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Glucocorticoids suppress basal (but not interleukin-1-supported) ovarian phospholipase A_2 activity[1]: Evidence for glucocorticoid receptor-mediated regulation

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Abstract

Ovulation may constitute a cyclic, inflammatory-like process, wherein the increased expression of interleukin (IL)-1 and the biosynthesis of prostaglandins may be established corollaries. In this communication we hypothesize that glucocorticoids, potent anti-inflammatory principles, may exert an antiovulatory effect by interfering with ovarian IL-1-driven prostaglandin biosynthesis. To test this hypothesis, we examined the effect of treatment with dexamethasone on the activity of ovarian phospholipase A_2 (PLA_2), the event-limiting enzyme in prostaglandin biosynthesis, and on the gene expression pattern of secretory and cytosolic PLA_2 ($sPLA_2$ and $cPLA_2$, respectively). Whole ovarian dispersates from immature rats were cultured under serum-free conditions for 48 h in the absence or presence of dexamethasone. At the conclusion of this culture period, PLA_2 activity was determined in cell sonicates and conditioned media. Parallel probing for $sPLA_2$ and $cPLA_2$ transcripts was also undertaken using a solution hybridization/RNAse protection assay. Treatment of whole ovarian dispersates with dexamethasone produced a significant ($P < 0.005$) decrease in basal cellular and extracellular PLA_2 activity to 27 and 40% of controls, respectively. A 5-fold decrease in the basal steady state levels of $sPLA_2$ (but not $cPLA_2$) transcripts was also noted. Co-treatment with dexamethasone produced complete inhibition of IL-1-stimulated $cPLA_2$ transcripts but not of IL-1-supported cellular and extracellular PLA_2 activity or $sPLA_2$ transcripts. A glucocorticoid receptor antagonist (RU486), blocked the ability of dexamethasone to inhibit basal $sPLA_2$ transcripts and extracellular PLA_2 activity. The inhibitory effect of dexamethasone proved glucocorticoid-specific in that aldosterone and 17β-estradiol were without effect. Taken together, these observations suggest that dexamethasone is capable of inhibiting basal (but not IL-1-supported) ovarian PLA_2 activity, a glucocorticoid receptor-mediated effect due, in part, to a decrease in $sPLA_2$ gene expression. Our findings further suggest that $sPLA_2$ and $cPLA_2$ are differentially regulated and that they may well differ in their relative contribution to ovarian prostaglandin biosynthesis in general and to PLA_2 activity in particular. To the extent that IL-1 plays a central role in the ovulatory process, these findings argue against the view that the chronic anovulatory state induced by glucocorticoid excess is due, if only in part, to suppression of ovarian IL-1-dependent PLA_2 activity. © 1998 Published by Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Interleukin-1; Phospholipase; Ovary; Glucocorticoid

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1. Introduction

The notion that ovulation may constitute a cyclic inflammatory-like reaction, originally postulated by Espey (1980, 1994), has been the subject of significant interest. Central to this hypothesis is the recognition that prostanoids, established mediators of inflammation play an indispensable and indeed obligatory role in the ovulatory process (Tsafriri et al., 1973; Hamada et al., 1977; Espey et al., 1986; Schmidt et al., 1986; Sogn et al., 1987). More recently, the suggestion has been made that Interleukin (IL)-1 may constitute yet another key intermediary in the ovulatory process. Importantly, IL-1 has been documented to exert a potent stimulatory effect on ovarian prostaglandin biosynthesis.

Although the biosynthesis of prostanoids constitutes a complex process, there is little doubt as to the central importance of the initial rate limiting event (Dennis, 1994) wherein arachidonic acid substrate is released from the *sn-2* position of membrane phospholipids by the enzyme phospholipase A_2. It is undoubtedly that recognition which prompted a flurry of interest in the ovarian expression, cellular localization, and hormonal regulation of ovarian PLA_2 (Kol et al., 1997a,b). Evidence to date (Glaser et al., 1993; Mayer and Marshall, 1993; Mukherjee et al., 1994) suggests that mammalian PLA_2 constitute a heterogeneous family of enzymes representative of two classes of proteins: secretory, low molecular weight (14 kDa, $sPLA_2$), and cytosolic, high molecular weight (85–110 kDa, PLA_2).

Glucocorticoids may, when in excess, exert an anti-ovulatory effect (Iannaccone et al., 1959; Hagino et al., 1969; Hagino, 1972; Baldwin and Sawyer, 1974; Cunningham et al., 1975; Ewy et al., 1985). This communication examines the possibility that the anti-ovulatory action of glucocorticoids may be due, in part, to interference with prostanoid biosynthesis. Specifically, we examined the effect of treatment with dexamethasone on the expression and activity of ovarian PLA_2. Our findings reveal that dexamethasone is capable of inhibiting basal (but not IL-1-supported) ovarian PLA_2 activity, an effect due, in part, to a decrease in $sPLA_2$ gene expression. These observations are incompatible with the view that the antiovulatory action of glucocorticoids is due, in part, to suppression of ovarian IL-1-dependent liberation of arachidonic acid.

