Rabbit ovarian production of interleukin-6 and its potential effects on gonadotropin-induced progesterone secretion in granulosa and theca cells

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Abstract

Recent studies suggest that non-steroid factors, such as cytokines, may play a role in ovarian processes. The purpose of this study was to explore cellular sites of interleukin (IL)-6 biosynthesis in rabbit follicles and to investigate IL-6 modulation in granulosa and theca cell functions. In this report development of rabbit preovulatory follicles was induced by 200 mIU equine chorionic gonadotropin (eCG) daily for 2 days. Seventy-two hours after the last injection ovaries were excised and granulosa and theca cells isolated. The two types of cells were preincubated for 24 h in Minimum Essential Medium (MEM) with 5% fetal calf serum (FCS), and then incubated for 24 h in MEM–2.5% FCS with appropriate

stimulants. Results showed that rabbit granulosa and theca cell culture supernatants contained IL-6 bioactivity and that its production was inhibited by FSH and human CG and stimulated by IL-1. IL-6 inhibited gonadotropin-induced progesterone production, but not basal secretion, in both cell types, without a cytotoxic effect. IL-6 affected cAMP generation and steps distal to cAMP formation, but the mechanism of IL-6 action on progesterone differed in granulosa and theca cells. Taken together our results suggest that gonadotropins, by inhibiting IL-6 production, could control, in our model, IL-6 modulation of gonadotropin action on steroidogenesis.

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Introduction

In recent years, accumulating evidence has suggested that the immune system is an additional local regulator of ovarian functions, which are essentially modulated by the gonadotropins. Specifically, numerous studies have shown that a variety of cytokines modulate the reproductive process and are implicated as regulators of gonadal steroid secretion, corpus luteum function, embryo development and implantation.

Interleukin-6 (IL-6), a glycoprotein of 23–30 kDa, is one of these substances, initially identified as a T-cell derived cytokine which specifically promoted T-cell activation, B-cell differentiation and antibody production. It is synthesized by a large variety of cell types including fibroblasts, mast cells, endothelial cells, monocytes/macrophages, anterior pituitary cells and keratinocytes (Spangelo *et al.* 1990a,b, Van Snick 1990). The regulation of IL-6 production has been an area of intense investigation and IL-1, tumor necrosis factor- α (TNF α), diacylglycerols, calcium ionophore and serum all have been reported to stimulate IL-6 production *in vitro* (Kishimoto 1989).

Evidence of IL-6 release by the ovary was demonstrated by Watson *et al.* (1990), who showed that basal production

of IL-6 occurs in cultures of primary ovarian tumors and ovarian cancer cell lines. Then it was demonstrated that granulosa cells of many species are an active site of IL-6 biosynthesis (Gorospe *et al.* 1992, Fong *et al.* 1994, Machelon *et al.* 1994a,b). But, although involvement of IL-6 in granulosa steroidogenesis regulation is well documented (Gorospe *et al.* 1992, Alpizar & Spicer 1993, Gorospe & Spangelo 1993, Pitzel *et al.* 1993, Hughes *et al.* 1994, Machelon *et al.* 1994b, 1995), no studies of its effects on theca steroidogenesis have been reported.

The aim of the present study was to investigate *in vitro* IL-6 production by hyperstimulated rabbit ovary, from which both cell types can be efficiently obtained, and to assess *in vitro* IL-6 regulatory effects on cell number and progesterone production.

Materials and Methods

Reagents

Minimum Essential Medium (MEM) with Earle's salts, RPMI 1640, and fetal calf serum (FCS) were obtained from Eurobio (Les Ulis, France). Purified ovine follicle-stimulating hormone (FSH) (USDA-o-FSH 18, 1640 IU/mg) was provided by the National Hormone and Pituitary

Program (National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD, USA).

Forskolin, cholera toxin, collagenase type II, protease type XIV, hyaluronidase type II, N_6O_2 (butyryl)₂ cyclic AMP (Db-cAMP), 2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide (XTT), phenazine methosulfate, BSA, trichloroacetic acid and EGTA were obtained from Sigma-Chimie (L'Isle d'Abeau, France). Human recombinant interleukin-6 (100 IU/ng) and human recombinant interleukin-1 β (50 IU/ng) were purchased from Boehringer-Mannheim (Meylan, France). A commercial preparation of human chorionic gonadotropin (hCG) (Pregnyl; 3500–4000 IU/mg) was supplied by Organon (Serfontaine, France); equine gonadotropin (eCG) (Chrono-gest) was obtained from Intervet (Angers, France). Embutramide T61 was obtained from Distrivet (Paris, France).

