Vol. 149, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1987 Pages 112-117

PROSTAGLANDIN $F_{2\alpha}$ INITIATES POLYPHOSPHATIDYLINOSITOL HYDROLYSIS AND MEMBRANE TRANSLOCATION OF PROTEIN KINASE C IN SWINE OVARIAN CELLS

Johannes D. Veldhuis

With the Technical Assistance

of Diana Juchter

and James Garmey

Division of Endocrinology and Metabolism

Department of Internal Medicine

University of Virginia School of Medicine

Charlottesville, VA 22908

Received October 12, 1987

The biochemical mechanisms subserving the inhibitory actions of prostaglandin $F_{2\alpha}$ on ovarian cells are not known. Since the protein kinase C pathway is coupled to steroidogenesis in an inhibitory fashion in pig granulosa cells, we have tested the hypothesis that prostaglandin $F_{2\alpha}$ activates this phospholipid-dependent, calcium-stimulated effector pathway. Using monolayer cultures of swine granulosa cells, we now report that prostaglandin $F_{2\alpha}$ is capable of activating critical components of the protein kinase C pathway, including the production of water-soluble inositol phosphates, liberation of free arachidonic acid, release of endogenous diacylglycerol, and translocation of cytosolic protein kinase C to the phospholipid-enriched membrane microenvironment.

Prostaglandin $F_{2\alpha}$ exerts potent inhibitory effects upon ovarian (luteal) cells in a variety of species (1-3). However, to date the exact sub-cellular and biochemical mechanisms of the inhibitory actions of prostaglandin $F_{2\alpha}$ on steroid hormone biosynthesis have not been delineated in detail. Recent studies of a phospholipid-dependent, calcium-activated protein kinase C effector pathway system in the ovary suggest that this

Abbreviations: IP_3, inositol-1,4,5-triphosphate; IP_2, inositol-1,4-biphosphate; IP_1, inositol-1-phosphate.

0006-291X/87 $1.50

mediator system can be coupled to steroidogenesis in an inhibitory fashion (4,5). This pathway is activated in the presence of phospholipid diacylglycerol, and calcium ions. Accordingly, in the present studies we have tested the hypothesis that prostaglandin $F_{2\alpha}$ activates one or more components of the protein kinase C system in ovarian cells. To this end, we investigated the ability of prostaglandin $F_{2\alpha}$ to initiate the rapid release of water-soluble inositol phosphates and endogenous diacylglycerol, and to instigate the translocation of protein kinase C from cytosol to the phospholipid-enriched microenvironment of the cell membrane.

