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Sustained Activity of Luteal Cytosolic Phospholipase A_2 During Luteolysis in Pseudopregnant Rats

Its Possible Implication in Tissue Involution

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We investigated the expression and activity of cytosolic phospholipase A_2 ($cPLA_2$) in the corpus luteum during spontaneous and induced luteolysis in pseudopregnant rats. In both models, luteal PLA_2 activity rose in association with functional regression and persisted during the following structural regression. Tissue concentration of prostaglandin $F_{2\alpha}$ with a luteolytic potency showed a similar fluctuation. The enzyme activity was almost completely suppressed by a $cPLA_2$-specific inhibitor. Expression of $cPLA_2$, analyzed by immunohistochemistry, became enhanced during luteolysis with preferential localization to phagocytotic and fibrotic replacement sites. Taken together with our previous finding, the data indicate a persistent elevation in luteal $cPLA_2$ expression and activity that may affect tissue involution in vivo.

Key Words: Cytosolic phospholipase A_2; luteolysis; rat; immunohistochemistry.

Introduction

The corpus luteum is a temporary endocrine tissue that regresses to maintain ovarian homeostasis and cyclicity when pregnancy fails or terminates *(1,2)*. The regression of corpus luteum (luteolysis) consists of two phases: functional luteolysis defined by the reduction in progesterone (P_4) secretory activity and the following structural luteolysis characterized by loss of tissue weight and size. These two phases are overlapped in most mammalian species but are clearly separate at least in rodents *(2)*. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) induces luteolysis in mammals, and the major source of the luteolytic agent is the uterus in ruminants *(1,2)*. However, corpus luteum itself in all mammalian species examined to date has the inherent capacity to produce prostaglandin *(3)*, whose biologic significances are not fully understood.

Phospholipase A_2 (PLA_2) is a critical regulator of prostaglandin synthesis and consists of a still growing superfamily *(4)*. Using a rat model, we have presented the first evidence for the expression and a primary role of type IV cytosolic PLA_2 ($cPLA_2$) in corpus luteum *(5–7)*. Accumulating evidence provided by us and others clearly shows that luteal PLA_2 activity *(5,7,8)* and thus $PGF_{2\alpha}$ level *(8–11)* increased during functional luteolysis in pseudopregnancy (PSP) and pregnancy in rats. In pseudopregnant rats, pharmacologic inhibition by dexamethasone of ovarian PLA_2 activity *(12)* prolonged P_4 secretion in vivo *(13,14)*, strongly suggesting that luteal $cPLA_2$ promotes functional luteolysis through local $PGF_{2\alpha}$ synthesis. In addition, gene knockout of $cPLA_2$ in mice resulted in the failure of parturition owing to obstructed functional luteolysis *(15,16)*, clearly demonstrating its crucial role in the induction of luteolysis.

However, it is still obscure whether luteal PLA_2 activation is associated with structural luteolysis. To address this issue, the current study examined changes in PLA_2 activity and $PGF_{2\alpha}$ level and identified the distribution of immunoreactive $cPLA_2$ in structurally regressing corpus luteum of pseudopregnant rats.

Results

Table 1 summarizes changes in luteal PLA_2 activity and $PGF_{2\alpha}$ level during spontaneous luteolysis in adult pseudopregnant rats. Plasma P_4 level gradually declined after d 8 of PSP (PSP8; PSP1 = the first day when the vaginal smear showed primarily leukocytic infiltration) and reached basal levels on PSP12, indicating the accomplishment of functional luteolysis on PSP12 in this model. Luteal cytosol PLA_2 activity increased from PSP9 to PSP11 with a 1.9-fold increase in activity over that detected on PSP6. The increased enzyme activity persisted until PSP17. Luteal $PGF_{2\alpha}$ level showed a marked (4.8-fold) increase from PSP6 to PSP9 and also remained elevated until PSP17.

As shown in Fig. 1, the analysis of plasma P_4 confirmed that functional regression occurred following a dopamine agonist bromocryptine (Brom) treatment that inhibited the

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Table 1
Changes in Luteal PLA_2 Activity and $PGF_{2\alpha}$ Level During Spontaneous Luteolysis in Adult Pseudopregnant Rats[a]

Day PSP	P_4 (ng/mL)	PLA_2 activity (pmol/[mg protein·min])	$PGF_{2\alpha}$ level (pg/mg protein)
6	155.2 ± 17.2*	8.24 ± 0.36*	3.73 ± 0.85*
8	119.0 ± 16.4*,†		
9	93.1 ± 20.7*,†,‡	9.20 ± 0.64*	25.06 ± 6.29†
10	77.6 ± 10.3†,‡		
11	48.3 ± 2.6‡	16.04 ± 1.58†	29.15 ± 8.00*,†
12	29.3 ± 5.2§		
13	20.7 ± 4.1§	14.97 ± 0.47†	24.53 ± 3.73†
15	32.8 ± 6.0§	17.75 ± 1.50†	21.51 ± 1.96†
17	22.4 ± 5.2§	17.54 ± 1.28†	25.95 ± 6.40†

