THE INDUCTION OF OVARIAN LH-RECEPTORS BY FSH IS MEDIATED BY CYCLIC AMP

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1. Introduction

Development of ovarian follicles is associated with an extensive modulation of the density of luteinizing hormone (LH) receptors on the granulosa cells (GC) [1,2]. These cells are considered to be the target for the pre-ovulatory surge of LH. The receptors for LH appear on GC only during the final stages of follicular growth [2,3], and this process is believed to be induced by follicle stimulating hormone (FSH) [4,5]. This notion is supported by the observation that FSH induces the appearance of LH-receptors on GC of preantral follicles both in vivo and in vitro [5–7].

The gonadotrophic hormones stimulate steroidogenesis in their target cells through activation of adenylate cyclase, leading to an increase in the cellular concentration of the second messenger, namely cyclic AMP [8]. However, it is not clear whether the cyclic nucleotide is specifically required to mediate this effect of FSH on the induction of the LH-receptors. In cultures of GC from preantral follicles, addition of serum to the medium abolished the induction of LH-receptors by FSH, while the stimulation of steroidogenesis by FSH was not affected [6]. The inhibitory effect of serum could be overcome by exposing whole follicles in organ culture to FSH [6]. However, addition of 8-bromo cAMP to cultured preantral follicles did not mimick the FSH-induction of LH-receptors. These findings have cast some doubt on the involvement of cAMP in the LH-receptor induction process. In the present study, we demonstrate that this FSH effect can be mimicked in GC cultures either by exogenous cAMP, or by cholera toxin – a potent stimulator of endogenous cAMP levels.

2. Materials and methods

Granulosa cells from hypophysectomized immature rats treated with diethyl stilbestrol (Hx-DES) were cultured as described in [9], except that the medium was devoid of serum and contained 50 ng ml^{-1} of cortisol. In addition, the culture plates (Falcon Multiwell No. 3008) were pre-coated with serum constituents by incubating them with fetal calf serum for at least 3 h at 37°C. The plates were rinsed twice with phosphate-buffered saline (PBS) and once with McCoy's 5a medium before use. Rat FSH (rFSH, NIH-I-4, kindly supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases, NIAMDD, Bethesda, MD), 8-bromo cyclic AMP (Sigma, St Louis) or cholera toxin (Schwartz-Mann, Orangeburg, NY) were added where indicated. Human chorionic gonadotrophin (hCG;NIAMDD) was iodinated by the lactoperoxidase method [10]. Binding studies were performed by incubating GC monolayes after 48 h of culture with labelled hCG: incubation was performed for 3 h at 37°C in 0.3 ml of McCoy's 5a medium with 0.1% bovine serum albumin (BSA) containing 150 000 cpm (2.5 nmol) of ^{125}I-labelled hCG. Non-specific binding was determined by incubating replicate cultures with labelled hormone diluted with an exess (60 U/ml) of non-iodinated hCG (Pregnyl, Organon). At the end of the 3-h incubation period, the monolayers were washed 3 times with 1 ml of PBS containing 0.1% BSA, then dissolved in 0.6 ml of 1 M NaOH and taken for radioactivity counting and DNA determination [11].

The content of 20α-dihydroprogesterone (20α-OH-P) was measured by radioimmunoassay [12] in the spent medium following 48 h of incubation.

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3. Results and discussion

The use of tissue-culture plates treated with serum enabled us to maintain GC in a good condition in serum-deficient medium. Under these culture conditions, the plating efficiency was similar to that obtained in the presence of FCS, and cell death was minimal. Addition of rat FSH (3–30 ng ml) for 48 h resulted in a marked induction of new binding sites for hCG (fig.1a). A maximal response was obtained with 10 and 30 ng/ml rFSH ($P < 0.01$ by Student's t-test; control *vs* FSH).

