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STIMULATION BY PROSTAGLANDIN $F_{2\alpha}$ OF PHOSPHATIDIC ACID-PHOSPHATIDYLINOSITOL TURNOVER IN RAT LUTEAL CELLS

Vincent Raymond, Peter C.K. Leung*, and Fernand Labrie

MRC Group in Molecular Endocrinology,
Le Centre Hospitalier de l'Université Laval,
Québec, G1V 4G2, Canada

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SUMMARY: Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) causes a rapid and marked increase of [^{32}P]-orthophosphate incorporation into phosphatidylinositol (PI) and phosphatidic acid (PA) in rat luteal cells in culture. The incorporation of radioactivity is increased as early as 2 and 5 min after $PGF_{2\alpha}$ addition into PA and PI, respectively, and by 10 min has reached a 2-fold stimulation over control in both lipid moieties. The labeling of other phospholipids is not affected. $PGF_{2\alpha}$ exerts its stimulatory effect at an ED_{50} value of approximately 200 and 60 nM on PI and PA labeling, respectively. By contrast, human chorionic gonadotropin has no effect alone and does not interfere with the $PGF_{2\alpha}$-induced stimulation of PA-PI labeling. The striking similarity between the effects of $PGF_{2\alpha}$ and LHRH on PA-PI labeling suggests that the two agents may exert their direct action on the corpus luteum via a common intracellular mechanism involving acidic phospholipid metabolism.

There is increasing evidence supporting the role of changes of phospholipid metabolism as an initial event in response to specific regulatory agents. Following the first report by Hokin and Hokin (1), alterations in acidic phospholipid metabolism, particularly an increase in PI[1] hydrolysis generating 1,2-DG and PA, have been proposed as transducing mechanisms for different intracellular signals (2-4). It has also been suggested that increased PI turnover may be involved in the control of Ca^{2+} fluxes by hormones (2, 5).

*Present address: Department of Obstetrics and Gynaecology, University of British Columbia, Vancouver, B.C. V6H 3V5

[1] Abbreviations used: PA, phosphatidic acid; PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; 1,2-DG,1,2-diacylglycerol; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; LHRH, luteinizing hormone-releasing hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; TRH, thyrotropin-releasing hormone; [^{32}P]Pi, [^{32}P]orthophosphate; ED_{50}, effective dose-fifty; cyclic AMP, adenosine 3',5'-monophosphate.

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At the anterior pituitary level, increased PA-PI turnover has been described in normal cells in primary culture following addition of LHRH (6, 7) and TRH (8) as well as in GH tumor cells stimulated by TRH (9-11) and bombesin (11). At the ovarian level, we have recently observed an increased PI turnover within minutes following the exposure of luteal cells to LHRH (12). A similar effect of the peptide has also been observed in granulosa cells in culture (13, 14). Since it is well recognized that $PGF_{2\alpha}$, in analogy with LHRH, acts directly at the level of the corpus luteum to inhibit LH-induced cAMP and progesterone production (15-17), we have examined the possible involvement of acidic phospholipid metabolism in the action of $PGF_{2\alpha}$ in luteal cells in culture.

MATERIALS AND METHODS

Cell culture. Adult female Sprague-Dawley rats (Crl:CD(SD)BR), 150-250 g body weight, obtained from Charles River Canada Inc., were injected subcutaneously with 50 IU pregnant mare's serum gonadotropins (Ayerst) followed, 48-60h later, by the injection of 20 IU hCG (courtesy of J.P. Raynaud, Roussel-UCLAF, Romainville). Ovaries were removed 4 days after treatment with hCG, minced and digested with collagenase-DNAse, as described previously (18). The dispersed cells were then washed with serum-free McCoy's 5A medium (Gibco) before plating in 35 mm Corning petri dishes (approximately 1 x 10^6 luteal cells/1.5 ml/dish) in McCoy's 5A medium supplemented with human fibronectin (5 µg/ml, Collaborative Research), insulin (2 µg/ml), transferrin (5 µg/ml), cortisol (200 nM), non-essential amino acids (1%), penicillin (100 U/ml) and streptomycin (50 µg/ml).