Progesterone antibody was supplied by the Fondation de Recherche en Hormonologie, France. [³H]Progesterone (80–110 Ci/mmol) and a cAMP RIA kit were obtained from Amersham (Les Ulis, France).

Animals

For this study, we used HY white female rabbits, 12–15 weeks old.

The development of preovulatory follicles was induced in immature rabbits by intramuscular injection of 200 IU eCG daily for 2 days. The rabbits were killed by intracardiac injection of 2 ml narcotic (embutramide T61), 72 h after the last injection of eCG. The ovaries were excised and placed in culture medium. For each experiment, 80–100 follicles (0.8–1.5 mm diameter) were dissected out and cleared of interstitial tissue stereomicroscopically.

Cell preparation

Dispersed granulosa and theca cells were obtained as described previously (Féral *et al.* 1990). Briefly, follicles were cut in half in MEM–0.1% BSA containing 20 mM Hepes, 50 IU/ml penicillin, 50 µg/ml streptomycin, 0.1% BSA, and 6.8 mM EGTA for 15 min. The released granulosa cells and the follicles were then centrifuged together for 10 min at 150 g and resuspended in MEM–BSA containing 0.5 M sucrose and 1.8 mM EGTA for 5 min before being centrifuged again for 10 min at 150 g. Granulosa cells were then gently scraped away from the theca with a microspatula under a dissecting microscope. The dispersed granulosa cells were then pelleted by centrifugation at 150 g, subjected to mechanical dissociation, washed twice in MEM–0.1% BSA, and suspended in MEM supplemented with 5% FCS.

In order to eliminate the few granulosa cells remaining on the theca, the pieces of tissue were placed in MEM containing 0.1% collagenase and 0.1% hyaluronidase

(type II) for 10 min at 37 °C. After a short centrifugation (600 g, 30 s) of thecal tissue, thecal cells were gently dissociated by incubation for 10 min at 37 °C in MEM containing 0.2% hyaluronidase and 0.1% protease (type XIV). They were then washed three times in MEM–0.1% BSA, and suspended in MEM–5% FCS.

Granulosa and theca cells culture

Cells were cultured in Costar 48-well tissue culture plates. Cell concentrations were 10⁵ cells/well for the study of progesterone production or cell number determination and 2.5 × 10⁵ cells/well for the measurement of IL-6 activity. Granulosa and theca cells were preincubated for 24 h at 37 °C in a total volume of 0.5 ml MEM–5% FCS, under an atmosphere of 5% CO₂ and 95% air. Supernatants were then removed and cells were incubated at 37 °C in MEM–2.5% FCS containing the appropriate stimulants, with or without IL-6, for a further 24 h. Then cell-free supernatants were collected and frozen until progesterone and IL-6 bioactivity measurement. Cell layers were used to determine cell number. The viability of the suspensions of both cell types at seeding was higher than 90%, and we found no statistical difference in granulosa and theca cells numbers at seeding or after culture without hormones or substances.

Cell number determination

Granulosa and theca cell numbers from each culture well were estimated by measuring DNA content using the fluorometric technique described by Labarca & Paigen (1980) and also by a colorimetric estimation of mitochondrial activity evaluated by conversion of the tetrazolium salt XTT into its formazan product, according to Mosmann (1983). This colorimetric assay allows quantification of living metabolically active cells. This assay has previously been used in granulosa cells by Best *et al.* (1994). Briefly, at the end of the 24 h culture period, supernatants were removed and granulosa or theca cells were incubated in 200 µl serum-free MEM with XTT (0.25 mg/ml) and phenazine methosulfate (1.9 µg/ml) for 3 h. The production of formazan by the cells was quantified by measuring absorbance at 570 nm with a multiwell ELISA reader.

