Identification of G-Protein α-Subunits in Ovaries

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 11, pp. 582-585, November, 1997
Original article submitted December 26, 1996

The presence of G-protein α-subunits in somatic ovarian cells of albino rats and changes in their distribution during follicle maturation are demonstrated. A possible role of these proteins in signal transduction to ovarian structures is discussed.

Key Words: *ovary; folliculocyte; thecocyte; α-subunit; G-protein*

Function of all cells, tissues, and organs is subjected to neurohumoral regulation. Humoral factors play an outstanding role in the regulation of female reproductive organs, since cyclic morphofunctional reorganization in these organs and changes directed toward conception are strictly controlled by the hypothalamo-hypophyseal system. This classic scheme of gonadotropin-mediated humoral regulation has been recently supplemented by a discovery of a great number of humoral modulators of this system (thyroid hormones, adrenocorticotropic hormone, insulin, etc.).

Delicate mechanisms mediating the action of hormonal stimuli on effector cells in reproductive organs, in particular, cells of ovarian follicles, representing an oocyte microenvironment, remain poorly understood. Evaluation of the role of G-proteins, members of the GTPase superfamily, is a promising direction in the study of signal transduction system in the ovarian-follicular assembly. There is evidence that GTPases act as transmitters in the interactions between receptor proteins and intracellular target enzymes [1-8].

G-Proteins are a peculiar group of peripheral cytoplasmic proteins belonging to high-affinity GTPases, consisting of three subunits (α, β, and γ). The active center of the molecule is formed by α-subunits. High-affinity binding and hydrolysis of GTP molecule in the active center is accompanied by its inactivation, which affects its interactions with other macromolecules and modulates general and specific cellular processes. There are known different forms of G-proteins mediating both activating and inhibiting hormonal stimuli to the cell effector systems. These differences are determined by their α-subunits.

Study of G-protein α-subunits and their possible role in hormonal signal transduction to target cells in ovarian follicles can substantially supplement the fundamental theory of follicular selection.

Our objective was to visualize and investigate the distribution of G-proteins in developing ovarian follicles.

MATERIALS AND METHODS

Ovaries of mature albino random-bred rats were studied. Cryosections (7-μ thick) of ovaries were slightly dried at room temperature, fixed in absolute acetone for 10 min, and washed with 10 mM phosphate buffer (pH 7.6) containing 0.5 M NaCl and 1% Tween-20. Polyclonal rabbit immunoglobulin to G-protein α-subunit served as primary antibodies. Bound primary antibody were visualized using the avidin-biotin-peroxidase complex.

In order to verify the specificity of the reaction control sections were not incubated with primary antibodies. Microscopy and photography was performed using an Opton microscope.

RESULTS

In the ovaries, G-protein α-subunits were found in somatic follicular and stromal cells. The maximum

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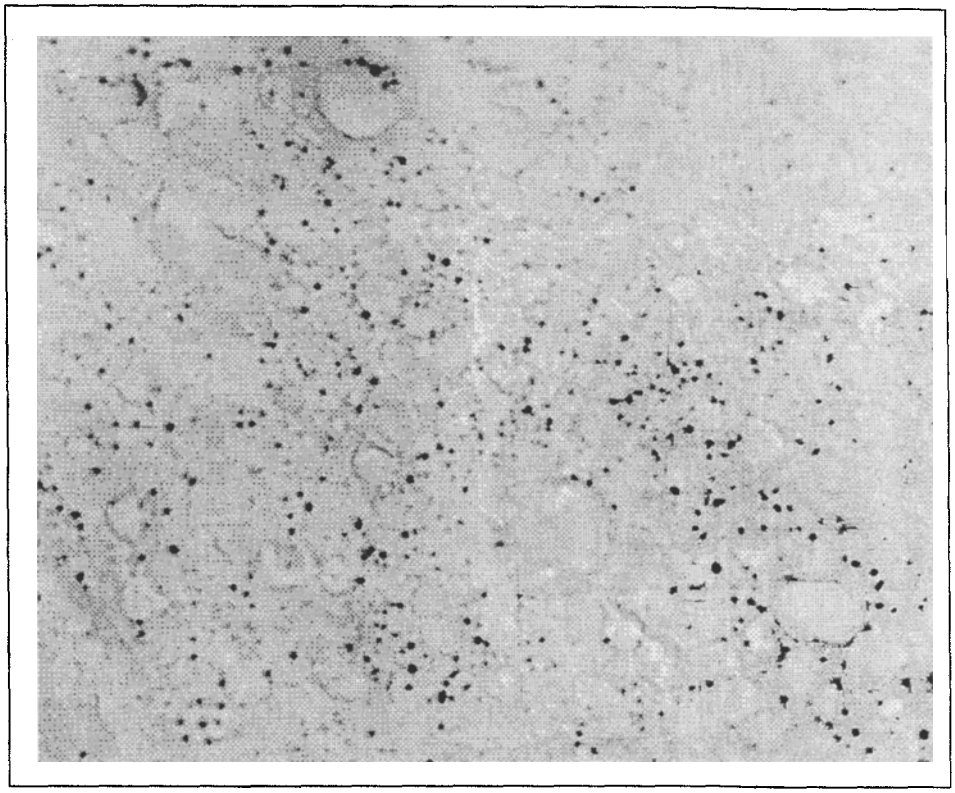

