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LH-Stimulation of the Adenylyl Cyclase System in Follicles and Oocytes of *Xenopus laevis*

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The presence of an adenylyl cyclase sensitive to LH in *Xenopus laevis* is studied. The assay for adenylyl cyclase in membranes and homogenates from *Xenopus laevis* follicles and oocytes is characterized and the aim is centered on the appearance of LH-response through oogenesis. The results show a stimulation by LH in whole follicle and oocytes surrounded by the follicle cells. The oocytes become responsive to LH from stage III onwards, suggesting an action of the gonadotropin on the monolayer of follicular cells in early stages of follicle development.

Key words: Adenylyl cyclase, LH, Amphibian ovary.

Gonadotropic hormones and steroid hormones affect the amphibian ovarian follicle to initiate maturation and ovulation (20, 21). It is well established that the blockade of meiosis in amphibian oocytes is released by the direct action on the oocyte surface of a progesterone-like hormone (16), which is secreted by the follicular cells in response to a gonadotropic signal (10-13).

Recent experimental evidence indicates that progesterone acts on the cell surface to trigger protein synthesis and to reinitiate the first meiotic division in *Xenopus laevis* oocytes (19). Cyclic AMP has been implicated in the mechanism of progesterone action and there is an almost immediate decrease in cAMP levels in oocytes after *in vivo* addition of progesterone (15). During the past few years, it has become evident that the mechanism by which progesterone lowers cAMP levels involves inhibition of the oocyte plasma membrane adenylyl cyclase (3, 5, 7, 17).

The oocytes removed from their follicular envelopes cannot display the maturation process after exposure to gonadotropins (10, 22), but still respond to progesterone (20) and follicular layers synthesize maturating steroid hormones in response to LH-like hormones (12, 21).

On the basis of these evidences, the work is conducted to determine whether or not LH acts on the amphibian follicle by a mechanism similar to that of mammalian ovary by stimulating follicular ad-

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enylyl cyclase and increasing cAMP levels, which in turns, give the initial signal for the events of follicular steroidogenesis.

In this report the existence of a LH-sensitive adenylyl cyclase in the amphibian follicle is shown. Furthermore, the enzymatic activity is stimulated *in vitro* by LH in the whole follicle and in oocytes surrounded by the monolayer of follicular cells.

Materials and Methods

Animals. — Adult female *Xenopus laevis* were obtained from NASCO and maintained under laboratory conditions.

Obtention of follicles and oocytes. — Animals were anesthetized by hypothermia and pieces of ovary were surgically removed and placed in Barth modified medium (BMM) pH 7.6.

Ovarian follicles, which contained full-grown oocytes (1.2-1.4 mm in diameter) were dissected out of the ovary under sterotaxic microscope. Oocytes surrounded by the monolayer of follicular cells were manually isolated from external enveloping layers with watchmaker's forceps (Dumont n.° 5). In some experiments, ovarian follicles containing oocytes at different stages of development were separated using the criteria of size and morphology described by DUMONT (2).

Preparation of homogenates and particulate adenylyl cyclase. — Follicles and oocytes were homogenized in a volume of a solution containing: 50 mM Tris-HCl pH 7.8, 1 mM EDTA, 0.44 sucrose using a Dounce homogenizer and 10 strokes with pestle A and 10 strokes with pestle B. The homogenate was centrifuged at 117 × *g* for 5 min and the supernatant was recentrifuged at 11,700 × *g* for 30 min. The pellet was resuspended in the same volume of the initial homogenization buffer and was used as the enzymatic source in the adenylyl cyclase experiments.

Adenylyl cyclase assay. — Adenylyl cyclase activity was assayed in triplicate by the procedure of HUNZICKER-DUNN and BIRNBAUMER (4) with some modifications: Incubations were carried out at 32.5° C in 50 µl of medium containing: 0.1 mM [^{32}P] – ATP (10 – 15 × 10^6 cpm), 2 mM or 5 mM $MgCl_2$, 1 mM EDTA, 1 mM [^{3}H] – cAMP (15,000 cpm), 20 mM creatine phosphate, 0.2 mg/ml creatine-kinase, 0.02 mg/ml myokinase and 25 mM Tris-HCl.