IL-6 assay

IL-6 activity was determined by the capacity of granulosa and theca cell supernatant to enhance 7TD1 hybridoma cell proliferation (Gorospe & Spangelo 1993). Briefly, 2500 cells/well were placed in RPMI 1640–10% FCS, containing 50 µM β-mercaptoethanol and antibiotics. These cells were cultured for 72 h in the presence of 50 µl RPMI containing increasing concentration of IL-6 (1–1000 pg/ml) or 50 µl granulosa or theca cell culture

supernatant. RPMI (50 μ l) containing XTT (0.25 mg/ml) and phenazine methosulfate (1.9 μ g/ml) was then added for a final 4 h incubation. Cell number was then estimated by measuring absorbance at 570 nm with a multiwell micro-ELISA reader. 7TD1 hybridoma cells are very sensitive and specific for IL-6, and they fail to grow in response to any other cytokines or hormones tested (Spangelo *et al.* 1990a).

Progesterone production

Progesterone levels were directly measured in granulosa or theca cell media by RIA using the specific progesterone antibody (Benhaim *et al.* 1987). The sensitivity of the assay was 6 pg/tube. The intra- and interassay coefficients of variation were 5 and 9% respectively. The cross reactivity with 17 α -hydroxyprogesterone was 0.01%.

Statistics

Data are the mean $\pm$ S.E.M. of at least three experiments performed in triplicate. One rabbit was used for each experiment. Results for progesterone secretion and cell number determination were expressed as the percentage change from control. The control results were obtained with cells cultured in MEM-2.5% FCS, without added substances, and run in parallel with test wells. Statistical differences between treatment groups were assessed by two-way ANOVA, followed by Fisher's test and considered significant at $P < 0.05$. Data in Tables 1 and 2 were assessed by Student's *t*-test analysis.

Results

Biological activity was regulated by gonadotropins and IL-1 β in granulosa and theca cells

As shown in Fig. 1, IL-6 bioactivity was detected in rabbit granulosa and theca cell culture medium. Medium supplemented with 10% FCS did not contain measurable amounts of IL-6 (data not shown).

In the absence of gonadotropins, IL-6 bioactivity was similar in media from granulosa and theca cells (11.5 and 11.7 pg/2.5 $\times 10^5$ cells respectively). IL-6 production was inhibited in a dose-dependent manner, for both types of cells, by high doses of gonadotropins (Fig. 1).

When IL-1 β was added to granulosa and theca cell cultures, IL-6 production was significantly enhanced and this enhancement was statistically significant in granulosa cells (Fig. 2). In the presence of both IL-1 β and gonadotropins we no longer observed the inhibitory effect of gonadotropins on granulosa and theca IL-6 production.

Estimation of granulosa and theca cell number

We estimated the amount of DNA per well after cell culture and the results demonstrated that the higher dose

Figure 1 Dose-dependent effect of gonadotropins on IL-6 secretion by granulosa cells (A) or theca cells (B). Results are the mean $\pm$ S.E.M. of *n* experiments performed in triplicate (A, *n*=6; B, *n*=4). * $P < 0.05$; ** $P < 0.01$ compared with the absence of gonadotropins.

of IL-6 (25 ng/ml), in the presence or absence of low doses of gonadotropins (1 ng/ml for FSH and 10 mIU/ml for hCG), did not affect significantly the amount of DNA (data not shown). By using a more sensitive assay, the colorimetric method of Mosmann (1983), a slight dose-dependent enhancement of the number of the two types of cells was obtained when increasing concentrations of IL-6 (1–25 ng/ml) were added to the culture medium in

Figure 2 Dose-dependent effect of IL-1, in the presence or absence of gonadotropins, on IL-6 secreted by granulosa cells (A) or theca cells (B). Results are the mean $\pm$ S.E.M. of n experiments performed in triplicate (A, $n=6$; B, $n=4$). * $P<0.05$ compared with control, without IL-1 and gonadotropins.

Figure 3 Effect of increasing concentrations of IL-6 on the number of granulosa cells cultured in the presence of FSH (1 ng/ml) (A), or of theca cells cultured in the presence of hCG (10 mIU/ml) (B). Results are the mean $\pm$ S.E.M. of n experiments performed in triplicate (A, $n=3$; B, $n=4$). * $P<0.05$; ** $P<0.01$; *** $P<0.001$ compared with control, without gonadotropins.

addition to FSH or hCG (Fig. 3). However, no significant effect was observed with IL-6 alone or gonadotropins alone (Fig. 3).