[a]Adult pseudopregnant rats were killed at indicated times. P_4 level was assayed by RIA. Corpus luteum were separated and measured for cytosolic PLA_2 activity and PGF_{2a} levels. Data are mean ± SEM ($n = 4$ or 5). Within each column, values with different superscript symbols mean significant differences ($p < 0.05$).

pituitary secretion of prolactin (PRL). This hormone is luteotropic for the corpus luteum that is still functional, and it failed to cause luteolysis when given alone from PSP7 to PSP9 in this study. However, PRL exerts a luteolytic action for the functionally regressing corpus luteum. Structural regression, which was examined by measuring the wet wt of corpus luteum, was further caused by the challenge of PRL in combination with pretreatment with Brom. In these experiments, PLA_2 activities in both functionally and structurally regressed corpus luteums were significantly greater than those in control and PRL-only groups (Fig. 1C). The $PGF_{2\alpha}$ level was also high in the Brom and Brom/PRL groups (Fig. 1D).

Next, we characterized luteal PLA_2 activity using some inhibitors (Table 2). Enzyme activities on PSP9 and PSP17 were suppressed in the presence of arachidonyl trifluoromethyl ketone ($AACOCF_3$), which preferentially inhibited $cPLA_2$ *(4)* to 11.1 ± 1.3 and 37.1 ± 6.1% of each control (both $p < 0.01$), respectively. Those of the Brom and Brom/PRL groups were decreased by the inhibitor to 11.6 ± 1.5 and 5.3 ± 2.0% of each control (both $p < 0.01$), respectively. Those enhanced activities in regressing corpus luteum of immature and adult pseudopregnant rats were less sensitive to bromoenol lactone (BEL), which preferentially inhibits calcium-independent intracellular PLA_2 ($iPLA_2$) *(4)*, and to dithiothreitol (DTT), an inactivator of secreted type PLA_2s ($sPLA_2$) *(17)*.

Finally, $cPLA_2$ expression in regressing corpus luteum was examined by immunohistochemistry (Fig. 2). While no positive signal (dark brown) was seen using nonspecific rabbit serum (Fig. 2A), luteal cells of the control group of immature rats were moderately immunoreactive to $cPLA_2$ (Fig. 2B). Immunoreactive signals became intense in the Brom (data not shown) and Brom/PRL groups (Fig. 2C). Structural luteolysis involves the infiltration of immune cells such as macrophages and T-lymphocytes *(1,18–20)* and replacement by fibroblasts *(1,2,21)*, and similar histologic results were obtained in this examination. Intense signals were localized to the central portion of corpus luteum of the Brom/PRL group in which fibrotic replacement was taking place (Fig. 2D). In addition, the sites where leukocyte-like cells assembled as the clumps in adult rat corpus luteum on PSP17 were also highly immunoreactive (Fig. 2E).

Discussion

Using two different models of luteolysis in pseudopregnant rats, this study clearly demonstrated that luteal $cPLA_2$ activity, and thus $PGF_{2\alpha}$ levels, showed a persistent increase during luteolysis. Immunohistochemical analysis revealed a preferential expression of $cPLA_2$ in phagocytotic and fibrotic replacement sites of structurally regressing corpus luteum. Studies with $cPLA_2$-deficient mice have demonstrated its indispensable role in the induction of luteolysis *(15,16)*, and our results reported here and previously *(8)* strongly suggest that it also has an important role in the accomplishment of luteolysis.

We previously found that increases in the expression *(5, 6)* and activity *(5,7)* of $cPLA_2$ were associated with a decline in circulating P_4 in pseudopregnant rats. This study not only confirmed an elevated PLA_2 activity from PSP6 to PSP12 but also revealed that the enhanced activity persisted as late as PSP17, i.e., for at least 5 d after the occurrence of functional luteolysis. Luteal $PGF_{2\alpha}$ content exhibited a similar fluctuation to that of PLA_2 activity. Since the elevation of luteal $PGF_{2\alpha}$ preceded that of PLA_2 activity, the regulated cyclooxygenase activity may also be involved. $cPLA_2$ was supposed to be the primary isoform in functionally regressing corpus luteum of pseudopregnant rats *(5,7)*. Present pharmacologic characterization of PLA_2 activity strongly suggested that $cPLA_2$ is still a principal PLA_2 that functions in corpus luteum undergoing tissue involution, even though $iPLA_2$ and $sPLA_2$ might participate to some extent. These data provide a novel finding that local prostaglandin synthesis ensured in part by $cPLA_2$ activity shows a persistent elevation even after functional luteolysis occurred. In the adult rat model, it was difficult to distinguish corpus luteum of the latest generation from those of previous cycles after PSP18, because (1) the adult rat ovary contains several generations of corpus luteum and (2) some individual rats will ovulate and have a new generation of corpus luteum. Therefore, we next utilized a more appropriate model of immature pseudopregnant rats having a single generation of corpus luteum to pursue a possible association between PLA_2 and structural luteolysis.