2. Materials and methods

2.1. Animals

Immature Sprague-Dawley female rats from Zivic-Miller Laboratories (Zelienople, PA) were sacrificed by CO_2 asphyxiation on day 25 of life. The project was approved by the Institutional Animal Care and Use Committee.

2.2. Reagents and hormones

Phosphatidylcholine L-α-1-stearoyl-2-arachidonyl {arachidonyl-5,6,8,9,11,12,14,15-^{3}H(N), PCSA; specific activity 88 Ci/mmol}, Phosphatidylcholine L-α-dipalmitoyl-{2-palmitoyl-9,10-^{3}H(N), PCDP; specific activity 42 Ci/mmol}; palmitic acid {9,10^{3}H (PA); specific activity 39 Ci/mmol} and [α-^{32}P]UTP (specific activity 800 Ci/mmol) were purchased from DuPont, New England Nuclear (Boston, MA). The corresponding unlabeled principles and RNase A were purchased from Sigma (St. Louis, MO). McCoy's 5a (serum-free) medium, penicillin-streptomycin solution, bovine serum albumin (BSA), DNase, and trypan blue stain were obtained from Life Technologies (Grand Island, NY). Collagenase (Clostridium Histolyticum; CLS type I; 144 U/mg was from Worthington Biochemical (Freehold, NJ). Hexane, methanol and 2-propanol were from J.T. Baker (Phillipsburg, NJ). Chloroform was from Fisher Scientific (Fair Lawn, NJ). RNA marker set kit was from Ambion (Austin, TX). RNase T1 were from Pharmacia (Piscataway, NJ). T7 RNA polymerase, pGEM7Zf+ and other molecular grade reagents were from Promega (Madison, WI).

RU486 was a generous gift from Rousell UCLAF (Romainville Cedex, France). Dexamethasone, hydrocortisone, estradiol-17β, and aldosterone and other chemicals were from Sigma (St. Louis, MO). Recombinant human IL-β (2×10^7 units/mg) was generously provided by Drs Errol B. De Souza and C.E. Newton, DuPont-Merck Pharmaceutical (Wilmington, DE).

2.3. Tissue culture procedures

Whole ovarian dispersates were prepared and cultured as previously described (Hurwitz et al., 1991).

2.4. Cell-free enzyme assays

Cellular and extracellular PLA_2 activities were determined by measuring the release of ^{3}H-labeled fatty acid from the *sn-2* position of ^{3}H-labeled PCSA or PCDP substrates (arachidonic or palmitic acid, respectively) as previously described (Kol et al., 1997b).

2.5. Nucleic acid probes

The rat type II $sPLA_2$, cytosolic PLA_2 and ribosomal protein large 19 (RPL19) probes were generated and employed as previously described (Kol et al., 1997b).

2.6. RNA extraction

RNA of cultured cells was extracted with RNAZOL-B (Tel Test, Friendswood, TX) according to the manufacturer's protocol.

2.7. RNAse protection assay

Linearized DNA templates were transcribed with T7 RNA polymerase to specific activities of 800Ci/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 that are shorter than the full length probes as previously described (Kol et al., 1996). The assay was carried out as previously described (Lowe et al., 1987). 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 mRNA signal was used to normalize the $cPLA_2$ and $sPLA_2$ mRNA data for possible variation in RNA loads. Specifically, the net protected signal (respective background subtracted) to net RPL19 signal ratio was calculated for each sample and gene of interest.

2.8. Statistical analysis

Except as noted, each experiment was replicated a minimum of 3 ×. Data points are presented as mean ± SE and statistical significance (Fisher's protected least significance difference) determined by ANOVA and Student's t test. Statistical values were calculated using Statview 512+ for MacIntosh (Brain Power, Calabasas, CA).

3. Results

3.1. Basal and IL-1-stimulated extracellular PLA_2 activity in media conditioned by whole ovarian dispersates: effect of dexamethasone

To evaluate the effect of dexamethasone on extracellular PLA_2 activity, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (10 ng/ml), with or without dexamethasone (5 μM). At the conclusion of this period, media were collected and subjected to a cell-free PLA_2 activity as described, with PCDP as substrate. The latter is highly-selective for $sPLA_2$ and is barely cleaved by $cPLA_2$ (Dennis, 1994). As shown (Fig. 1), treatment with dexamethasone resulted in a significant ($P < 0.01$), decrease (60%) in basal PLA_2 activity. Treatment with IL-1β produced a 2-fold increase in PLA_2 activity ($P < 0.05$). Moreover, co-treatment with IL-1β completely reversed the inhibitory dexamethasone effect to a point beyond that noted for controls ($P < 0.05$ for IL-1β + dexamethasone vs control).