Effect of IL-6 on gonadotropin-induced progesterone secretion in cultured granulosa and theca cells

As shown in Fig. 4A, basal progesterone secreted by granulosa cells (510 ± 11 pg/ 10^5 cells) was not modified

by the presence of various doses of IL-6. FSH (1 ng/ml) induced a 15-fold increase in progesterone production. Increasing concentrations of IL-6 (1–50 ng/ml) inhibited FSH-stimulated progesterone secretion in granulosa cells in a dose-dependent manner ($P<0.001$). The response to higher doses of FSH (5 or 10 ng/ml), which could induce an increase of progesterone secretion as high as 25 times the basal level, was not changed by IL-6 (data not shown).

Figure 4 Effect of increasing doses of IL-6 on progesterone secretion in granulosa cells under basal or FSH (1 ng/ml)-stimulated conditions in the absence (A) or presence (B) of IBMX (100 ng/ml). Results are the mean $\pm$ S.E.M. of *n* experiments performed in triplicate. Values are expressed as percentage of mean value of progesterone secreted by untreated cells. *** $P < 0.001$ compared with FSH alone. (A, $n=5$, control: 510 ± 11 pg/ 10^5 cells; B, $n=3$, control: 682 ± 35 pg/ 10^5 cells.)

In the presence of 3-isobutyl-1-methylxanthine (IBMX), an inhibitor of phosphodiesterase activity, the inhibitory effect of IL-6 (25 ng/ml) on FSH-stimulated progesterone secretion was no longer seen (Fig. 4B).

Basal progesterone production in theca cells (334 ± 14 pg/ 10^5 cells) was not significantly modified in the presence of IL-6 (Fig. 5A). Treatment of theca cells with

Figure 5 Effect of increasing doses of IL-6 on progesterone secretion in theca cells under basal or hCG (10 mIU/ml)-stimulated conditions in the absence (A) or presence (B) of IBMX (100 ng/ml). Results are the mean $\pm$ S.E.M. of three experiments performed in triplicate. Values are expressed as percentage of mean value of progesterone secreted by untreated cells. *** $P < 0.001$ compared with hCG alone (A) and * $P < 0.05$ compared with hCG+IBMX (B). (A, control: 334 ± 14 pg/ 10^5 cells; B, control: 390 ± 15 pg/ 10^5 cells.)

10 mIU/ml hCG resulted in a 7.4-fold increase in progesterone secretion. Concomitant treatment with increasing doses of IL-6 caused a dose-dependent inhibition of hCG-stimulated progesterone production. Addition of IBMX (100 ng/ml) did not modify the inhibitory effect of 25 ng/ml IL-6 (Fig. 5B).

Table 1 Effect of IL-6 on progesterone production by granulosa cells induced by Db-cAMP (0.1 mM) or forskolin (0.25 µg/ml). Results are the mean ± S.E.M. of triplicate well cultures. Values are pg/10⁵ cells

	Experiment	Control	Agent alone	Plus IL-6	
				10 ng/ml	25 ng/ml
Db-cAMP	1	474 ± 46	5016 ± 265	5625 ± 227 ^{NS}	5700 ± 147 ^a
	2	713 ± 69	2995 ± 50	3370 ± 107 ^a	3420 ± 93 ^b
	3	335 ± 17	3907 ± 228	4757 ± 285 ^a	5545 ± 415 ^b
Forskolin	4	445 ± 24	1690 ± 80	2543 ± 182 ^b	2970 ± 205 ^c
	5	636 ± 16	5037 ± 165	5046 ± 496 ^{NS}	6963 ± 165 ^c

^aP<0.05; ^bP<0.01; ^cP<0.001 versus Db-cAMP or forskolin alone; NS: not significant.

Effect of IL-6 on granulosa and theca cells treated with Db-cAMP and adenylate cyclase-stimulating agents

In order to determine if the IL-6 inhibition of gonadotropin-induced progesterone production occurred downstream or upstream of the adenylate cyclase activation, granulosa and theca cells were cultured in the presence of Db-cAMP or adenylate cyclase-stimulating agents.

In the presence of 0.1 mM Db-cAMP, stimulated progesterone production was slightly enhanced by IL-6 in the three experiments performed (Table 1). A more important IL-6 stimulatory effect was observed when granulosa cells were cultured in the presence of forskolin (0.25 µg/ml), an agent that stimulates adenylate cyclase (Table 1). However, in the presence of cholera toxin, progesterone secretion by granulosa cells was not modified by addition of 10 or 25 ng/ml of IL-6 (Fig. 6).