In addition to findings obtained from naturally regressing preparation of corpus luteum *(5,7,8)*, elevated PLA_2 activity in ovarian microsome during $PGF_{2\alpha}$-induced lute-

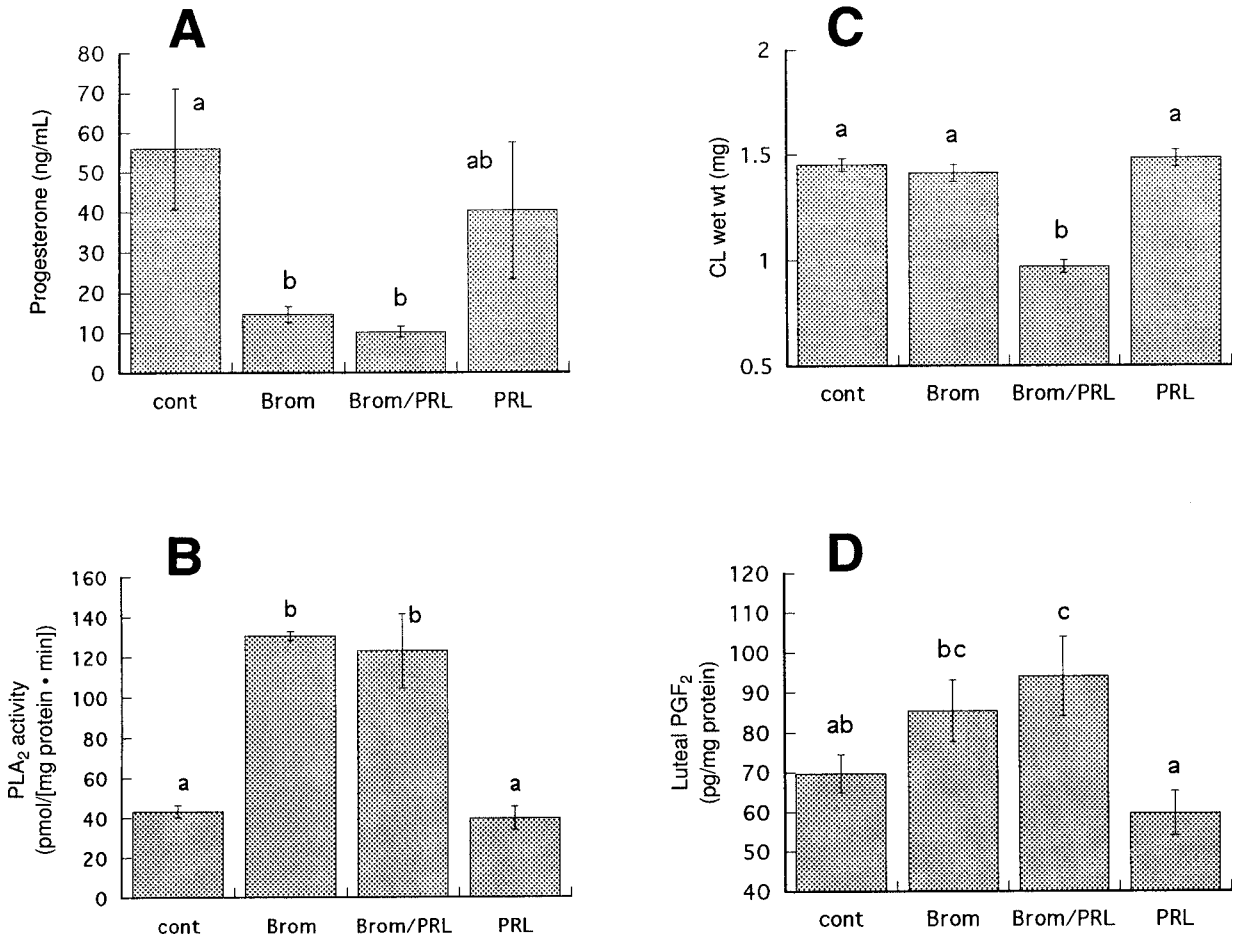

Fig. 1. Changes in PLA_2 activity, and $PGF_{2\alpha}$ level of corpus luteum during functional and structural luteolysis induced in combination with Brom and PRL. Immature pseudopregnant rats were injected with Brom or vehicle twice daily from PSP4 and further injected with PRL or saline from PSP7. These animals were killed on PSP10 and then were analyzed for parameters described. Plasma P_4 level (**A**) was assayed by radioimmunoassay (RIA). Corpus luteums were separated, weighed (**B**), and measured for cytosolic PLA_2 activity (**C**) and $PGF_{2\alpha}$ levels (**D**) as described in Materials and Methods. Data are mean ± SEM ($n = 6$ or 7 in [A] and [B], 4 in [C], and 4–7 in [D]). Within each column, values with different letters mean significant differences ($p < 0.05$).

Table 2
Effects of Several Inhibitors
on Cytosolic PLA_2 Activity in Regressing Corpus Luteum

Period	Cont	DTT (5 m*M*)	$AACOCF_3$ (10 μ*M*)	BEL (50 μ*M*)
PSP9	100.0 ± 9.0	108.2 ± 4.3	11.1 ± 1.3**	60.8 ± 7.8*
PSP17	100.0 ± 4.1	79.1 ± 3.4*	37.1 ± 6.1**	67.0 ± 9.2*
Brom	100.0 ± 9.9	31.4 ± 2.9**	11.6 ± 1.5**	114.8 ± 4.2
Brom/PRL	100.0 ± 7.1	32.2 ± 5.3**	5.3 ± 2.0**	87.7 ± 6.9