Fig. 1. A fragment of ovarian stroma. Positive labeling for G-protein α-subunit in interstitial endocrinocytes, ×600.

label incorporation was observed in stromal (interstitial) endocrinocytes (Fig. 1). This fact together with high steroid-producing activity of the stromal interstitial tissue in rodents suggests the existence of a direct relationship between the presence of G-proteins and synthesis of sex steroids, and consequently, involvement of these proteins into signal transduction to interstitial endocrinocytes.

In follicles of different pools, G-protein α-subunits were not identified in gametes, while in somatic cells their presence and content depended on the stage of follicle maturation. These proteins were ab-

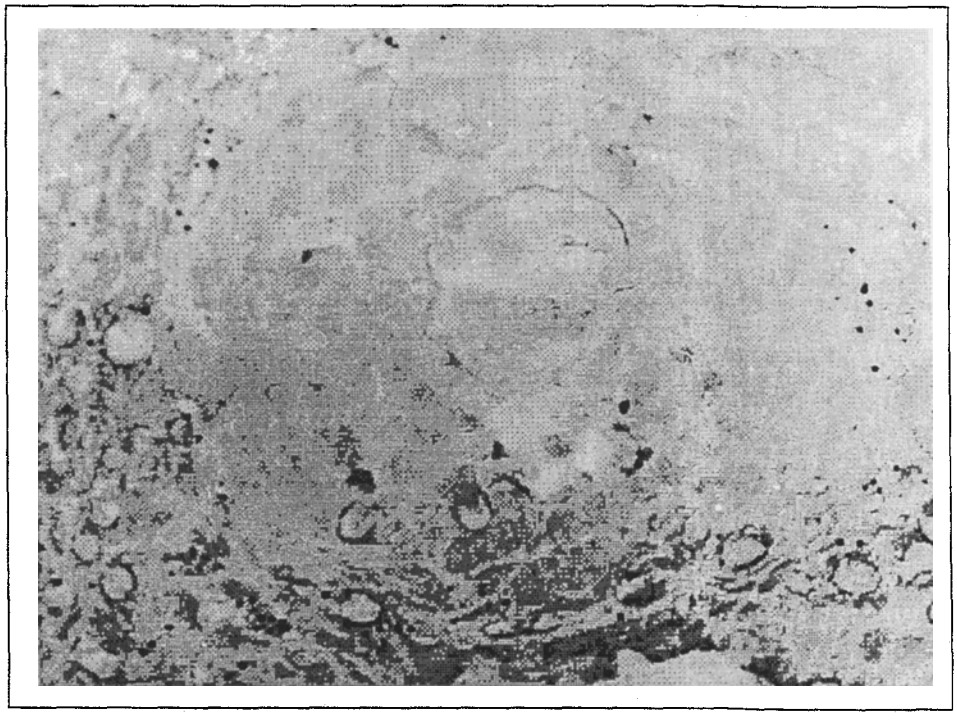

Fig. 2. A growing ovarian follicle. The presence of specific label in some folliculocytes, ×600.