^{32}P-cAMP formed was isolated by the method of SALOMON *et al.* (18) as modified by BOCKAERT *et al.* (1). Protein concentration was determined by the method of LOWRY *et al.* (9) using BSA (Sigma) as standard.

Sources of materials. — ^{32}P-ATP was prepared as described by WALSETH and JOHNSON (23). ^{3}H-cAMP was purchased from ICN Radiochemicals; cAMP, GTP and GMP-P(NH)-P were from Boehringer-Manheim; NaF and forskolin were obtained from Fisher; ATP, creatine-phosphate, creatine-phosphokinase and myokinase were from Sigma. The batch of LH used was NIADKK-oLH-25.

Results

Whether the ovarian adenylyl cyclase in amphibia would respond to LH was the main task. Initial experiments were designed to set up an optimal assay for the LH response. In figures 1A and 1B, the linearity of basal and LH-stimulated adenylyl cyclase as a function of protein content and incubation time are shown.

Figure 2A illustrates the dependance of follicular adenylyl cyclase on ATP concentration in basal and LH-stimulated conditions. The activity progressively increased and, above 1 mM ATP was constant. A concentration of 0.1 mM ATP was used in subsequent experiments for two reasons: the LH relative stimulation

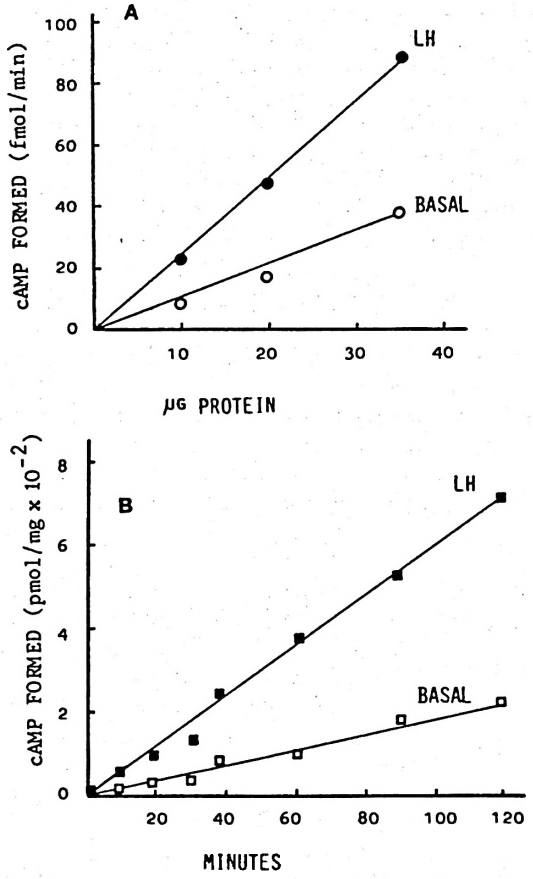

Fig. 1. *Effect of incubation time and protein concentration on adenylyl cyclase activity.*
(A) Linearity of basal and LH-stimulated adenylyl cyclase activity as a function of protein content. (B) Linearity of basal and LH-stimulated activity as a function of assay time. LH concentration was 10 µg/ml.