3.2. Extracellular PLA_2 activity in media conditioned by untreated and dexamethasone-treated whole ovarian dispersates: IL-1 dose-dependence

To further confirm the ability of IL-1β to reverse the dexamethasone-induced inhibition of basal extracellular PLA_2 activity, whole ovarian dispersates were cultured in the absence or presence of dexamethasone (5 μM), IL-1β (10 ng/ml), or dexamethasone plus increasing concentrations of IL-1β (0.5–50 ng/ml). As shown (Fig. 2), treatment with the lowest concentration of IL-1β (0.5 ng/ml) all but reversed the dexamethasone-induced inhibition of basal extracellular PLA_2 activity. Treatment with higher concentrations of IL-1β resulted in further increases (above control) in extracellular PLA_2 activity ($P < 0.05$ for 5ng/ml of IL-1β + dexamethasone vs control).

3.3. $sPLA_2$ gene expression by untreated and dexamethasone-treated whole ovarian dispersates: IL-1 dose-dependence

To examine the role of dexamethasone in the regulation of ovarian $sPLA_2$ gene expression and to further explore the opposing property of IL-1β in this regard, whole ovarian dispersates were cultured for 48 h in the absence or presence of dexamethasone (5 μM), IL-1β (10 ng/ml) or dexamethasone plus increasing concentrations (0.5–50 ng/ml) of IL-1β. Thereafter, total cellular RNA was extracted and subjected to an RNAse protection assay with antisense riboprobes corresponding to

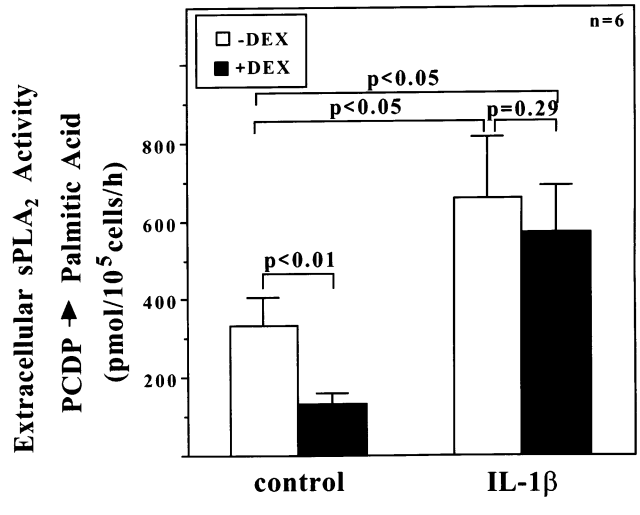

Fig. 1. Extracellular PLA_2 activity in media conditioned by untreated and IL-1-treated whole ovarian dispersates: effects of dexamethasone. Whole ovarian dispersates (5×10^5 viable cells/tube) were cultured for 48 h in the absence or presence of IL-1β (10 ng/ml), with or without dexamethasone (DEX: 5 μM). At the conclusion of this culture period, media were collected and subjected to a cell-free PLA_2 activity assay as described, with PCDP as substrate. Data depicted in bar graph form the mean ± SE of six independent experiments.

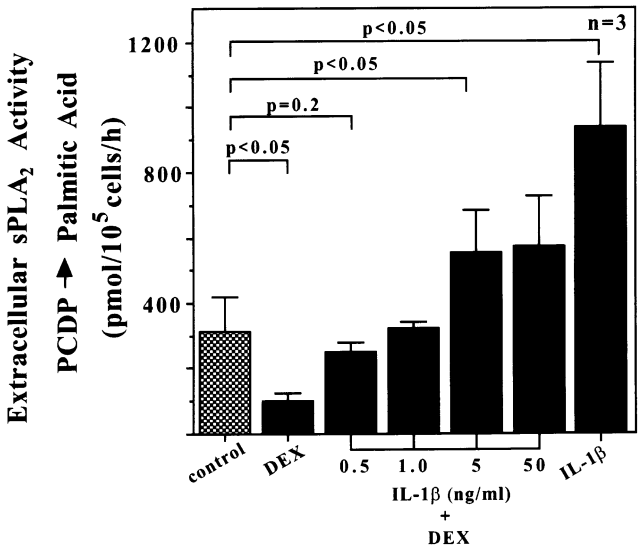

Fig. 2. Extracellular PLA_2, activity in media conditioned by untreated and dexamethasone-treated whole ovarian dispersates: IL-1 dose-dependence. Whole ovarian dispersates (5×10^5 viable cells/tube) were cultured for 48 h in the absence or presence of dexamethasone (5 μM), IL-1β (10 ng/ml) or dexamethasone plus increasing concentrations of IL-1β (0.5–50 ng/ml). At the conclusion of the culture period, media were collected and subjected to a cell-free PLA_2 activity assay as described, with PCDP as substrate. Data depicted in bar graph form the mean $\pm$ SE of three independent experiments.

rat $sPLA_2$ and RPL19. As shown (Fig. 3), treatment with dexamethasone resulted in a 5-fold decrease ($P < 0.001$) in $sPLA_2$ transcripts as compared with untreated controls. However, the concurrent provision of even the lowest dose of IL-1β (0.5ng/ml) resulted in a complete reversal of the inhibitory dexamethasone effect to a level beyond that displayed by control.

