REVISTA ESPAÑOLA DE FISIOLOGÍA, 48 (3), 167-170, 1992

R5020 Enhances PGE_2 Stimulated Steroidogenesis in Cultured Rat Granulosa Cells

L. F. Fanjul, J. González, P. Santana, F. Estévez, J. Quintana and C. M. Ruiz de Galarreta*

Departamento de Endocrinología Celular y Molecular
Facultad de Ciencias Médicas y de la Salud
Universidad de Las Palmas de G.C.
35086 Las Palmas (Spain)

(Received on February 24, 1992)

L. F. FANJUL, J. GONZÁLEZ, P. SANTANA, F. ESTÉVEZ, J. QUINTANA and C. M. RUIZ DE GALARRETA. *R5020 Enhances PGE_2 Stimulated Steroidogenesis in Cultured Rat Granulosa Cells.* Rev. esp. Fisiol., 48 (3), 167-170, 1992.

Progesterone biosynthesis and metabolization to 20α-hydroxyprogesterone was stimulated in granulosa cells cultured in the presence of 20 ng/ml of follicle stimulating hormone (FSH) or increasing concentrations of PGE_2 (10^{-9}-10^{-7} M). Concurrent treatment with the synthetic progestin R5020 (10^{-6} M) enhanced the FSH or PGE_2 stimulated progesterone and 20α-hydroxyprogesterone accumulation in culture media, as well as Δ^5-3β-hydroxysteroid dehydrogenase activity in granulosa cell homogenates. These findings may represent another example of an autocrine control mechanism in which the steroidogenic product of the granulosa cell exerts an ultra-short loop regulation of its own production.

Key words: Prostaglandins, R5020, Steroidogenesis, Granulosa cells.

Maturation of ovarian follicles results to a great extent from the FSH induced differentiation of granulosa cells. After binding to specific receptors in the granulosa cell membrane, FSH induces intracellular cAMP accumulation, increases membrane luteinizing hormone (LH) and prolactin receptor content and stimulates the secretion of estrogens, progestins and other non steroidal secretory products, important for optimal folliculogenesis and oocyte maturation (2, 5, 17).

Ovarian prostaglandin synthesis (F and E series) is stimulated by gonadotropins, and its concentration increases in the follicular fluid of several species as ovulation approaches (1, 6, 9, 18). Early studies based on the ability of prostaglandin synthesis inhibitors to prevent ovulation, suggested a role for these molecules as regulators of ovarian function (4, 10, 11). An explanation of the possible mechanism of prostaglandin regulation of follicle rup-

* To whom all correspondence should be addressed.

ture and ovulation was provided by experimental evidence showing the ability of PGE_2 to stimulate plasminogen activator secretion by granulosa cells (7, 16).

Nevertheless PGE_2 stimulation of granulosa cell progesterone secretion *in vitro* (12) and *in vivo* (8, 15) raises the interesting posibility that high intrafollicular prostaglandin concentration may be important to regulate ovarian functions others than ovulation.

Since progestins have been shown to enhance FSH stimulated progesterone biosynthesis in granulosa cells (3, 13), it was of interest to examine whether gonadotropin independent PGE_2 stimulated steroidogenesis in these cells is also sensitive to progestin modulation.

Materials and Methods

FSH (NIADDK-oFSH-16) was donated by the National Hormone and Pituitary Agency (Baltimore, MD). The steroid hormones and Prostaglandin E_2 were purchased from Sigma. All tissue culture reagents were obtained from Gibco (Grand Island NY) and the culture dishes and polystyrene tubes were from Becton-Dickinson (Oxnard, CA). Granulosa cells obtained from the ovaries of immature (21-23 days) estrogen treated rats from the Sprague-Dawley strain were inoculated ($\sim 2 \times 10^5$ viable cells/dish) onto 35 × 10 mm tissue culture dishes containing 1 ml of MacCoy's 5a medium (modified without serum). All experimental agents (diluted from stock solutions in sterile culture medium) were added in 50 µl aliquots. Cell cultures were maintained for 48 h at 37 °C under a water saturated atmosphere of 5 % CO_2 and 95 % air. At the end of the experiments collected media were stored at − 20 °C until assayed for steroid hormone content. Progesterone and 20α-hydroxyprogesterone were measured in unextracted media as previously described (13). In selected experiments cells were scraped from the dishes and assayed for Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSD activity as the rate of conversion of [^{3}H]pregnenolone (50 µM, ~ 100.000 dpm) to [^{3}H]progesterone in the presence of 5 mM NAD^+. Enzyme activity was expressed as nmol progesterone formed/30 min/mg protein (3, 14). Results are expressed as mean ± SEM from triplicate or quadruplicate cultures and the experiments were replicated three times. Experimental data were analysed by analysis of variance (ANOVA) and Student's t test.