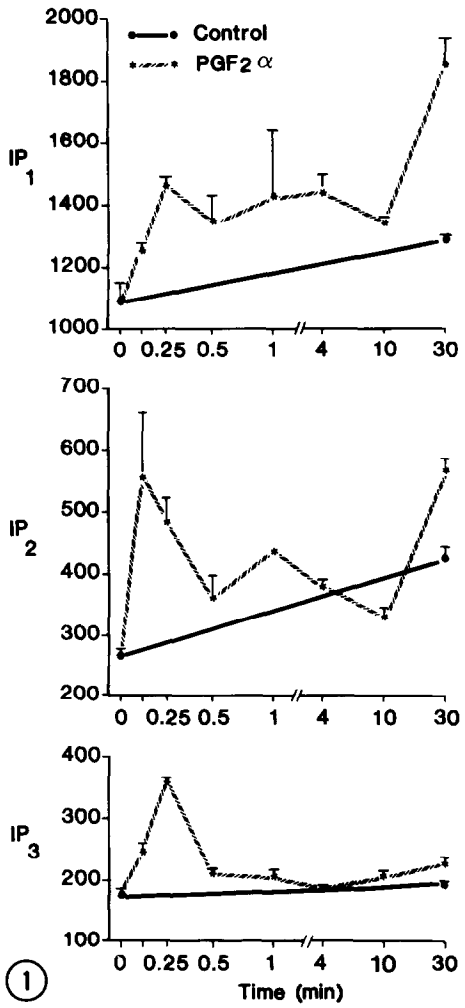
Methods

Granulosa cells were harvested by fine needle aseptic aspiration of porcine follicles of 1-5 mm diameter obtained from immature swine. Cells were washed three times in serum-free Eagle's Minimal Essential Media, and monolayer cultures established in 3 percent bovine fetal serum at a density of $1X10^7$ viable cells/10 cm Falcon tissue culture dishes (4). After 36 hr, the medium was removed, and the specific experiments initiated. For equilibrium prelabeling with [^{3}H]myoinositol, cultures were exposed to Hams F-12 medium in the presence of 5 μCi/ml [^{3}H]myoinositol (L-myo-[1,2-^{3}H(N)]inositol 60Ci/mmol) with no unlabeled myoinositol for 18 hr. Thereafter, the cells were washed twice in balanced salt solution, and incubated for one hr in the presence of 10 mM LiCl and 1 mM unlabeled myoinositol as a "cold chase" to dilute pools of unincorporated [^{3}H]myoinositol. The medium was then removed, and replenished with or without prostaglandin $F_{2\alpha}$ (1 μg/ml). At designated intervals, medium was rapidly removed and cellular processes terminated by the addition of chloroform/methanol (1:2, v/v) to permit the subsequent extraction of water-soluble [^{3}H]inositol phosphates. Individual [^{3}H]inositol phosphates (inositol-1,4,5-trisphosphate, inositol-1,4-bisphosphate, and inositol-1-phosphate) were separated by anion-exchange chromatography, as described in the laboratory of Berridge (6). To simultaneously monitor the release of free arachidonic acid and diacylglycerol, cultures were also incubated with 1 μC/ml [^{14}C]arachidonic acid and the organic extract of the cells subjected to thin-layer chromatography using a solvent system of hexane:diethylether:acetic acid (65:35:4, v/v/v) to separate radiolabeled arachidonic acid and diacylglycerol (7). In the translocation experiments, cells were exposed to prostaglandin $F_{2\alpha}$ or control solvent and harvested at indicated intervals. Cell pellets were washed rapidly by microfuge centrifugation, and the cell pellets lysed in hypotonic buffer (20 mm TRIS HCl pH 7.4) to prepare cytosolic (100,000 X G X 1 hr) and crude membrane fractions (PDB). The cytosol and membranes were then tested for [^{3}H]phorbol-12,13-dibutyrate binding (20 nM), which was quantitated in the presence or absence of 1,000-fold excess unlabeled 12-O-tetradecanoyl-13-acetate (TPA) to assess nonspecific and specific binding (4).

Data were subjected to analysis of variance to ascertain significant effects, which were discerned individually by the Newman-Keuls procedure (8). Each experiment was performed at least twice with a separate batch of cells derived from 200 ovaries. Radiolabeled compounds were purchased from New England Nuclear Corp. (Boston, MA) and prostaglandin $F_{2\alpha}$ from Upjohn Co. (Kalamazoo, MI).

Results and Discussion

As shown in Figure 1, the addition of prostaglandin $F_{2\alpha}$ to monolayer cultures of swine granulosa cells prelabeled to equilibrium with



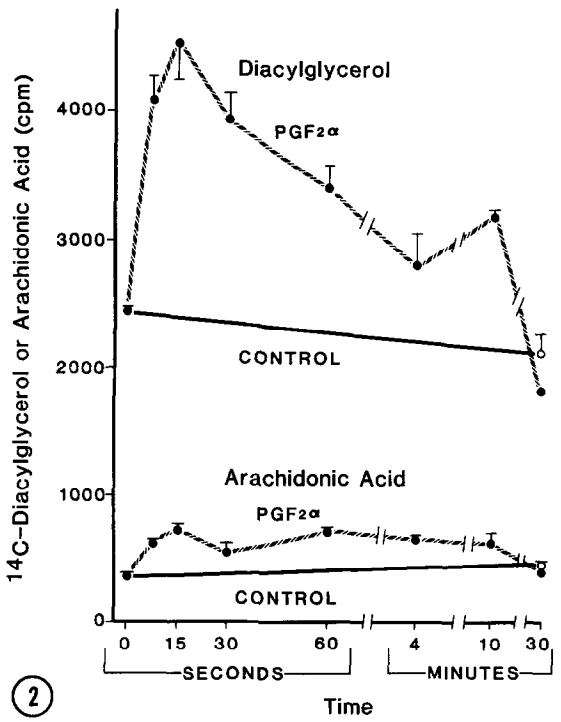


Figure 1. Time-dependent liberation of water-soluble inositol phosphates from granulosa cells equilibrium prelabeled with [^{3}H]myoinositol. Granulosa cells were prelabeled with [^{3}H]myoinositol for 18 hr, and after a 1 hr pulse chase were exposed to prostaglandin $F_{2\alpha}$ (1 μg/ml) or control solvent. The subsequent liberation of inositol phosphates was assessed by anion exchange chromatography. IP_3 = inositol-1,4,5-trisphosphate; IP_2 = inositol-1,4-bisphosphate; IP_1 = inositol-1-phosphate. Data are means ± SD representing three independent determinations.