3.4. Cellular PLA_2 activity of untreated and IL-1-treated whole ovarian dispersates: effect of dexamethasone

To explore the possibility that dexamethasone may also inhibit cellular PLA_2 activity, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (10 ng/ml), with or without dexamethasone (5 μM). Thereafter, the cells were sonicated, and sonicates (representing 1.5×10^5 cells) subjected to a cell-free PLA_2 activity assay as described, with PCSA as the substrate. The latter serves as a substrate for both $sPLA_2$ and $cPLA_2$ (Dennis, 1994). As shown (Fig. 4) treatment with dexamethasone resulted in a 4-fold decrease in basal cellular PLA_2 activity ($P < 0.01$). Treatment with IL-1β alone produced a 2.4-fold increase in PLA_2 activity ($P = 0.1$). The concurrent provision of IL-1β resulted in a complete reversal of the inhibitory dexamethasone effect well beyond levels displayed by controls ($P < 0.05$ for IL-1β + dexamethasone vs control).

3.5. Extracellular PLA_2 activity in media conditioned by untreated whole ovarian dispersates: glucocorticoid receptor mediation

To determine if the dexamethasone effect is glucocorticoid receptor-mediated, whole ovarian dispersates were cultured with or without increasing concentrations of dexamethasone, hydrocortisone, aldosterone, or estradiol-17β. Media were collected and subjected to a cell-free PLA_2 activity assay as described, with PCDP as substrate. As shown (Fig. 5), dexamethasone proved to be the most potent inhibitor of basal extracellular PLA_2 activity, followed by hydrocortisone and aldosterone, suggesting that the dexamethasone effect is glucocorticoid receptor-selective. Estradiol-17β was without effect on basal extracellular PLA_2 activity.

3.6. Extracellular PLA_2 activity in media conditioned by untreated and dexamethasone-treated whole ovarian dispersates: effect of RU486

To further examine the possibility that dexamethasone-mediated inhibition of extracellular PLA_2 activity is receptor-mediated, whole ovarian dispersates were cultured in the absence of treatment, in the pres-

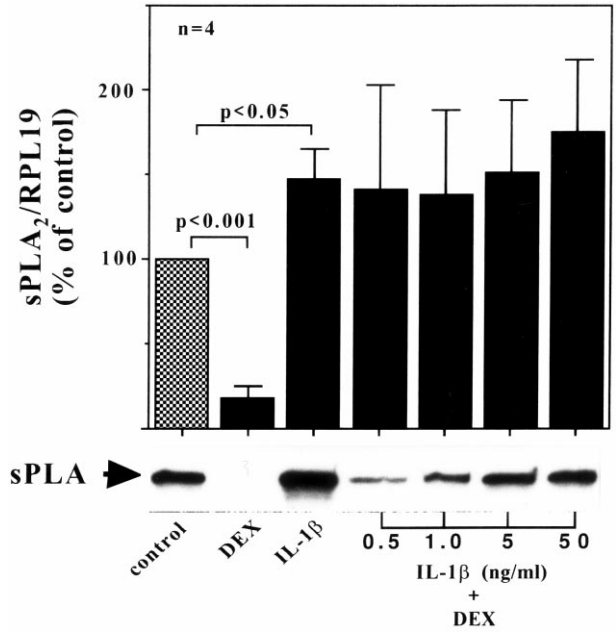

Fig. 3. $sPLA_2$ gene expression by untreated and dexamethasone-treated whole ovarian dispersates: IL-1 dose-dependence. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in the absence or presence of dexamethasone (5 μM), IL-1β (10 ng/ml) or dexamethasone plus increasing concentrations of IL-1β (0.5–50 ng/ml). Thereafter, total cellular RNA was extracted and subjected to an RNAse protection assay with antisense riboprobes corresponding to rat $sPLA_2$ and RPL19. The intensity of the protected fragments was quantified and normalized as described. The top panel depicts in bar graph form the mean $\pm$ SE of four independent experiments. The lower panel depicts a representative autoradiograph.