Results and Discussion

In the present study, the effect of the synthetic progestin R5020 on PGE_2 stimulated progesterone biosynthesis was investigated. Since progesterone and R5020 enhance gonadotropin stimulated steroidogenesis (3, 13), granulosa cells were also cultured in the presence or absence of R5020 alone or in combination with FSH. The effect of progestins on the stimulatory action of FSH has been extensively studied in rat granulosa cells, and involves activation of 3β-HSD and 20α-hydroxysteroid dehydrogenase enzymes (3, 13). Results presented herein demonstrate that the dose dependent PGE_2 stimulation of progestin biosynthesis is exerted at the same enzyme step as steroidogenesis (fig. 1). These results are not surprising since the 3β-HSD enzyme is a rate limiting step of steroidogenesis (14) and FSH or PGE_2 stimulated intracellular cAMP accumulation in the granulosa cell (5, 8), and/or activation of granulosa cell progestin secretion by but_2-cAMP or cAMP-inducing agents, involves activation of both hydroxysteroid dehydrogenase enzymes (3, 13).

The steroidogenic responses to FSH and PGE_2, however, are not identical in granulosa cells (fig. 1). Treatment with FSH or PGE_2 elicited similar responses in

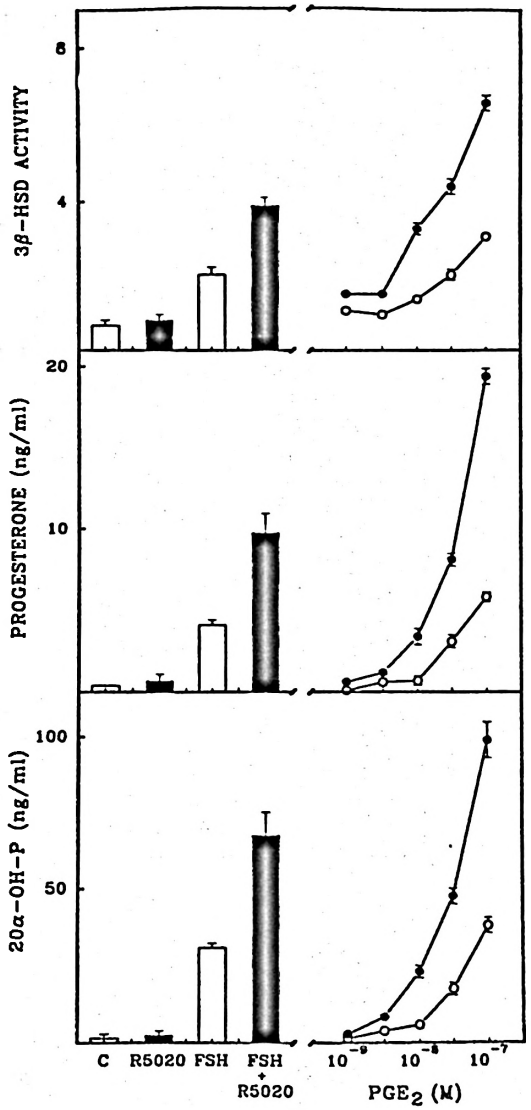

Fig. 1. *Effect of R5020 on PGE_2 stimulated granulosa cell steroidogenesis.*
Granulosa cells (~ 2×10^5 viable cells/dish) were cultured for 48 h in the absence (open bars or circles) or presence (solid bars or circles) of R5020 (10^{-6} M) with and without FSH (20 ng/ml) or increasing concentrations of PGE_2 (10^{-9}-10^{-7} M). Enzyme activity is expressed as nmol progesterone formed/30 min/mg protein. The results are the means ± SEM of triplicate incubations of an experiment representative of three others. In several cases the standard error is less than data point drawn.

terms of progesterone, 20α-hydroxyprogesterone and 3β-HSD activity, whereas in the presence of R5020, progestin biosynthesis was higher in PGE_2 treated cells.

The reason for this different response of granulosa cell is unknown but could be related to the increased PGE_2 concentration in follicular fluid after the LH peak of proestrus when FSH levels decline and granulosa cell progesterone biosynthesis increases.

These *in vitro* findings provide supporting evidence for a physiological role of PGE_2 in maintaining progesterone secretion during ovulation, and may be related to the autonomy of the luteal cell progesterone biosynthesis (3, 13, 18). In addition, results presented herein provide another example of an autocrine control mechanism in which the steroidogenic product of the granulosa cell exerts an ultra-short loop regulation on its own production.

Acknowledgements

This work was supported by CICYT research project PM 88/203 and DGUEI-GAC 77/86. The authors are grateful to Mr. J. Sarmiento for expert technical assistance.

Resumen

El tratamiento de células de la granulosa ovárica mantenidas en cultivo primario con 20 ng/ml de hormona folículo estimulante (FSH) o dosis crecientes (10^{-9}-10^{-7} M) de prostaglandina E (PGE_2) estimula la biosíntesis de progesterona y su metabolización a 20α-hidroxiprogesterona. El tratamiento simultáneo con el progestágeno sintético R5020 (10^{-6} M) aumenta el efecto estimulatorio de la FSH y PGE_2 sobre la producción de progesterona, 20α-hidroxiprogesterona y la actividad del enzima Δ^5-3β-hidroxiesteroide deshidrogenasa determinada en homogeneizados celulares. Estas respuestas constituyen otro ejemplo de un

mecanismo ultra-corto de control autocrino por el que el producto de la esteroidogénesis de la célula granulosa regula su propia síntesis.

Palabras clave: Prostaglandinas, R5020, Esteroidogénesis, Célula granulosa.

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