Figure 2. Time-dependent release of [^{14}C]arachidonic-acid labeled diacylglycerol or free arachidonic acid from swine granulosa cells in response to stimulation with prostaglandin $F_{2\alpha}$. Monolayer cultures of swine granulosa cells were equilibrium prelabeled with [^{14}C]arachidonic acid and then exposed to prostaglandin $F_{2\alpha}$ (1 μg/ml) or control solvent at time 0. The cells were subsequently extracted with organic solvents and [^{14}C]arachidonyl diacylglycerol and free arachidonic acid quantitated by thin layer chromatography. Data are otherwise as presented in the legend of Fig. 1.

[^{3}H]myoinositol resulted in the immediate liberation of water-soluble inositol phosphates. In particular, treated cultures exhibited a significant increase in inositol-1,4,5-trisphosphate (IP_3) accumulation within 15 and 30 seconds of stimulation with prostaglandin $F_{2\alpha}$. The increase in IP_3 accumulation was sustained over several min and then returned toward baseline. There was also a significant increase in inositol-1,4-bisphosphate with a more gradual and sustained pattern of accumulation. A similar delayed pattern was observed for inositol-1-phosphate.

Coincident with the rapid liberation of IP_3, prostaglandin $F_{2\alpha}$ elicited a transient significant increase in the cellular content of [^{14}C]arachidonyl-labeled diacylglycerol (Figure 2). The increase in intracellular diacylglycerol was transient and similar in time-course to that of IP_3.

The ability of prostaglandin $F_{2\alpha}$ to evoke the immediate release of both IP_3 and endogenous diacylglycerol could provide a mechanism for activation of the protein kinase C effector pathway. This consideration is based on the recognized ability of IP_3 to mobilize intracellular calcium in a variety of secretory tissues (9). In conjunction with increased intracellular concentrations of calcium ions, the availability of diacylglycerol in the presence of phospholipid to a functional protein kinase C enzyme could result in effective activation of this effector pathway.

Prostaglandin $F_{2\alpha}$ also elicited a rapid, reversible and time-dependent translocation of cytosolic protein kinase C to the membrane fraction of ovarian cells (Figure 3). Although the exact physiological significance of the translocation process has not been fully defined, the present observations with prostaglandin $F_{2\alpha}$ are congruent with the ability of diverse effector agents that activate protein kinase C to initiate the membrane translocation of this enzyme (10,11). Accordingly, the present observations provide important evidence that prostaglandin $F_{2\alpha}$ is capable of initiating cellular responses in ovarian cells typical of those accompanying activation of the protein kinase C effector pathway in other tissues.