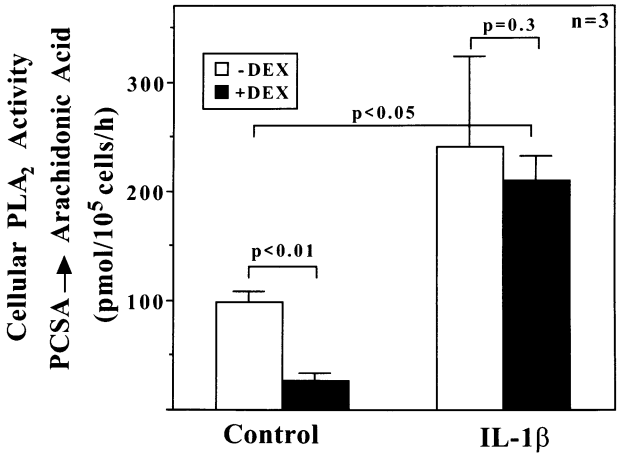

Fig. 4. Cellular PLA_2 activity in untreated and IL-1-treated whole ovarian dispersates: effects of dexamethasone. Whole ovarian dispersates (5×10^5 viable cells/tube) were cultured for 48 h in the absence or presence of IL-1β (10 ng/ml), with or without dexamethasone (5 μM). Thereafter, media were collected, the cells sonicated and sonicates (representing 1.5×10^5 cells) was subjected to a cell-free PLA_2 activity assay with PCSA as the substrate. Data depicted in bar graph form the mean $\pm$ SE of three independent experiments.

ence of dexamethasone (5 μM), or in the presence of dexamethasone plus the glucocorticoid receptor antagonist (RU486). As shown (Fig. 6), treatment with dexamethasone resulted in significant ($P < 0.05$) inhibition (80%) of extracellular PLA_2 activity ($P < 0.05$). However, the concurrent provision of RU486 (1 μM) resulted in substantial (albeit incomplete) reversal of that effect to a level indistinguishable from controls ($P = 0.08$) thereby suggesting that the inhibitory dexamethasone effect is glucocorticoid receptor-mediated.

3.7. $sPLA_2$ gene expression by untreated and dexamethasone-treated whole ovarian dispersates: effect of RU486

To further confirm that the dexamethasone effect is glucocorticoid receptor-mediated, whole ovarian dispersates were cultured for 48 h in the absence of treatment, in the presence of dexamethasone (5 μM), or in the presence of dexamethasone plus RU486 (1 mM). At the conclusion of this period, total cellular RNA was extracted and subjected to an RNAse protection assay with antisense riboprobes corresponding to rat $sPLA_2$ and RPL19. As shown (Fig. 7) the dexamethasone-induced inhibition (79%) of $sPLA_2$ gene expression ($P < 0.001$) was reversed by concurrently-provided RU486 thereby further suggesting glucocorticoid receptor mediation.

3.8. $cPLA_2$ gene expression by untreated and IL-1-treated cultured whole ovarian dispersates: effect of dexamethasone

To examine the role of dexamethasone in the regulation of ovarian $cPLA_2$ gene expression and to further explore a possible opposing property of IL-1β in this regard, whole ovarian dispersates were cultured for 48 h in the absence or presence of IL-1β (10 ng/ml), with or without dexamethasone (1 μM). Thereafter, total RNA was extracted and subjected to an RNAse protection assay with antisense riboprobes corresponding to rat $cPLA_2$ and RPL19. As shown (Fig. 8), treatment with dexamethasone by itself was without significant effect on the basal steady state of $cPLA_2$ transcripts. Treatment with IL-1β resulted in a 5-fold increase ($P < 0.001$) in $cPLA_2$ transcripts. The latter effect was completely reversed by co-treatment with dexamethasone ($P < 0.001$).

4. Discussion

A growing body of literature supports the proposition that ovulation may constitute an inflammatory-like process (Espey, 1980, 1994). Indeed, ovulation appears to display the key attributes of the classic inflammatory process inclusive of heat, swelling, redness, temporary loss of function, and perhaps even pain. A central component of ovulation is the biosynthesis of prostaglandins (Tsafriri et al., 1973; Hamada et al., 1977; Espey et al., 1986; Schmidt et al., 1986; Sogn et al., 1987). Although the precise role of prostaglandins in the ovulatory cascade remains uncertain, it is highly likely that these mediators of inflammation may serve as coordinating messengers for a series of ovulation-associated phenomena such as the induction of periovulatory hyperemia (Lee and Novy, 1978; Murdoch and Myers, 1983; Abisogun et al., 1988) and of collagenolysis (Reich et al., 1985, 1991).

Glucocorticoids have been used for decades as clinical tools to suppress both the immune response and the process of inflammation (Cupps and Fauci, 1982). However, the precise molecular and cellular mechanism(s) underlying the immunosuppressive property of glucocorticoids remains poorly understood. In part, glucocorticoids may markedly decrease cytokine secretion and thus effectively block the activation of the immune system (Knudsen et al., 1987). In this context, the inhibition of nuclear factor kappa B (NF-κB), a regulator of immune system and inflammation genes has been shown to be targeted for decrease by glucocorticoids (Mukaida et al., 1994; Ray and Prefontaine, 1994; Scheinman et al., 1995).