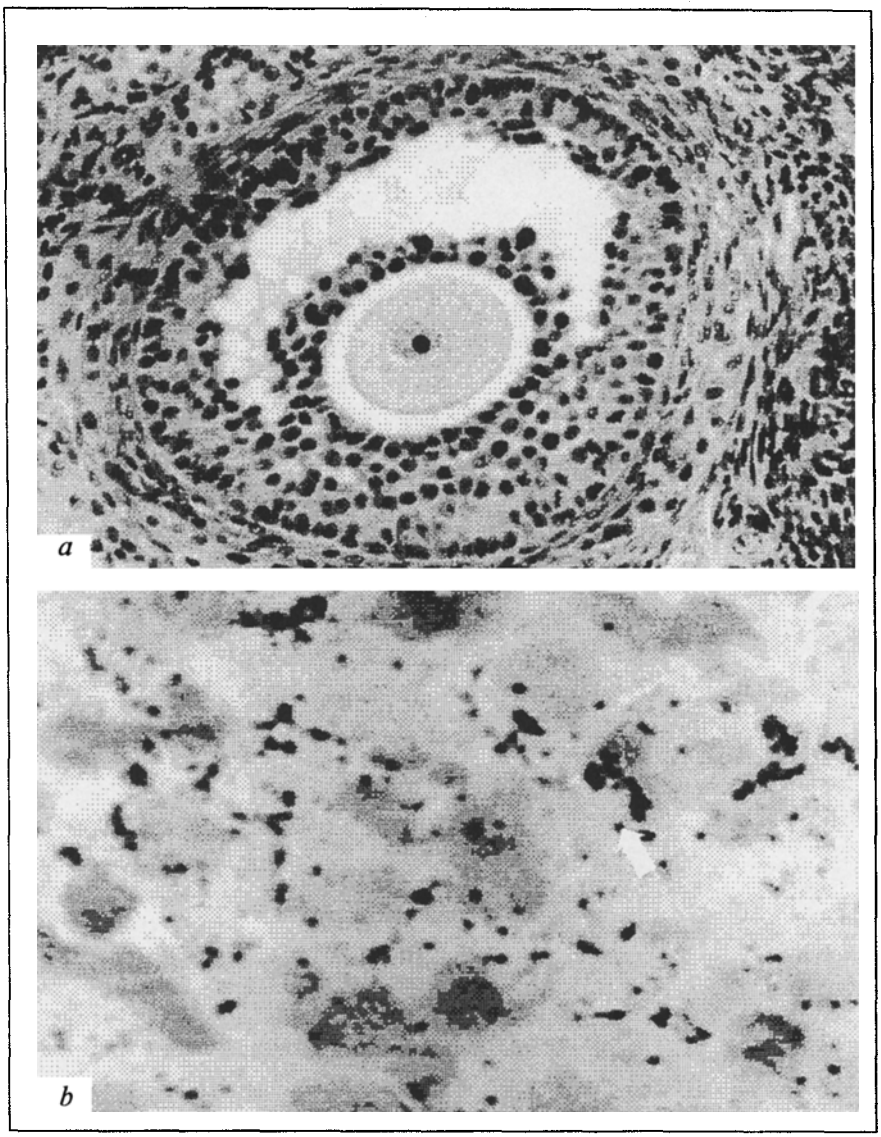

Fig. 3. Antral follicles in rat ovaries. *a*) hematoxylin and eosin staining, ×125; *b*) a fragment of membrane of antral follicle, G-protein α-subunit-positive labeling in basal folliculocytes and thecocytes, ×1000. Arrow indicates the label.

sent from primordial and primary follicles (hormone-independent stages), while in secondary (growing) follicles, positive staining was noted only in some folliculocytes of the granulation layer (Fig. 2).

It can be assumed that at the early stages of follicle growth mature and functionally active G-protein complexes are not always present in folliculocytes, but the number of cells containing these proteins increases in parallel with expression of hormone receptors.

In tertiary (antral) follicles, the label is more abundant and diffuse than in the growing follicles. The maximum positive staining is seen in basal folliculocytes of the granulation layer (Fig. 3). Marked labeling is also seen in the internal thecal layer (presumably in thecocytes, since the external thecocyte-free thecal layer exhibits no staining). The higher label incorporation may reflect hormone-induced activation of hormone production. The double labeling in follicles (folliculocytes and thecocytes) at this stage is consistent with the onset of bicellular sex steroid synthesis realized due to multiple hormone regulation.

These data on the presence and distribution of G-protein α-subunits in somatic cells of growing and antral follicles agrees with our previous data on activation of 3-β-steroid dehydrogenase (specific enzyme of steroidogenesis), which is most pronounced in thecocytes and folliculocytes of antral follicles (Fig. 4).

Thus, cells of somatic oocyte microenvironment (folliculocytes and thecocytes) express G-proteins. The dynamics of the content and distribution of G-protein α-subunits suggest that these proteins play a role in transduction of hormonal signals.

Fig. 4. A group of antral follicles, 3-β-steroid dehydrogenase-positive staining, ×75.

The study was supported by the Russian Foundation for Basic Research (Grant 96/04/49450).

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