[a]Corpus luteum cytosols were incubated in 0.1 *M* Tris-HCl (pH 9.0), 4 m*M* $CaCl_2$, 1 mg/mL of bovine serum albumin (BSA), and 2 μ*M* phosphatidylcholine (PC) substrate with or without each inhibitor for 1 h at 37°C. Free ^{3}H-arachidonic acid released by the reaction was extracted and counted for radioactivity. Data are mean ± SEM ($n = 4$). *$p < 0.05$; **$p < 0.01$ vs each control (no inhibitor).

olysis was also documented *(22,23)*. Here we present further evidence for its enhanced activity during PRL depletion-induced functional luteolysis that occurs in a different mechanism from $PGF_{2\alpha}$-induced luteolysis *(24)*. PRL can exert both luteotropic and luteolytic activities in rats. The former effect is expressed to the corpus luteum when it is still functional, and the latter is done to the regressing corpus luteum. Here, we further found the sustained activation of $cPLA_2$ during structural luteolysis induced by additional PRL. The $PGF_{2\alpha}$ level in regressing corpus luteum pretreated with Brom increased significantly in response to PRL stimuli, while the prostaglandin content in the hormone stimulated-functional corpus luteum rather decreased compared with that of control corpus luteum. Therefore, the bipotential of PRL in the regulation of corpus luteum may be associated with its influence on luteal prostaglandin production. Because PRL also stimulated $PGF_{2\alpha}$ synthesis more prominently than PLA_2 activity in regressing corpus luteum of postpartum rats *(8)*, this hormone may stimulate the site(s) distal to the PLA_2 process during structural luteolysis. Luteal $cPLA_2$ may be upregulated by some factor(s) such as immune cell–derived cytokines other than PRL. Collectively, our data provide evidence that structural luteolysis involves