The ability of glucocorticoids to inhibit prostaglandin biosynthesis may be due to the suppression of PLA_2 activity (Nakano et al., 1990; O'Banion et al., 1992; Hoeck et al., 1993). Previous studies suggested (but since questioned) that this inhibitory effect may be mediated indirectly by lipocortins (Wu et al.,

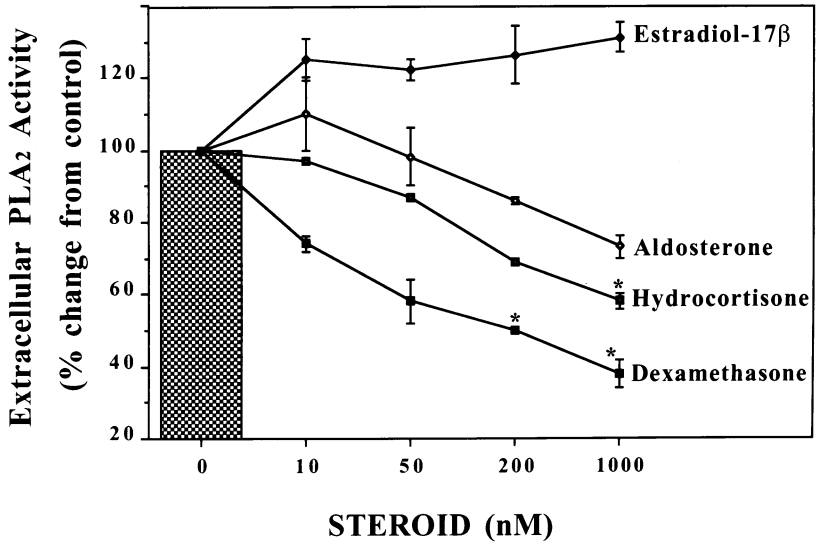

Fig. 5. Extracellular PLA_2 activity in media conditioned by untreated whole ovarian dispersates: glucocorticoid receptor mediation. Whole ovarian dispersates (5×10^5 viable cells/tube) were cultured with or without increasing concentrations of dexamethasone, hydrocortisone, aldosterone or estradiol-17β. Media were collected and subjected to a cell-free PLA_2 activity assay as described with PCDP as substrate. Data depicted in bar graph form the mean $\pm$ SE of three independent experiments.

1995), intracellular peptides which inhibit PLA_2 enzyme activity. Glucocorticoids could also exert a direct transcriptional effect thereby diminishing the steady state levels of $cPLA_2$ and $sPLA_2$ transcripts. Either way, blockage of PLA_2 results not only in the attenuation of prostaglandin biosynthesis, but also in the blockade of other potent mediators of inflammation (e.g. leukotrienes). Consequently, glucocorticoid-mediated suppression of PLA_2 activity may have a profound effect on the generation of inflammatory mediators. This communication concerns itself with the possibility that the anti-ovulatory effect of glucocorticoids is attributable to their ability to inhibit inflammation in general and in the ovary in particular. Specifically, the ability of glucocorticoids to suppress one component of the inflammatory response, i.e. prostaglandin biosynthesis was assessed. Our present findings establish the ability of dexamethasone to exert a significant inhibitory effect on both cellular and extracellular basal ovarian PLA_2 activity (Figs. 1, 2 and 4–6). These phenomena were associated with a marked decrease in the basal steady state levels of $sPLA_2$ (but not $cPLA_2$) transcripts (Figs. 3, 7 and 8). As might be expected, most of the above effects were markedly reversed by IL-1β, an established mediator of inflammation (Dinarello and Sheldon, 1993). Indeed, previous studies have clearly established the ability of IL-1 to upregulate PLA_2 transcripts and activity (Kol et al., 1997a,b). A comparable phenomenon was reported for a number of extraovarian sites (Burch et al., 1988; Nakazato et al., 1991; Scalkwijk et al., 1992). Moreover, IL-1 is a potent stimulator of ovarian prostaglandin biosynthesis (Kokia et al., 1992; Brannstrom et al., 1993; Ben-Shlomo et al., 1994; Townson and Pate, 1994). Taken together, these observations suggest that at the level of the ovary, dexamethasone and IL-1 exert diametrically opposed effects.

