $PGE_2$ and $PGF_{2\alpha}$ release by human luteal cells. Luteal cells were cultured for 24 h with medium alone (CTR) or containing hCG (100 ng/ml) or hCG and IL-1β (0.1 ng/ml). Data are expressed as the mean ± SEM of 12 experiments. Significance *vs.* CTR: **, $P < 0.01$; ***, $P < 0.001$. Significance *vs.* IL-1β: °, $P < 0.05$; °°°, $P < 0.001$.

### *Effect of IL-1β on P synthesis by human luteal cells*

Luteal cells were incubated for 24 h with graded concentrations of IL-1β (0.01–10 ng/ml). The cytokine induced a concentration-dependent increase in P production, which attained statistical significance from 1 ng/ml onward; increases obtained with IL-1β are shown in comparison with that obtained after exposure of cells to 100 ng/ml hCG (Fig. 6), but the maximal increase attainable with IL-1β (+45.8% *vs.* controls) was lower than that elicited by hCG (+95.5%).

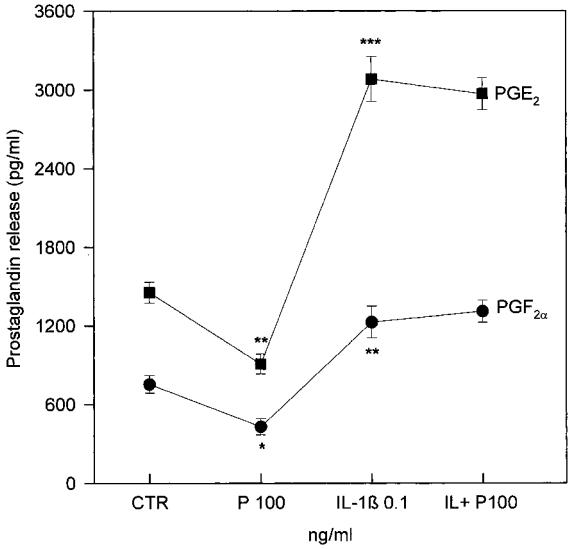


FIG. 5. Effect of P on IL-1β-induced $PGE_2$ and $PGF_{2\alpha}$ release by human luteal cells. Luteal cells were cultured for 24 h with medium alone (CTR) or containing P (100 ng/ml), IL-1β (0.1 ng/ml), or their combination. Data are expressed as the mean ± SEM of six experiments. Significance *vs.* CTR: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

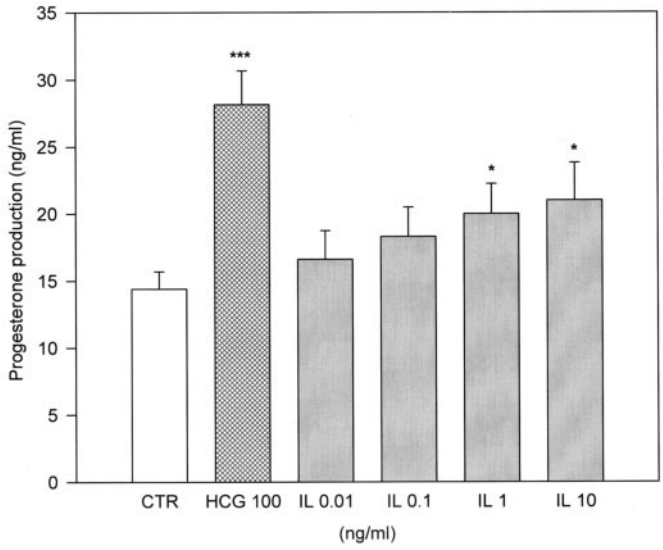


FIG. 6. Dose-dependent effects of IL-1β on P production by human luteal cells. Luteal cells were cultured for 24 h with medium alone (CTR) or containing hCG (100 ng/ml) or increasing concentrations of IL-1β (0.01–10 ng/ml). Data are expressed as the mean ± SEM of 12 experiments. Significance *vs.* CTR: *, $P < 0.05$; ***, $P < 0.001$.

Experiments with the nonselective COX inhibitor indomethacin were performed to investigate the involvement of prostanoids in IL-1β stimulation of P biosynthesis. Indomethacin (1 μg/ml) completely counteracted the increase in P production elicited by IL-1β, but had no effect when given alone (Fig. 7).

## Discussion

In this study, we found that IL-1β consistently and concentration-dependently stimulates P synthesis and secretion from human luteal cells. This effect appears to be mediated