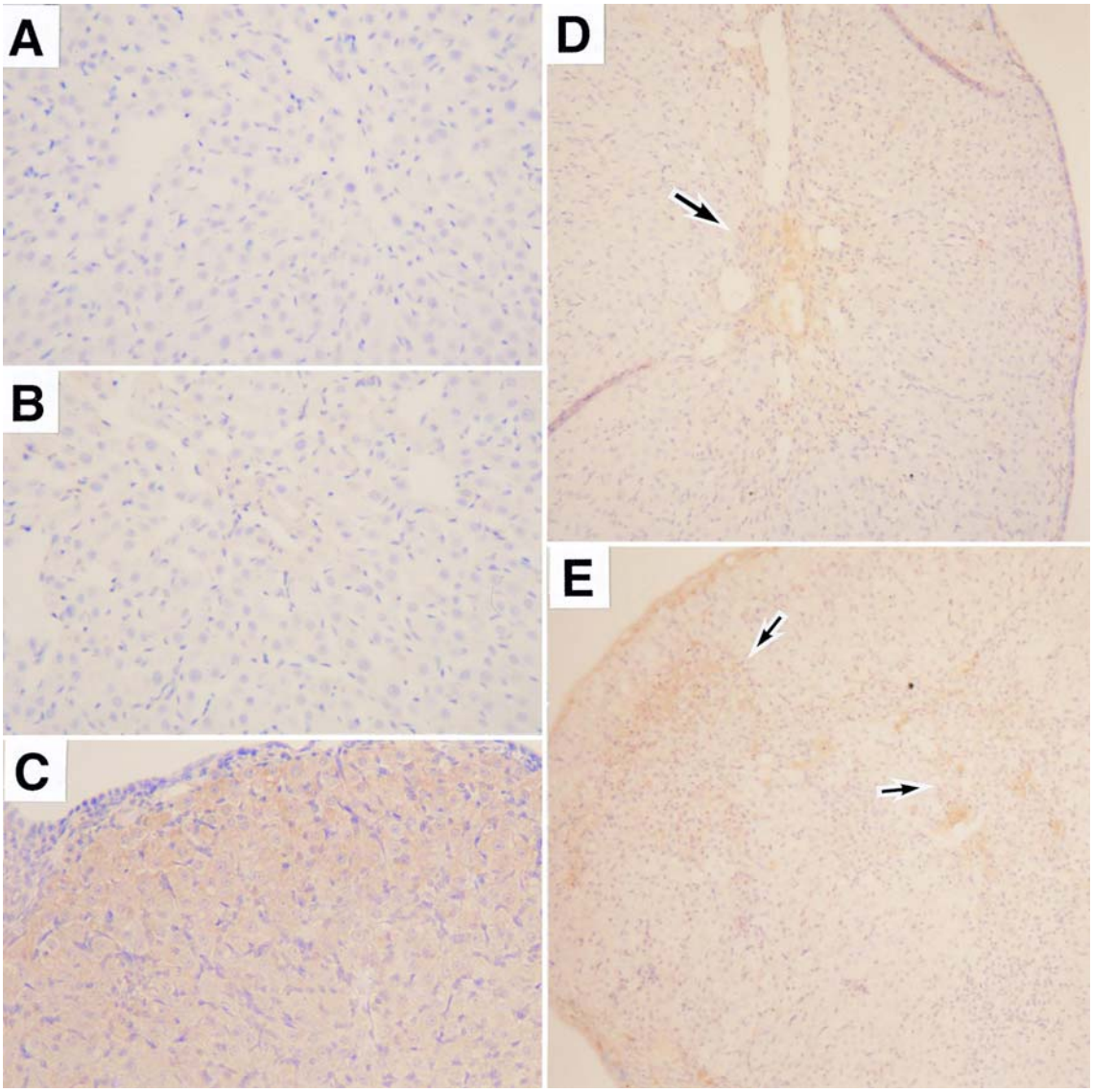

Fig. 2. Immunohistochemical localization of $cPLA_2$ in regressing corpus luteum. Whereas no positive reactions were seen in the section stained by nonspecific rabbit serum (**A**), the moderate and distinct immunoreactions were seen in sections stained by anti-$cPLA_2$ antibody (**B–E**). (**B**) Moderate immunoreactivity for $cPLA_2$ in corpus luteum of control group of immature rats; (**C**) enhanced immunoreactivity in corpus luteum of Brom/PRL group; (**D**) preferential localization of immunoreactive $cPLA_2$ to the fibrotic replacement site (arrow) appearing in the corpus luteum central portion of Brom/PRL group; (**E**) association of $cPLA_2$ immunoreactivity with sites of immune cells assembling as clumps (arrows) in adult rat corpus luteum on PSP17. Magnification: ×100 (D,E) and ×200 (A–C).

the enhanced prostaglandin synthesis, which is ensured at least in part by $cPLA_2$ activation.

Structural luteolysis consists of a multiple biologic process, including physiologic cell death of steroidogenic cells and endothelial cells, their phagocytosis by macrophages and other immune cells, and replacement by fibroblasts forming corpus albicans *(1,2,18–21)*. We finally investigated the localization of $cPLA_2$ activity in involuting corpus luteum. In this tissue, almost all the cell types were positive for $cPLA_2$ immunoreactivity, which became enhanced during luteolysis. This result is consistent with our previous finding from other physiologic states of rat corpus luteum *(6)*. More importantly, we found $cPLA_2$ activity to be localized in phagocytotic sites and fibrotic replacement sites. Since macrophages and other immune cells that phagocytose degenerative cells are supposed to activate luteal $PGF_{2\alpha}$ synthesis through secretion of cytokines such as tumor necrosis factor-α and interferon-γ *(21,25)*, $cPLA_2$ may mediate this effect. On the other hand, this enzyme may be implicated in fibrotic replacement, because $cPLA_2$ and its metabolite arachidonic acid have been shown to be crucial for proliferation of fibroblast cell lines *(26,27)*. These findings suggest a significant role of this synthase of inflammatory lipid mediators *(4)* in tissue involution and remodeling. There have been many, but contradictory, reports that implicate $cPLA_2$ activity in apoptosis using a single type of cultured cells *(28–31)*. Our results show the sustained expression and activity of $cPLA_2$ during corpus luteum

involution at least at the tissue level in vivo. Further investigation will be needed to clarify the possible involvement of $cPLA_2$ in the physiologic cell death of luteal constituent cells.