Phospholipid turnover. Three days after plating, the cells were washed and subsequently incubated in Eagle's minimum essential medium without phosphate (Flow) supplemented with [^{32}P]Pi orthophosphate (Amersham) (200 µCi/ml). At the end of an initial 20-min period, hormone additions were performed and the incubation was continued for the indicated time intervals. The incubations were stopped by washing the cells twice with 1 ml HEPES buffer (25 mM, pH 7.2) and adding 2x 1 ml of ice-cold 10% trichloroacetic acid. The cells were then scraped and collected by centrifugation at 27,000 g for 10 min. The radioactive phospholipids were extracted directly with chloroform/methanol/water (1:2:0.8, v/v/v) according to Bligh and Dyer (19), isolated by thin-layer chromatography on silica gel 60F-254 plates (Merck) using a two-dimensional chromatography system (first dimension: chloroform/methanol/28% ammonia, 65:35:5, v/v/v; second dimension: chloroform/acetone/methanol/glacial acetic acid/water, 6:8:2:2:1, v/v/v/v/v) and the radioactivity measured (12). Statistical analysis was performed as described previously (20, 21).

Chemicals. $PGF_{2\alpha}$ was obtained from Upjohn as $PGF_{2\alpha}$-tromethamine salt. Synthetic LHRH and hCG (No. CR-121) were obtained from Beckman and the Center for Population Research, NIH, respectively.

RESULTS

As illustrated in Fig. 1, the addition of 1 μM $PGF_{2\alpha}$, a dose which maximally suppresses LH-induced progesterone secretion in luteal cells (16), markedly increased [^{32}P]Pi incorporation into both PI and PA. Thus, following a 10-min incubation, the level of radioactivity was 344% ($p < 0.01$) and 295% ($p < 0.01$) above control in the PI and PA fractions, respectively. The stimulatory effect of $PGF_{2\alpha}$ on [^{32}P]Pi incorporation was limited to PI and PA, no significant effect of the prostaglandin being observed in PE, PC and lysoPC under the same experimental conditions.

Since LH is the main gonadotropin involved in the control of luteal cell function and $PGF_{2\alpha}$ blocks the stimulatory action of LH or hCG on cAMP and steroid formation, we have examined the possible effect of hCG on $PGF_{2\alpha}$-induced PI labeling. It can be seen in Fig. 1 that 10 nM hCG, a dose previously shown to stimulate cAMP and progesterone accumulation in this system (18), failed to affect the

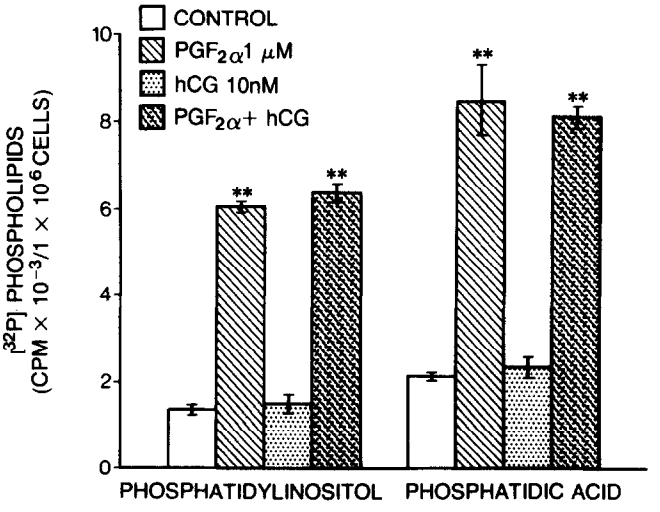


Fig. 1. Effect of $PGF_{2\alpha}$ and hCG alone or in combination on the incorporation of [^{32}P]Pi into PI and PA in rat luteal cells in primary culture. Cells were incubated for 10 min in the presence or absence of 10 nM hCG, 1 μM $PGF_{2\alpha}$ or both compounds. Data are expressed as means ± SEM of triplicate determinations and analyzed using the multiple-range test of Duncan-Kramer (21). **, $p < 0.01$, exp. vs control.