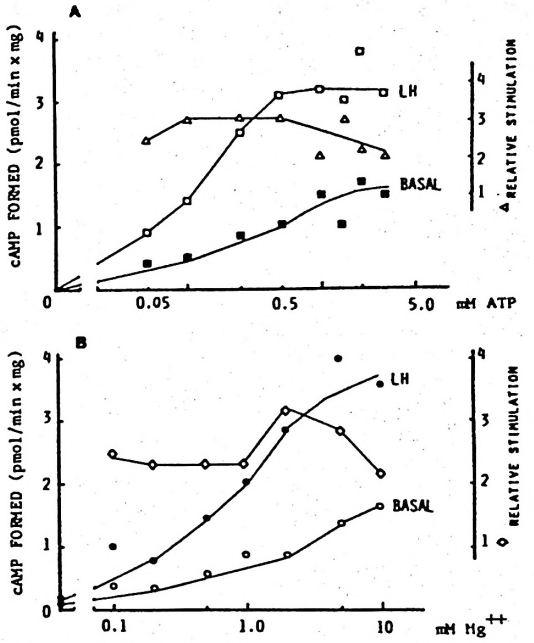

Fig. 2. *Effect of ATP concentration (A) and the divalent cation Mg^{++} (B) on adenylyl cyclase activity.*
Aliquots of follicle membranes were assayed under standard conditions with 10 µg/ml LH.

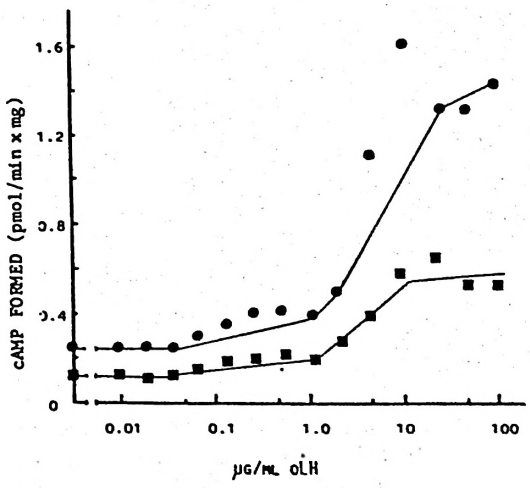

Fig. 3. *Effect of oLH concentration on adenylyl cyclase activity.*
The enzymatic activity was measured in membranes obtained from follicles (●) or oocytes (■) in the presence of 2 mM $MgCl_2$.

was maximal, and the ratio ATP ^{32}P-ATP allowed the number of counts incorporated to cAMP to be maximal. The effect of the divalent cation Mg^{++} is shown in figure 2B. At 5 mM Mg^{++} the adenylyl cyclase activity was maximal and at 2 mM Mg^{++} the maximal LH relative stimulation was obtained.