The sustained enhancement of the local mechanism of $PGF_{2\alpha}$ synthesis and action may be critical for corpus luteum involution. Accumulation of immunoreactive $PGF_{2\alpha}$ receptor in regressing corpus luteum and corpus albicans of the rat *(32)* may support this concept. Very recently, we found that dexamethasone and the cyclooxygenase inhibitor indomethacin partially reversed the decrease in corpus luteum weight induced by PRL in immature pseudopregnant rats and in postpartum rats (unpublished data). Similarly, Sanchez-Criado et al. *(33)* also demonstrated that indomethacin treatment has such an effect in the pituitary autografted rat model. These data support the hypothesis by Rothchild *(34)* that the sustained mechanism of luteal $PGF_{2\alpha}$ synthesis, i.e., the intrinsic luteolytic system, promotes structural luteolysis.

In summary, the present study provides further evidence that luteal PLA_2 activity, which increases during both spontaneous and induced functional luteolysis, does persisit during the following structural luteolysis. The sustained activity may ensure a substantial amount of $PGF_{2\alpha}$, which is probably involved in tissue involution and the remodeling process in vivo.

Materials and Methods

Reagents

Antihuman $cPLA_2$ antibody was kindly donated by Genetics Institute (Cambridge, MA) *(35)*. Gonadotropins equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) were obtained from Shionogi (Osaka, Japan) and Sankyo (Tokyo, Japan), respectively. Radiolabeled PC (1-stearoyl-2-[5,6,8,9,11,12,14,15-^{3}H]-arachidonyl PC) was obtained from DuPont-NEN (Boston, MA). $AACOCF_3$, BEL, and an enzyme immunoassay (EIA) kit for $PGF_{2\alpha}$ were purchased from Cayman (Ann Arbor, MI). Ovine PRL and Brom were obtained from Sigma (St. Louis, MO). All other reagents, including DTT, were of analytical grade.

Animals

Adult and immature female rats of the Wistar-Imamichi strain were used. For adult rats, vaginal smears were examined every morning. Pseudopregnancy in adult rats was induced by mating with infertile male rats on the proestrus. These rats were decapitated on PSP6, PSP9, PSP11, PSP13, PSP15, and PSP17 (n = 4 to 5 rats for each group), and ovaries and blood plasma were sampled. Blood was further collected from experimental animals on PSP8, PSP10, and PSP12. All procedures employed in this study were done following the guidelines for animal treatment of Kitasato University. Plasma samples were stored at –20°C until P_4 assay. Only large and round-shaped corpus luteum, probably of the latest generation, were instantly isolated from the ovary and were stored frozen (–80°C) until analyses of PLA_2 activity and $PGF_{2\alpha}$ content.

Pseudopregnancy in immature rats was induced by priming of 25 to 26-d-old animals with eCG (10 IU) followed by hCG (10 IU) 48 h later, as previously described *(5,7)*. They hold a single generation of corpus luteum and were injected with 10 IU of PRL on PSP1 and PSP2 to ensure the functional corpus luteum. Artificial induction of functional and subsequent structural luteolysis was performed according to the method of Kiya et al. *(36)*. From PSP4 to PSP9, Brom (0.5 mg subcutaneously in 0.3% tartaric acid and 10% ethanol) or its vehicle was given twice daily (8:00 AM and 6:00 PM). PRL (10 IU) or its vehicle (0.2 mL of physiologic saline) was further challenged twice daily from PSP7 to PSP9. Animals were divided into four experimental groups: control group, receiving vehicle; Brom group, receiving only Brom; Brom/PRL group, receiving both Brom and PRL; and PRL group receiving only PRL. These experimental rats were sacrificed on PSP10, and blood plasma and ovaries were sampled. Corpus luteum were instantly isolated and measured for their wet weights. They were stored frozen until analyses.