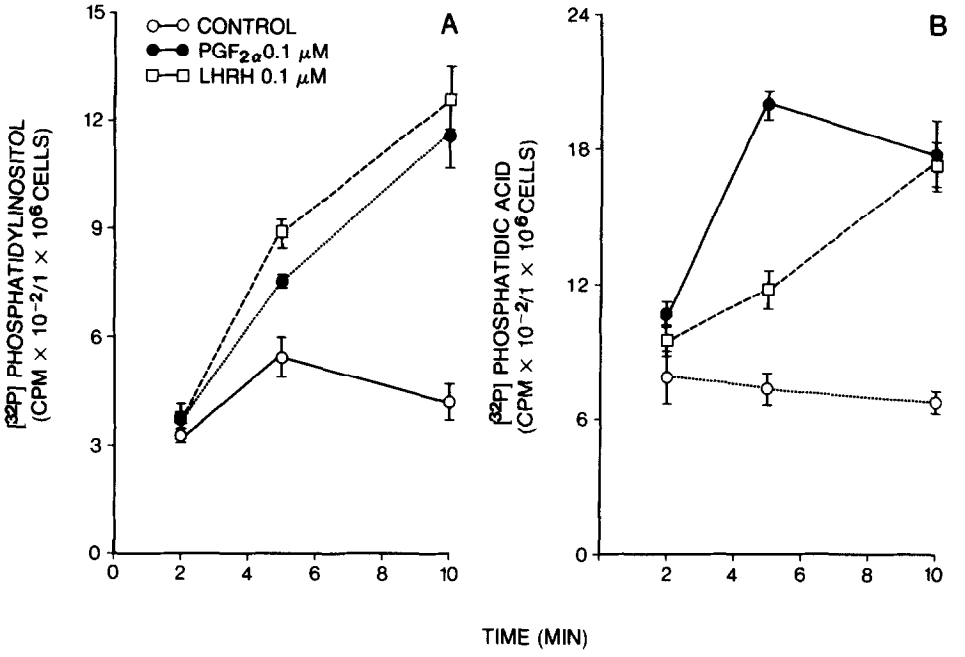
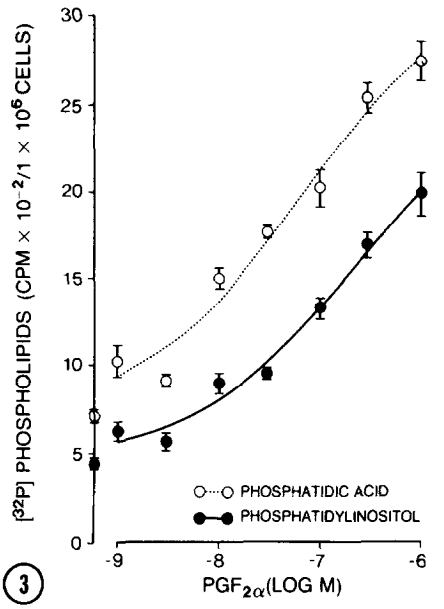


Fig. 2. Time course of the effect of $PGF_{2\alpha}$ or LHRH on [^{32}P]Pi incorporation into PI (A) and PA (B) in rat luteal cells in primary culture. Cells were incubated for the indicated time intervals in the absence (control) or presence of 0.1 μM $PGF_{2\alpha}$ or 0.1 μM LHRH. Data are expressed as means ± SEM of triplicate determinations.

labeling of PI and PA when present alone and did not interfere with $PGF_{2\alpha}$-induced PA-PI labeling.

The stimulation by $PGF_{2\alpha}$ of PA-PI turnover in luteal cells was rapid. As shown in Fig. 2, the stimulation of [^{32}P]Pi incorporation into PA was already significant ($p < 0.05$) as early as 2 min after $PGF_{2\alpha}$ addition (the earliest time interval studied), while radioactivity incorporation into PI was increased ($p < 0.05$) at 5 min. Although the time course is slightly different, LHRH caused an increase in [^{32}P]Pi incorporation into both PA and PI. In fact, at the end of 10 min, the LHRH-induced increase in radioactivity incorporated into PA and PI was not significantly different from that caused by $PGF_{2\alpha}$ (Fig. 2). The sensitivity of the effect of $PGF_{2\alpha}$ is illustrated in Fig. 3. After 10 min of incubation, 1 μM $PGF_{2\alpha}$ led to 4-fold maximal enhancement of radioactivity incorporation