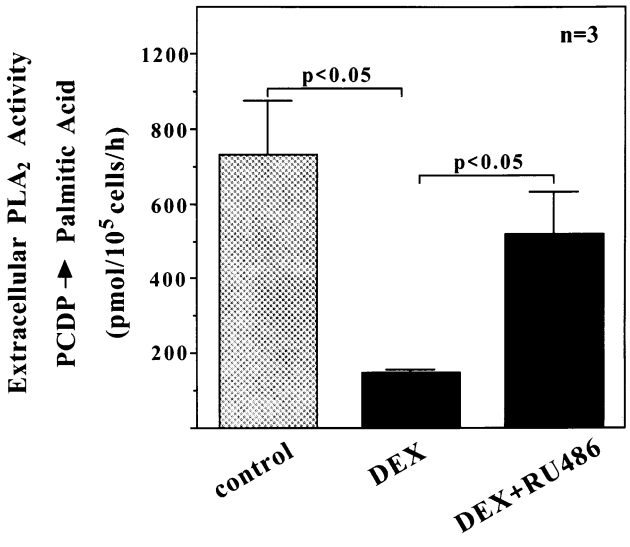

Fig. 6. Extracellular PLA_2 activity in media conditioned by untreated and dexamethasone-treated whole ovarian dispersates: effect of RU486. Whole ovarian dispersates (5×10^5 viable cells/tube) were cultured for 48 h in the absence of treatment, in the presence of dexamethasone (5 μM), or in the presence of dexamethasone plus the glucocorticoid receptor antagonist (RU486, 1 μM). At the conclusion of the culture period, media were collected and subjected to a cell-free PLA_2 activity assay as described with PCDP as substrate. Data depicted in bar graph form the mean $\pm$ SE of three independent experiments.

It is generally agreed that glucocorticoids bind to a nuclear glucocorticoid receptor, a member of the

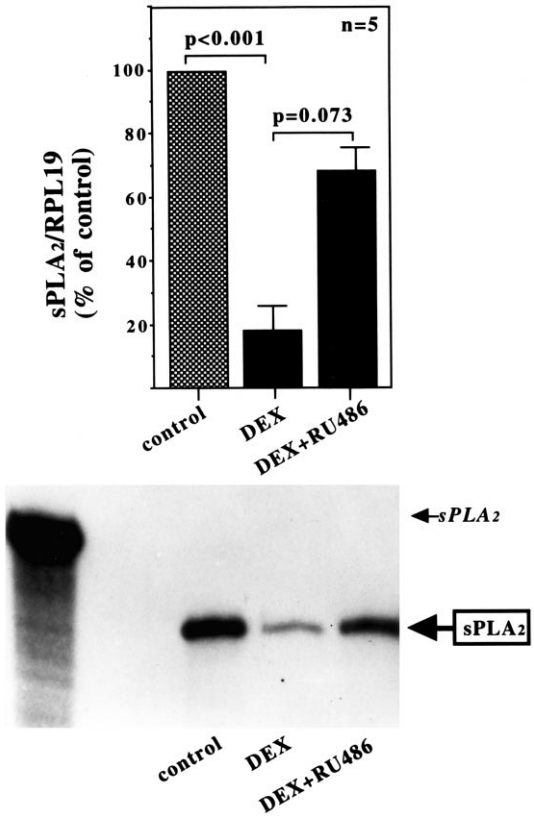

Fig. 7. $sPLA_2$ gene expression by untreated and dexamethasone-treated whole ovarian dispersates: effect of RU486. Whole ovarian dispersates (5×10^5 viable cells/tube) were cultured for 48 h in the absence of treatment, in the presence of dexamethasone (5 mM), or in the presence of dexamethasone plus the glucocorticoid receptor antagonist (RU486, 1 mM). At the conclusion of the culture period, total cellular RNA was extracted and subjected to an RNAse protection assay with antisense riboprobes corresponding to rat $sPLA_2$ and RPL19. The top panel depicts in bar graph form the mean $\pm$ SE of five independent experiments. The lower panel depicts a representative autoradiograph. The full-length $sPLA_2$ riboprobe is labeled in italics. The corresponding protected fragment is labeled in bold face letters.

steroid hormone receptor superfamily, which acts as a transcription factor (Truss and Beato, 1993). It must be assumed that the ability of dexamethasone to inhibit ovarian PLA_2 is glucocorticoid receptor-mediated. This presumption appears to be supported by the demonstration that the dexamethasone effect is characterized by an order of potency of dexamethasone > hydrocortisone > aldosterone (Fig. 5). In addition, co-treatment with RU486, an established glucocorticoid receptor antagonist (Bamberger and Chrousos, 1995), all but abolished the ability of dexamethasone to downregulate $sPLA_2$ transcripts (Fig. 7) and to inhibit extracellular PLA_2 activity (Fig. 6).

The association between Cushing's syndrome and ovarian dysfunction is well documented (Iannaccone et al., 1959). Autopsy of ovarian material revealed the absence of growing follicles and a decreased complement of primordial follicles. Moreover, glucocorticoid excess of an iatrogenic nature has been shown to produce ovulatory dysfunction in seven of eleven normally cycling women (Cunningham et al., 1975).