Assay of Plasma P_4 and Luteal PLA_2 Activity and $PGF_{2\alpha}$ Content

Plasma P_4 was extracted by *n*-hexane and assayed by RIA as previously described *(5,8,14)*. The P_4 antiserum was generated in an adult male Japanese White rabbit using pregn-4-ene-3,20-dione-3-carboxymethyloxime-BSA as the antigen. The specificity of this antiserum had <8.33% crossreactivity with pregnenolone, androst-4-en-3,17 dione, or testosterone. The crossreactivities of the antiserum with corticosterone and 20α-hydroxypregn-4-en-3-one were 0.86 and 2.0%, respectively, while the value with 5β-pregnanediol, cortisone, dehydroepiandrosterone, estrone, estradiol, or estriol was <0.25%. The workable range of the assay was 6.3–1000 pg/tube. The inter- and intraassay coefficients of variation were 6–8% and 12–14%, respectively.

PLA_2 activity in corpus luteum cytosol was measured by the previous method *(7,8)*. Briefly, corpus luteums were homogenized in 0.25 *M* sucrose, 0.25 m*M* EDTA, and 0.05 *M* Tris-HCl (pH 9.0). The homogenates were centrifuged at 105,000*g* for 1 h, and the supernatants (cytosol) were determined for protein concentration using a Bio-Rad assay kit. Liposomes for substrate were prepared with radiolabeled and PC and nonradiolabeled PC at a molar ratio of 1:4. The assay mixtures (200 µL in total volume) contained 0.1 *M* Tris-HCl (pH 9.0), 4 m*M* $CaCl_2$, 1 mg/mL of fatty acid–free BSA, 2 µ*M* PC, and cytosol. To characterize luteal PLA_2 activity pharmacologically, it was also measured in the presence of 10 µ*M* $AACOCF_3$, 5 m*M* DTT, or 50 µ*M* BEL. The reaction was performed at 37°C for 1 h and stopped by adding 1.0 mL Dole's reagent. The released fatty acid was extracted and measured for its radioactivity by liquid scintillation counting.

Luteal $PGF_{2\alpha}$ was determined according to Higuchi et al. *(37)* using a commercial EIA kit. Briefly, corpus luteum was homogenized in 0.05 *M* phosphate buffer (pH. 7.3) containing 0.1% gelatin and 0.01% merthiolate, and then it centrifuged at 10,000*g* for 20 min at 4°C. The supernatant was directly assayed for $PGF_{2\alpha}$ content without any extraction or separation from other eicosanoids or lipids.

Immunostaining of $cPLA_2$

Adult rats on PSP17 and four experimental groups of immature rats on PSP10 were killed. Three ovaries collected from three individual rats of each group were subject to histologic analysis to give standardized results. Ovaries were fixed in Bouin's fixative, dehydrated, and embedded in paraffin. Tissues were serially sectioned (6 µm in thickness), deparaffinized, and examined. Immunohistochemistry of $cPLA_2$ was performed by the previous methods *(8, 38)*. Endogenous peroxidase was blocked by pretreatment with 0.3% H_2O_2 in methanol. Sections were incubated with an anti-$cPLA_2$ (1:500) for 1.5 h at room temperature. The complex of the objective enzyme and the antibody was visualized using the Vectastain Elite ABC staining method and 3,3'-diaminobenzidine tetrahydrochrolide as peroxidase substrate. Sections were finally counterstained by hematoxylin.

Statistical Analyses

All data are presented as mean ± SEM of the indicated numbers of samples. The data were analyzed by one-way analysis of variance followed by student's *t*-test. A p value <0.05 was considered to be significant.