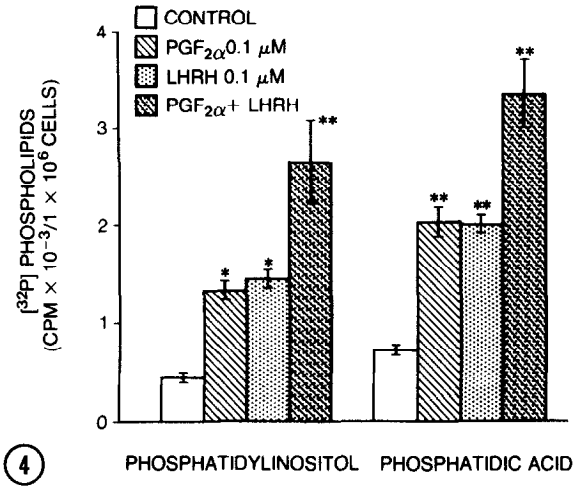


Fig. 3. Effect of increasing concentrations of $PGF_{2\alpha}$ on [^{32}P]Pi incorporation into PI and PA in rat luteal cells in primary culture. Cells were incubated for 10 min with increasing concentrations of $PGF_{2\alpha}$ or the vehicle alone. Data are expressed as means ± SEM of triplicate determinations.

Fig. 4. Effect of $PGF_{2\alpha}$ and LHRH alone or in combination on the incorporation of [^{32}P]Pi incorporation into PI and PA in rat luteal cells in primary culture. Cells were incubated for 10 min in the presence or absence of 0.1 μM $PGF_{2\alpha}$, 0.1 μM LHRH or both compounds. Data are expressed as means ± SEM of triplicate determinations and analysed using the multiple-range test of Duncan-Kramer (21). *, $p < 0.05$, exp. vs control; **, $p < 0.01$, exp. vs control.

into PI and PA. ED_{50} values of approximately 200 and 60 nM were calculated for the effect of LHRH on PI and PA labeling, respectively.

We next examined the interactions between $PGF_{2\alpha}$ and LHRH on the same parameters (Fig. 4). Following a 10-min incubation, 0.1 μM $PGF_{2\alpha}$ or LHRH caused an almost identical stimulation of radioactivity incorporation into both PI (200% and 228% over control, respectively, $p < 0.05$) and PA (184% and 180% over control, respectively, $p < 0.05$). Furthermore, the effects of $PGF_{2\alpha}$ and LHRH appeared to be additive, the incorporation of radioactivity into PI and PA reaching 500% and 370% over control, respectively, when both $PGF_{2\alpha}$ and LHRH were present.

DISCUSSION

The present data clearly demonstrate that $PGF_{2\alpha}$ causes a rapid and marked increase of [^{32}P]Pi incorporation into both PI and PA in rat luteal cells in primary culture. The finding of an increased labeling first into PA at 2 min preceding the stimulation of [^{32}P]Pi incorporation into PI measured at 5 min suggests that the mechanism likely to be involved is stimulation of a phospholipase C causing PI hydrolysis into 1,2-DG followed by conversion into PA before PI resynthesis. As recently reviewed by Farese (22), several alternative mechanisms which are metabolically distinct from PI breakdown may also lead to increased [^{32}P]Pi incorporation into PI. However, the specificity of phospholipid labeling, PA and PI being the only phospholipids showing $PGF_{2\alpha}$-induced stimulation of [^{32}P]Pi incorporation, and the rapidity of this stimulatory effect provide support for the involvement of increased PA-PI turnover as an initial event in the action of $PGF_{2\alpha}$ in the corpus luteum. With regard to the sensitivity of the effect of $PGF_{2\alpha}$ on PA-PI turnover, the observation of an ED_{50} value of approximately 60 nM for PA labeling is in close agreement with the potency of $PGF_{2\alpha}$ to inhibit LH-induced progesterone secretion as measured at an ED_{50} value of 40 nM (16). Such data support a role of changes in PA-PI turnover in the inhibitory action of $PGF_{2\alpha}$ on hCG-or LH-induced cyclic AMP and progesterone production in luteal cells (16).