Comparable observations have been reported for different mammalian species in association with the administration of exogenous glucocorticoids or ACTH (Hagino et al., 1969; Hagino, 1972; Baldwin and Sawyer, 1974; Cunningham et al., 1975; Ewy et al., 1985). Although the precise cellular and molecular mechanism(s) involved remain uncertain, consideration must be given to the hypothalamic/pituitary effect. However, the possibility of a direct ovarian effect cannot be excluded. In this context, previous observations have shown glucocorticoids to exert a variety of cytodifferentiative effects at the level of the rat granulosa cells (Adashi et al., 1981; Schoonmaker and Erickson, 1983). In vitro, dexamethasone was active at concentrations as low as 10 nM (Fig. 5) although statistically significant effects were only apparent at the 200 nM dose. Circulating levels of dexamethasone were 6.5 μM following the ingestion of 0.75–2 mg of the drug (Asnis et al., 1989). Higher circulating concentrations are likely following the use of immunosuppressive doses of dexamethasone. In principle then, the concentrations employed in vitro could be obtainable in vivo.

Given the ability of glucocorticoids to suppress basal ovarian $sPLA_2$ activity (and by extension prostaglandin biosynthesis), one might speculate that these phenomena account, if only in part, for the antiovulatory activity of glucocorticoids. Such observations are supported by the recognition that prostaglandin biosynthesis is obligatory to the ovulatory process (Tsafriri et al., 1973; Hamada et al., 1977; Espey et al., 1986; Schmidt et al., 1986; Sogn et al., 1987). However, IL-1β, a pro-inflammatory (Dinarello and Sheldon, 1993), and pro-ovulatory (Peterson et al., 1993; Takehara et al., 1994; Ben-Shlomo et al., 1997) principle proved capable of effectively reversing the dexamethasone effect. In fact, the antagonistic action of IL-1β is exerted at relatively low dosage (Figs. 2 and 3) in keeping with the observation that the upregulation of ovarian $sPLA_2$ transcripts is an exquisitely sensitive endpoint of IL-1β (Peterson et al., 1993; Takehara et al., 1994; Ben-Shlomo et al., 1997). In light of the above and the possibility that IL-1 may play a central role in the ovulatory process, our present observations argue against the possibility that the chronic anovulatory state induced by adrenal hyperactivity is due, if only in part, to suppression of ovarian PLA_2 activity.

Our present findings reveal distinct patterns for the regulation of $sPLA_2$ as compared with $cPLA_2$. Indeed, dexamethasone appeared capable of suppressing basal $sPLA_2$ but not $cPLA_2$ expression. Moreover, whereas dexamethasone produced profound inhibition of the ability of IL-1β to upregulate $cPLA_2$, no such effect

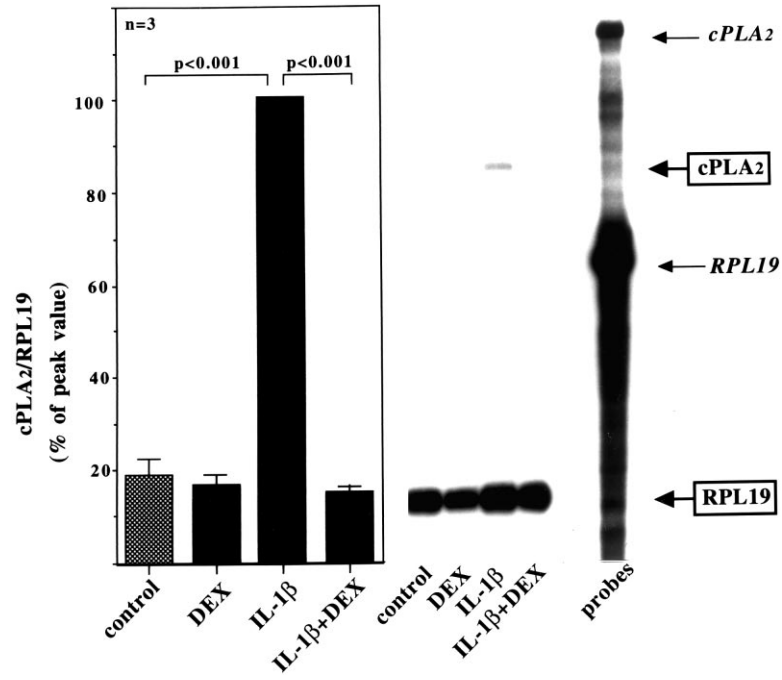

Fig. 8. $cPLA_2$ gene expression by untreated and IL-1 treated cultured whole ovarian dispersates: effect of dexamethasone. Whole ovarian dispersates (1.5×10^6 cells/dish) were cultured for 48 h in the absence or presence of dexamethasone (1 μM), with or without IL-1β (10 ng/ml). Thereafter, total cellular RNA was extracted and subjected to an RNAse protection assay with antisense riboprobes corresponding to rat $cPLA_2$ and RPL9. The left panel depicts in bar graph form the mean $\pm$ SE of three independent experiments. The right panel depicts a representative autoradiograph. The full-length riboprobes are labeled in italics. The corresponding protected fragments are labeled in bold face letters.

could be detected for $sPLA_2$. Although the precise significance of the above observations remain uncertain, it is highly likely that the two isoforms differ in their overall function and mission, as well as their relative contribution to ovarian prostaglandin biosynthesis.

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