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References

1. Niswender, G. D., Juengel, J. L., Silva, P. J., Rollyson, M. K., and McIntush, E. W. (2000). *Physiol. Rev.* **80,** 1–29.
2. McCracken, J. A., Custer, E. E., and Lamsa, J.C. (1999). *Physiol. Rev.* **79,** 263–323.
3. Olofsson, J. I. and Leung, P.C.K. (1996). *Biol. Signals* **5,** 90–100.
4. Balsinde, J.. Balboa, M. A., Insel, P. A., and Dennis, E. A. (1999). *Annu. Rev. Pharmacol. Toxicol.* **39,** 175–189.
5. Kurusu, S., Shingaki, S., Munekata, Y., Kawaminami, M., and Hashimoto, I. (1995). *J. Reprod. Dev.* **41,** 141–147.
6. Kurusu, S., Motegi, T., Kawaminami, M., and Hashimoto, I. (1998). *Prostagland. Leukot. Essent. Fatty Acids* **58,** 399–404.
7. Kurusu, S., Noguchi, T., Kawaminami, M., and Hashimoto, I. (1997). *Prostagland. Leukot. Essent. Fatty Acids* **57,** 119–124.
8. Kurusu, S., Kaizo, K., Ibashi, M., Kawaminami, M., and Hashimoto, I. (1999). *FEBS Lett.* **454,** 225–228.
9. Olofsson, J., Norjavaara E., and Selstam, G. (1990). *Biol. Reprod.* **42,** 792–800.
10. Cao, L. and Chan, W. Y. (1993). *J. Reprod. Fertil.* **99,** 181–186.
11. Stocco, C. O. and Deis, R. P. (1998). *J. Endocrinol.* **156,** 253–259.
12. Kol, S., Ben-Shlomo, I., Payne, D. W., Ando, M., Rohan, R. M., and Adashi, E. Y. (1998). *Mol. Cell. Endocrinol.* **137,** 117–125.
13. Wang, J., Riley, J. C. M., and Behrman, H. R. (1993). *Biol. Reprod.* **49,** 66–73.
14. Kurusu, S., Hirano, Y., Kawagishi, S., Kitabatake, T., Suzuki, A., Kawaminami, M., and Hashimoto, I. (1995). *J. Reprod. Dev.* **41,** 21–28.
15. Uozumi, N., Kume, K., Nagase, T., Nakatani, N., Ishii, S., Tashiro, F., Komagata, Y., Maki, K., Ikuta, K., Ouchi, Y., Miyazaki, J., and Shimizu, T. (1997). *Nature* **390,** 618–622.
16. Bonventre, J. V., Huang, Z., Reza Taheri, M., O'Leary, E., Li, E., Moskowitz, M. A., and Sapirstein, A. (1997). *Nature* **390,** 622–625.
17. Hara, S., Kudo, I., Chang, H. W., Matsuta, K., Miyamoto, T., and Inoue, K. (1989). *J. Biochem.* **105,** 395–399.
18. Murdoch, W. J., Steadman, L. E., and Belden, E. L. (1988). *Med. Hypoth.* **27,** 197–199.
19. Bowen, J. M., Keyes, P. L., Warren, J. S., and Townson, D. H. (1996). *Biol. Reprod.* **54,** 1120–1127.
20. Kuranaga, E., Kanuka, H., Bannai, M., Suzuki, M., Nishihara, M., and Takahashi, M. (1999). *Biochem. Biophys. Res. Commun.* **260,** 167–173.
21. Pate, J. L. (1995). *J. Anim. Sci.* **72,** 1884–1890.
22. Riley, J. C. M. and Carlson, J. C. (1987). *Endocrinology* **121,** 776–781.
23. Sawada, M. and Carlson, J. C. (1991). *Endocrinology* **128,** 2992–2998.
24. Bjurulf, E., Toffia, O., Selstam, G., and Olofsson, J. I. (1998). *Biol. Reprod.* **59,** 17–21.
25. Terranova, P. E. and Rice, V. M. (1997). *Am. J. Reprod. Immunol.* **37,** 50–63.
26. Mitchell, R. A., Metz, C. N., Peng, T., and Bucala, R. (1999). *J. Biol. Chem.* **274,** 18,100–18,106.
27. Lloret, S., Torrent, M., and Morreno, J. J. (1996). *Pflugers Arch.* **432,** 655–622.
28. Pirianov, M. C., Danielsson, C., Carlberg, C., James, S. Y., and Colston, K. W. (1999). *Cell Death Differ.* **6,** 890–901.
29. MacEwan, D. J. (1996). *FEBS Lett.* **379,** 77–81.
30. Atsumi, G., Tajima, M., Hadano, A., Nakatani, Y., Murakami, M., and Kudo, I. (1998). *J. Biol. Chem.* **273,** 13,870–13,877.
31. Adam-Klages, S., Schwandner, R., Luschen, S., Ussat, S., Kreder, D., and Kronke, M. (1998). *J. Immunol.* **161,** 5687–5694.
32. Orlicky, D. J., Fisher, L., Dunscomb, N., and Miller, G. J. (1992). *Prostagland. Leukotr. Essent. Fatty Acids* **46,** 223–229.
33. Sanchez-Criado, J. E., Ochiai, K., and Rothchild, I. (1987). *J. Endocrinol.* **112,** 317–322.
34. Rothchild, I. (1981). *Recent Prog. Horm. Res.* **37,** 183–295.
35. Clark, J. D., Lin, L.-L., Kriz, R. W., Ramesha, C. S., Sultzman, L. A., Lin, A. Y., Milona, N., and Knopf, J. L. (1991). *Cell* **65,** 1043–1051.
36. Kiya, T., Endo, T., Goto, T., Yamamoto, H., Ito, E., Kudo, R., and Behrman, H. R. (1998). *J. Endocrinol. Invest.* **21,** 276–283.
37. Higuchi, Y., Yoshimura, T., Tanaka, N., Ogino, H., Sumiyama, M., and Kawakami, S. (1995). *Prostaglandins* **49,** 131–140.
38. Kurusu, S., Endo, M., Madarame, H., Kawaminami, M., and Hashimoto, I. (1999). *FEBS Lett.* **444,** 235–238.