Figure 6 Effect of IL-6 on progesterone secretion by granulosa cells induced by cholera toxin (1 ng/ml). Results are the mean ± S.E.M. of three experiments performed in triplicate. Values are expressed as percentage of mean value of progesterone secreted by untreated cells. (Control: 541 ± 29 pg/10⁵ cells.)

In theca cells, the inhibitory effect of IL-6 on progesterone production induced by hCG appeared to result from a mechanism different from the one observed for granulosa cells. Indeed, IL-6 inhibited significantly, in a dose-dependent manner, progesterone production induced by Db-cAMP (1 mM) or forskolin (1 µg/ml) (Table 2). However, when steroidogenesis was enhanced by cholera toxin, IL-6 was without effect on progesterone production (Fig. 7).

Discussion

All of our results indicate that IL-6 may act as a paracrine or autocrine regulator of ovarian steroidogenesis. Indeed granulosa cells and, as we have observed for the first time, theca cells produced measurable amounts of IL-6 and this cytokine had the ability to inhibit *in vitro* differentiation processes induced by gonadotropins.

The capacity to produce IL-6 is common also to cell types other than granulosa and theca cells such as lymphocytes or macrophages. But lymphocytes do not adhere to culture dishes and thus are likely to have been removed by the end of the 24 h preincubation, and macrophages are known to undergo apoptosis within 24 h when placed in culture in the absence of added cytokines, such as IL-1β, TNFα, granulocyte-macrophage colony stimulating factor or interferon-γ (Mangan & Wahl 1991). Moreover, Machelon *et al.* (1994a) working on human granulosa cells demonstrated that levels of IL-6 transcripts were similar in preparations treated to remove immune cells and in untreated ones, and concluded that IL-6 biosynthesis by macrophages was a negligible source of IL-6. Taken together, these observations seem to exclude the possibility that cytokine bioactivity detected in supernatants of cell cultures in our study could originate from white blood cells (Bréard *et al.* 1998).

Our observation that production of IL-6 could be inhibited by gonadotropins both in granulosa and theca cells contrasts with previous observations in the rat. In that species, immunoreactive IL-6 was detected in the supernatant of granulosa cell cultures (Gorospe *et al.* 1992,

Table 2 Effect of IL-6 on progesterone production by theca cells induced by Db-cAMP (1 mM) or forskolin (1 µg/ml). Results are the mean ± S.E.M. of triplicate well cultures. Values are pg/10⁵ cells

	Experiment	Control	Agent alone	Plus IL-6		
				10 ng/ml	25 ng/ml	50 ng/ml
Db-cAMP	1	297 ± 17	4914 ± 241	3941 ± 169 ^b	2317 ± 107 ^c	1497 ± 45 ^c
	2	467 ± 15	4146 ± 159	3243 ± 196 ^b	2826 ± 252 ^c	2576 ± 93 ^c
	3	275 ± 8	3214 ± 129	3055 ± 261 ^{NS}	2926 ± 159 ^{NS}	740 ± 102 ^c
Forskolin	4	297 ± 17	4651 ± 122	3309 ± 80 ^c	1879 ± 53 ^c	1418 ± 65 ^c
	5	509 ± 13	3584 ± 208	3327 ± 137 ^{NS}	2857 ± 92 ^b	2021 ± 112 ^c

^bP<0.01; ^cP<0.001 versus Db-cAMP or forskolin alone; NS: not significant.

Gorospe & Spangelo 1993) and various doses of FSH were found to be able to induce a significant increase in IL-6 production. Results from this study also suggested that cAMP could be the second messenger of the gonadotropin action (Gorospe & Spangelo 1993). However, in other studies, FSH or hCG were not found to modify significantly the release of IL-6 by human or primate granulosa cells (Machelon *et al.* 1994a, 1995), and more recently it was observed that IL-6 production by primate granulosa cells was not detected in the presence of Db-cAMP (Machelon *et al.* 1995). Hence it appears that regulation by gonadotropins of IL-6 release in ovarian cells could be species dependent.

The regulatory effect of gonadotropin observed in our study could result from an indirect mechanism. We previously observed that estradiol was released in response to FSH and hCG by cultured granulosa and theca cells respectively (Féral *et al.* 1995) and estradiol has been

shown to inhibit the ovarian IL-6 expression (Pottratz *et al.* 1994). Moreover, IL-1 stimulated IL-6 production and we previously reported that, in the rabbit, IL-1 bioactivity production in cultured granulosa and theca cell supernatants was inhibited by gonadotropins (Bréard *et al.* 1998). So we can assume that gonadotropins could inhibit IL-6 release at least in part through both an increase in estradiol production and a decrease in IL-1 production.