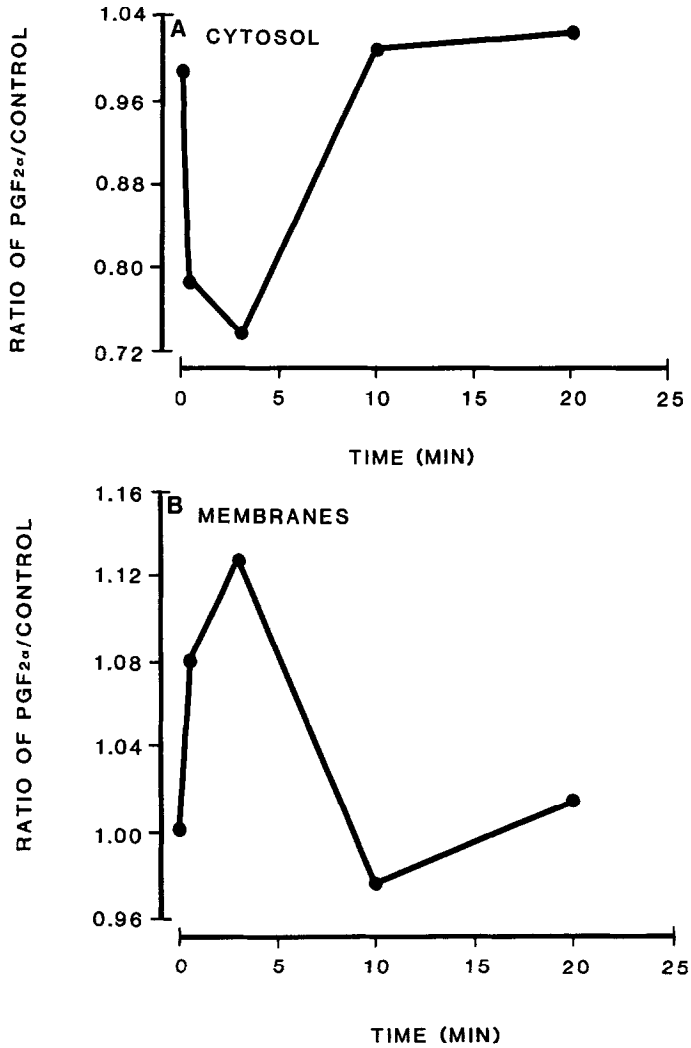


Figure 3. Time-dependent translocation of cytosolic protein kinase C by swine granulosa cells stimulated with prostaglandin $F_{2\alpha}$. Monolayer cultures of pig granulosa cells were treated with prostaglandin $F_{2\alpha}$ (1 μg/ml) or control solvent at time zero. Cells were subsequently harvested and the cytosolic and crude membrane fractions assayed for [^{3}H]phorbol-12,13-dibutyrate ([^{3}H]PDB) binding to identify the protein-kinase C phorbol-ester receptor. Data are mean ratios (N = 3 independent determinations). Ratio of PDB binding is shown in $PGF_{2\alpha}$/control cultures in cytosol (Panel A) and in membranes (Panel B).

The ability of prostaglandin $F_{2\alpha}$ to stimulate the liberation of inositol phosphates and free diacylglycerol with concomitant translocation of cytosolic protein kinase C to the membrane provides a mechanistic model for further studies of the cellular actions of this hormone. The demonstration that all three of the preceding events are activated by prostaglandin $F_{2\alpha}$ in granulosa cells complements and extends earlier observations on

polyphosphoinositide breakdown in the rodent corpus luteum (12). Moreover, our recent examination of steady-state calcium exchange in granulosa cells indicates that this specific prostaglandin also significantly alters equilibrium calcium exchange in isolated ovarian cells (13). Since prostaglandin $F_{2\alpha}$ is intimately associated with inhibition of steroidogenesis and regression of the corpus luteum, these overall results provide a rationale for investigating the role of the calcium-dependent protein kinase C pathway in prostaglandin $F_{2\alpha}$-mediated luteolysis.

Acknowledgements

We thank Chris McNett for the preparation of this manuscript, Paula Azimi for the artwork, Dr. Julianne Sando of the Department of Pharmacology for expert advice, and Dr. Robert MacLeod for his discussions in the conduct of experiments.

This work was supported by NIH RCDA 1 K04 HD 00634 and R01 HD16806.

References

1. Patek, C.E., and Watson, J. (1976) Prostaglandins 12, 97-109.
2. Buhr, M.M., Carlson, J.C., and Thompson, J.E. (1979) Endocrinology 105, 1330-1338.
3. Norjavaara, E., and Rosberg, S. (1986) Mol. Cell. Endocrinol. 48, 97-104.
4. Veldhuis, J.D., and Demers, L.M. (1986). Biochem. J. 239, 505-511.
5. Davis, J.S., and Clark, M.R. (1983) Biochem. J. 214, 569-574.
6. Berridge, M.J., Heslop, J.P., Irvine, R.F., and Brown, K.D. (1984) Biochem. J. 222, 195-201.
7. Kramer, J.K.G., Fouchard, R.C., and Farnworth, E.R. (1983) Lipids 18, 896-902.
8. Winer, B.J. Statistical Principles in Experimental Design. (1971) McGraw-Hill, New York, pp. 192-210.
9. May, W.S., Jr., Sahyoun, N., Wolf, M., and Cuatrecasas, P. (1985) Nature 317, 549-551.
10. Kraft, A.S., Anderson, W.B., Cooper, H.L., and Sando, J.J. (1982) J. Biol. Chem. 257, 13193-13196.
11. Farrar, W.L., Thomas, T.P., and Anderson, W.P. (1985) Nature 315, 235-237.
12. Leung, P.C.K., Minegishi, T., Ma, F., Zhou, F., and Ho-Yuen, B. (1986) Endocrinology 119, 12-18.
13. Veldhuis, J.D. (1987) Endocrinology 120, 445-449.