When 1 mM of 8-bromo cAMP was added to cells for 48 h, a significant increase ($P < 0.05$) in the number of hCG receptors was found (fig.1a). This effect appeared to be dose dependent. Higher amounts of 8-bromo cAMP could not be used in one dose, since it caused some detachment of cells from the plates. To overcome this, cells were first put into culture with 1 mM 8-bromo cAMP, and after 24 h a second amount of 1 mM was added. This experimental regimen resulted in an appearance of hCG binding that was similar to that obtained with FSH (fig.1a; $P > 0.05$ when compared to 30 ng ml FSH).

Addition of cholera toxin to ovarian cells is known to increase markedly the endogenous levels of cellular cAMP [13,14]. In the culture system described here, this treatment was accompanied by a significant increase in hCG/LH-receptor density. A graded response was obtained with doses of 0.16 and 0.8 μg/ml of the toxin, while maximal induction of the LH-receptor was obtained at 4 and 20 μg/ml (fig.1a). These maximal values were even higher than those obtained with FSH ($P < 0.05$).

The degree of stimulation of steroidogenic activity in the GC was determined by measuring progestin accumulation in the media. As shown (fig.1b), the accumulation of 20α-OH-P, the major metabolite of progesterone, followed a pattern that was almost identical to that of the induction of the LH-receptor. The apparent correlation between the two phenoma lends support to the notion that cAMP serves as the second messenger for both processes. The inability of 8-bromo cAMP to induce LH-receptors in GC of intact follicles [6] was probably the result of insufficient penetration of the cyclic nucleotide into the tissue. A continuous exposure to high levels of cAMP during the induction process is apparently required, since 8-bromo cAMP was more effective in GC monolayers when added in two daily doses than in a single dose, and since cholera

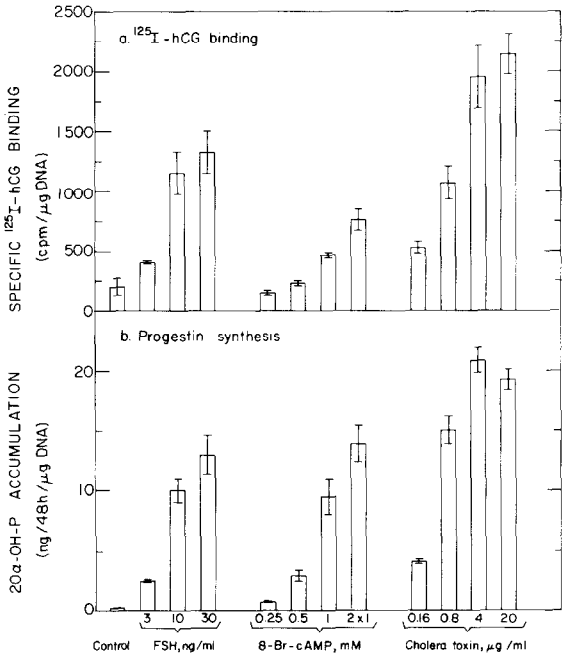

Fig.1. The effect of rat FSH, 8-bromo cAMP and cholera toxin on (a) ^{125}I-labelled hCG binding and (b) progestin accumulation in cultured granulosa cells. Cells (3×10^5/0.5 ml/well) were cultured for 48 h in a medium devoid of serum with the 3 compounds as specified in the graph. The media were then collected for determination of 20α-OH-P content, and the monolayers were washed and taken for measurement of ^{125}I-labelled hCG binding (see text). The bars and vertical brackets represent the mean ± S.E.M. of 4 replicate culture wells.

toxin was highly active in inducing the LH-receptor (fig.1a); the latter substance is known to stimulate the adenylate cyclase system in the cell continuously.

In conclusion, the results of the present study provide strong evidence supporting the concept that FSH induces the appearance of LH-receptors in GC through cyclic AMP as messenger. It is not clear whether this process involves de novo synthesis of receptor molecules, or the activation or unmasking of pre-formed inactive membrane component.

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