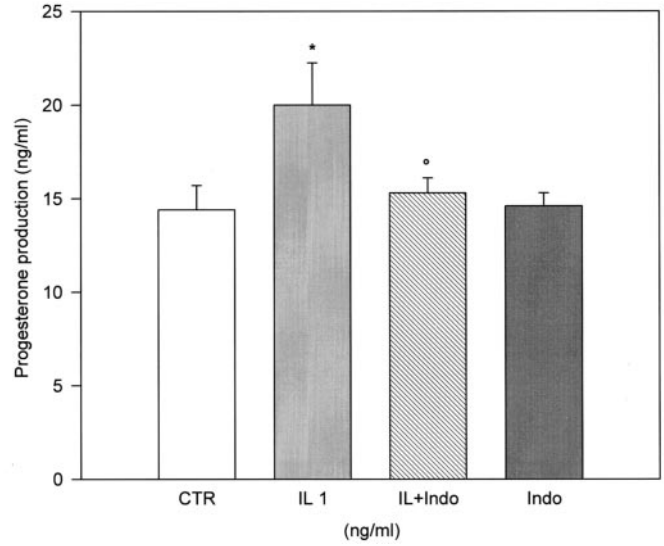


FIG. 7. Effect of indomethacin on IL-1β-induced P release by human luteal cells. Luteal cells were cultured for 24 h with medium alone (CTR) or containing indomethacin (1 μg/ml) or indomethacin plus IL-1β (1 ng/ml). Data are expressed as the mean ± SEM of six experiments. Significance *vs.* CTR: *, $P < 0.05$. Significance *vs.* IL-1β: °, $P < 0.05$.

by the COX pathway, because 1) the increase in P secretion elicited by IL-1β is paralleled by a robust increase in PG production; and 2) a nonselective COX blocker, indomethacin, prevented the stimulatory activity of the cytokine.

IL-1β stimulated the production of both $PGE_2$ and $PGF_{2\alpha}$, although it was more potent and more effective in stimulating $PGE_2$. Human CL produce both $PGE_2$ and $PGF_{2\alpha}$ (21–23). In various species, including humans, $PGF_{2\alpha}$ seems to be important in luteolysis (20, 24, 25), whereas a large body of evidence indicates $PGE_2$ as a luteotropic factor. As IL-1β stimulates P production, the positive effect of IL-1β on $PGF_{2\alpha}$ production was unexpected. However, in a classical yin-yang view of opposite actions exerted by the two prostanoids, the stimulation of P synthesis observed here may be explained by the 2- to 3-fold higher levels of $PGE_2$ compared with $PGF_{2\alpha}$, which would result in a prevailing luteotropic effect. Adopting a more sophisticated approach, Vaananen *et al.* (26), crossing two concentration-response curves of both PGs, recently demonstrated that neither $PGE_2$ nor $PGF_{2\alpha}$ was luteotropic *per se*, whereas specific concentrations of both prostanoids given together were. Moreover, the presence of a high concentration of either of these PGs attenuated the luteotropic effects of the other. Other combinations resulted in $PGF_{2\alpha}$ inhibition over $PGE_2$ luteotropic activity. In light of the presence of 3–7% leukocyte contaminants in our model, the possibility should be taken into account that a part of the total PG produced under IL-1 stimulation might not be of luteal origin. It is not possible in our system to estimate the amount of PGs produced by luteal cells and that released by leukocytes, although it might be reasonably assumed that the latter represents a small fraction of the total.

One other important factor influencing IL-1β activity was the degree of cell differentiation (17); indeed, our cultures were highly differentiated, and no mitosis was observed during the culture period (27). Taken together, the present

findings and the data from the literature indicate that IL-1β will stimulate P biosynthesis in granulosa-luteal cells *in vitro* provided that experiments are conducted using cell cultures with a high degree of purity and differentiation.

As expected, hCG also stimulated P secretion, but this effect did not appear to involve the COX pathway because it was not accompanied by a parallel increase in PG production, thus suggesting different signal transduction pathways for hCG and IL-1β in the regulation of P secretion.

The data presented here confirm the findings by Young and colleagues (10) in primate luteal cells that hCG significantly inhibits IL-1β-stimulated PG production. It was tempting to speculate that this effect was mediated by increased P production induced by hCG; this hypothesis was suggested by animal data showing the ability of P to inhibit $PGF_{2\alpha}$ production *in vitro* (28). Indeed, we found that P significantly reduces basal PG production, however, the steroid failed to antagonize the increase in PG synthesis stimulated by IL-1β. Therefore, it would seem that the inhibitory effects of hCG and P on PG release are exerted through different and separate metabolic pathways.

We know from the literature that two IL-1 receptors (types I and II) have been identified; however, current information indicates that IL-1β signaling occurs exclusively via the type I receptor. This receptor has been localized by immunohistochemistry in both somatic and immune cells of the CL and is expressed during all stages of the luteal phase (16). The finding with IL-1ra clearly indicates that IL-1 produced by cultured luteal cells is one of the factors contributing to PG production under resting conditions; reduction by IL-1ra of such baseline production suggests a mechanism involving the production and release of IL-1β, the secretory IL-1 isoform from luteal cells, and the subsequent activation, in a paracrine manner, of IL-1 type 1 receptors expressed by these cells (3, 16). In summary, here we have shown that IL-1β consistently stimulates P biosynthesis and release from primary cultures of human luteal cells via activation of the COX pathway. These results suggest a possible luteotropic activity of the cytokine and are somewhat in contrast with the older idea of IL-1 as the intraovarian luteinization inhibitor (1). A unifying picture that reconciles such opposing evidence should be based on the assumption that the actions of IL-1β at the ovarian level may vary widely depending on cell origin (whether it is the resident macrophage or the granulosa-luteal cell lineage), proliferation/differentiation balance of target cells, and the surrounding hormonal milieu.

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