Concerning the action of IL-6 on rabbit steroidogenesis, IL-6 had no effect on basal progesterone production by granulosa and theca cells cultured for 24 h as observed in monkey granulosa and porcine luteal cells (Pitzel *et al.* 1993, Machelon *et al.* 1995). When progesterone production was stimulated *in vitro* by physiological doses of gonadotropins, we observed an inhibition of its production by IL-6 in both types of follicular cells and this inhibition was not the result of an IL-6 cytotoxic effect. In fact, the ability of IL-6 to inhibit differentiation seems in keeping with its ability to promote an increase in cell number.

Our observation that IL-6 acted in an inhibitory way on steroidogenesis induced by gonadotropins in rabbit granulosa cells is in agreement with studies in several other species (rat (Gorospe *et al.* 1992, Gorospe & Spangelo 1993, Hughes *et al.* 1994), bovine (Alpizar & Spicer 1993)). However, in porcine and monkey granulosa cells (Pitzel *et al.* 1993, Machelon *et al.* 1995), no effect of IL-6 on hCG-stimulated progesterone secretion was observed, and Machelon *et al.* (1994a) recently reported that in human granulosa cells, IL-6 stimulated progesterone production induced by hCG.

When studying the action of IL-6 on steroidogenesis in rabbit granulosa cells, we unexpectedly observed that IL-6, while reducing progesterone production induced by FSH, increased at the same time progesterone synthesis induced by Db-cAMP or forskolin. This observation suggested that IL-6 could induce a post-cAMP step stimulatory effect associated with a pre-cAMP step inhibitory effect. This latter effect was apparently predominant when an intracellular cAMP rise was induced by FSH, since only the inhibitory effect was observed. Such antagonistic pre- and post-cAMP effects were previously observed when studying the role of IL-1 in the rabbit (Bréard *et al.* 1998) as well as in other species (Gottschall

Figure 7 Effect of IL-6 on progesterone secretion by theca cells stimulated by cholera toxin. Results are the mean ± S.E.M. of three experiments performed in triplicate. Values are expressed as percentage of mean value of progesterone secreted by untreated cells. (Control: 526 ± 17 pg/10⁵ cells.)

et al. 1988, Santana *et al.* 1996). However, in the present study, we could not document an inhibition by IL-6 of the FSH-induced cAMP accumulation (unpublished data), because, as previously reported (Bréard *et al.* 1998), the dose of FSH used was unable to stimulate granulosa cAMP accumulation, although this gonadotropin concentration stimulated 15-fold the basal progesterone production (an apparent discrepancy which has been largely reported in the past (Azhar & Menon 1979)). However, the pre-cAMP defect could be localized by the use of cholera toxin. In the presence of this substance, we observed neither the IL-6 inhibitory effect on granulosa progesterone production nor the stimulatory IL-6 effect which occurred at a post-cAMP step. This result demonstrates that the IL-6 inhibitory effect on a pre-cAMP step was decreased when adenylate cyclase was stimulated by cholera toxin, suggesting that IL-6 prevented in part the activation of adenylate cyclase by a G protein. In the same way, in the presence of IBMX, FSH-induced progesterone production was no longer inhibited by IL-6. This result suggests that the increase in the cAMP pool induced by IBMX could induce a post-cAMP effect overcoming the pre-cAMP inhibitory effect.

In theca cells, we observed a constant inhibitory effect of IL-6 on progesterone production stimulated by hCG, forskolin or Db-cAMP. These results indicate that, in contrast to its effect on granulosa cells, IL-6 exerted on theca cells an inhibitory effect at a post-cAMP step. We found no explanation for the fact that in the presence of cholera toxin IL-6 did not affect progesterone production in theca cells.

In summary, it appears from our results that IL-6 might be a factor inducing granulosa and theca cell dedifferentiation, reflected here by a decrease in progesterone synthesis. Surprisingly the post-cAMP mechanism of this modulation by IL-6 of steroidogenesis induced by gonadotropins did not seem to be identical in granulosa and theca cells. However, gonadotropins were apparently able to control the negative modulation of this cytokine on their steroidogenic action by inhibiting its production.

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