In figure 3 are represented the dose-re-

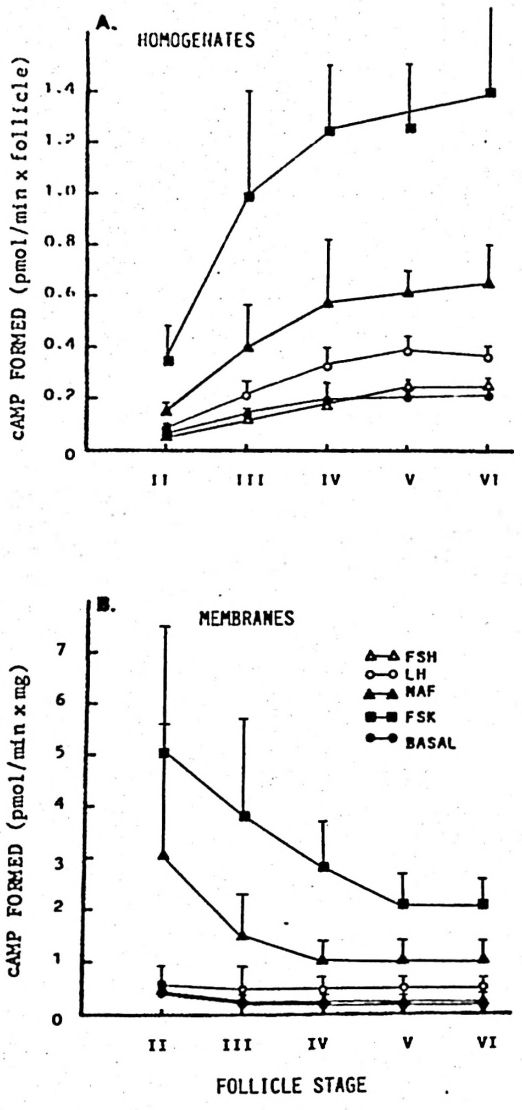

Fig. 4. *Changes in adenylyl cyclase activity during oogenesis.*

Aliquots of homogenates or membranes from isolated follicles, separated according to oocyte stage described by Dumont, were assayed under standard conditions. The results (mean ± SEM) were expressed as activity per follicle (A) or activity per mg of protein (B). Effector concentrations were: 10 μg/ml LH, 10 μg/ml FSH, 0.1 mM forskolin (FSK) and 10 mM NaF.

sponse curves at different concentrations of oLH in follicles and oocyte membranes. In both cases, the presence of hormone at concentrations above 1 μg/ml stimulates adenylyl cyclase and, at 10 μg/ml the LH relative stimulation was maximal. The EC_{50} for oocytes was 2.5 μg/ml and for follicles was 5 μg/ml.

The results in figure 4 summarize the changes in basal adenylyl cyclase and the response to LH, FSH, NaF and forskolin (FSK) during oogenesis. The graphics represent the enzymatic activity in homogenates (figure 4A) and membranes (figure 4B) of isolated follicles. The great increase of cellular protein content due to vitelogenin uptake by the stage III oocytes caused the decrease in the enzymatic activity.

Discussion

In this study, the existence of an LH-sensitive adenylyl cyclase in the amphibian follicle has been shown. LH stimulates the enzyme, the levels of cAMP being increased, which could be the signal for follicular steroidogenesis, leading to progesterone as final product (8, 10).

Several authors (3, 5) have demonstrated that the amphibian ovary contains an adenylyl cyclase system; the enzyme presents guanine nucleotide binding regulatory proteins since the enzymatic activity is highly activated by fluoride ions, nonhydrolizable GTP analogs, cholera toxin (6, 14, 15) and forskolin (7). Such findings are in agreement with results from preparations of isolated follicles and oocytes. Their characteristics are similar to those of other cyclase systems, although ATP concentrations above 0.1 mM do not affect the relative stimulation due to LH.

The enzyme is not stimulated by FSH, likely related to the absence of FSH response in follicles, which has been evident in all stages of oogenesis, and it is supported by the findings of MULNER and

OZON (13) since maturation does not occur when follicles are exposed *in vitro* to FSH.

The LH interesting response appears in stage III and it is maintained in the following stages of follicle development. This suggests an accumulation of cAMP in the follicle cells during early stages of oogenesis.

It is a general assumption that in the events of oocyte maturation gonadotropins act on follicle cells as steroidogenesis takes place (10-13). There is indirect evidence that intrafollicular levels of progesterone are controlled by cAMP in the follicle of *Rana pipiens* (8). Adenylyl cyclase would play a role in regulating cAMP and in mediating the action of FPH (frog pituitary hormone) on follicular progesterone levels; the stimulation of progesterone production caused by FPH would be mediated by elevating intracellular levels of cAMP. Therefore, the rise of cAMP levels affects intrafollicular steroid production and, subsequently, the steroid-induced maturation of the oocyte (13).

In conclusion, the results show that LH stimulates follicular adenylyl cyclase in *Xenopus laevis*, and that the link between the enzymatic system and steroidogenesis remains uncertain as well as the fate of cAMP inside the follicular cell.

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Resumen

Se estudia la existencia de un sistema adenilil ciclasa sensible a LH en homogenados y membranas de folículos y oocitos de *Xenopus laevis*, con objeto de determinar la respuesta a LH durante la oogénesis. Los resultados muestran una estimulación por LH en folículos enteros y en oocitos rodeados por la monocapa de células foliculares. La respuesta a LH aparece en un estadio temprano de desarrollo folicular, estadio III.

Palabras clave: Adenilil ciclasa, LH, Ovario de anfibios.

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