As mentioned earlier, the possible involvement of PI turnover as a potent intracellular mediator has been documented in various tissues following exposure to specific regulatory agents (3). Recently, we have found that LHRH, which also acts directly on the corpus luteum to exert antigonadotropic properties similar to $PGF_{2\alpha}$ causes a rapid and marked increase in [^{32}P]Pi incorporation into PI and PA in rat luteal cells in culture (12). While both LHRH and $PGF_{2\alpha}$ exert their antigonadotropic activity through binding to

different receptors, they may share common intracellular mechanisms, including calcium (17). Since PA has been suggested as a Ca^{2+} ionophore in biological membranes (23) and liposomes (24), the present finding of remarkably similar characteristics of stimulation of PA-PI turnover by $PGF_{2\alpha}$ and LHRH in rat luteal cells supports the suggestion that stimulation of acidic phospholipid metabolism could be a common transducing mechanism for the direct action of both inhibitory substances in the corpus luteum. Moreover, since 0.1 μM LHRH has been found to maximally increase [^{32}P]Pi incorporation into PI and PA (12) and 0.1 μM $PGF_{2\alpha}$ is a dose well over the ED_{50} value of $PGF_{2\alpha}$-induced PA labeling, it appears likely that LHRH and $PGF_{2\alpha}$, following binding to their respective membrane receptors, induce the turnover of two separate PI pools, thus which results in an additive effect on PA and PI labeling (Fig. 4).

REFERENCES

1. Hokin, M.R. and Hokin, L.E. (1953) J. Biol. Chem. 203, 967-977.
2. Michell, R.H. (1975) Biochim. Biophys. Acta 415, 81-147.
3. Berridge, M.J. (1981) Mol. Cell. Endocrinol. 24, 115-140.
4. Putney, J.W., Jr. (1981) Life Sci. 29, 1183-1194.
5. Michell, R.H. (1982) Nature 296, 492-493.
6. Raymond, V., Veilleux, R. and Leung, P.C.K. (1982) Proc. of the 64th Ann Meet Endocrine Soc., p 285, abst no 821.
7. Snyder, G., Bleasdale, J.E. (1982) Mol. Cell. Endocrinol. 28, 55-63.
8. Leung, P.C.K., Raymond, V. and Labrie, F. (1982) Life Sci. 31, 3037-3041.
9. Rebecchi, M.J., Monaco, M.E. and Gershengorn, M.C. (1981) Biochem. Biophys. Res. Commun. 101, 124-130.
10. Rebecchi, M.J., Kolesnick, R.N. and Gershengorn, M.C. (1983) J. Biol. Chem. 258, 227-234.
11. Sutton, C.A. and Martin, T.F.J. (1982) Endocrinology 11, 1273-1280.
12. Leung, P.C.K., Raymond, V. and Labrie, F. (1983) Endocrinology 112, 1138-1140.
13. Naor, Z. and Yavin, E. (1982) Endocrinology 111, 1615-1619.
14. Davis, J.S., Farese, R.V. and Clark, M.R. (1983) Proc. Natl. Acad. Sci. USA 80, 2049-2053.
15. Lahav, M., Freud, A. and Lindner, H.R. (1976) Biochem. Biophys. Res. Commun. 68, 1294-1299.
16. Thomas, J.P., Dorflinger, L.J. and Behrman, H.R. (1978) Proc. Natl. Acad. Sci. USA 75, 1344-1348.
17. Behrman, H.R., Preston, S.L. and Hall, A.K. (1980) Endocrinology 107, 656-664.
18. Massicotte, J., Borgus, J.P., Lachance, R. and Labrie, F. (1981) J. Steroid Biochem. 14, 239-242.

19.Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911-917.
20.Drouin, J., De Lêan, A., Rainville, D., Lachance, R. and Labrie, F. (1976) Endocrinology 98, 514-521.
21.Kramer, C.Y. (1956) Biometrics 12: 307-310.
22.Farese, R.V. (1983) Endocrine Rev. 4, 78-95.
23.Putney, J.W., Jr., Weiss, S.J., Van De Walle, C.M. and Haddas, R.A. (1980) Nature 284, 345-347.
24.Serhan, C.N., Fridovich, J., Goetz, E.J., Dunham, P.B. and Weissmann, G. (1982) J. Biol. Chem. 257, 4747-4752.