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Epidermal Growth Factor Induces Maturation of Rat Follicle-Enclosed Oocytes*

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ABSTRACT. Gonadotropin-induced differentiation of ovarian granulosa cells in culture is inhibited by epidermal growth factor (EGF). The present study was undertaken to test a possible inhibitory effect of EGF on LH-induced maturation of rat follicle-enclosed oocytes. We have found that EGF not only failed to affect LH action but served by itself as an inducer of maturation of follicle-enclosed oocytes. EGF action on the oocytes was dose and time dependent and could be prevented by $(Bu)_2$ cAMP. The response of the oocytes was specific to EGF and could not be elicited by other growth factors such as nerve growth factor and insulin. The response to EGF was not limited to the large antral follicles, as oocytes enclosed by small antral follicles (<0.4 mm) were induced to mature by EGF as well. In addition, we have demonstrated that oocytes, induced to mature by EGF, are concomitantly uncoupled from the follicular cells. Based on these results we suggest that EGF may terminate the transfer of a follicular inhibitor to the oocyte. It is also possible, however, that EGF induces oocyte maturation by a mechanism independent of its effect on communication between the cellular components of the follicle. (*Endocrinology* **116**: 406–409, 1985)

UNLIKE ITS effect on a variety of epidermal and nonepidermal cells (1), epidermal growth factor (EGF) has been shown to inhibit differentiation of ovarian granulosa cells in culture. Specifically, FSH-induced formation of both LH-receptors and estrogen biosynthesis cannot be demonstrated by granulosa cells cultured in the presence of EGF (2, 3). Is the negative modulation of gonadotropin-induced differentiation, by EGF, limited to the somatic components of the ovarian follicle? It is known that the follicular oocyte is maintained in a stage of meiotic arrest until shortly before ovulation. It is only after the preovulatory surge of gonadotropins that the oocyte resumes the meiotic maturation and becomes fertilizable (4). The nature of the intraovarian factor which is responsible for meiotic arrest is not clear as yet. It is possible that similar to its effect on the granulosa cells, EGF interferes also with LH action to inhibit oocyte maturation. In this case, EGF or other EGF-like factors could have had some important physiological role in controlling processes which interact with gonadotropin action to determine the overall course of follicular development. The present study was designed in an attempt to characterize the possible effect of EGF on the follicular oocyte.

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Materials and Methods

Ovarian fragments containing four to six follicles (>0.5 mm diameter) each were isolated from immature PMSG (15 IU)-primed rats and incubated in L-15 Leibovitz's tissue culture medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (Sera Lab, Sussex, UK), antibiotics (100 U/ml penicillin, 50 μg/ml streptomycin, Sigma, St. Louis, MO), and either ovine LH (NIH-LH S20), EGF (Sigma), *N,O'*-$(Bu)_2$ cAMP (Sigma), methylisobutylxanthine (MIX, Sigma), insulin (porcine, Eli Lilli, Indianapolis, IN), nerve growth factor [(NGF) mouse, kindly provided by Dr. J. Kimhi, Weizmann Institute of Science] or their combination, in 25-ml flasks gassed with O_2:N_2 (1:1), in a shaking water bath, at 37 C. Ovarian fragments containing small antral follicles (<0.4 mm diameter) were isolated from immature nonprimed rats and incubated as above. After the indicated times the follicles were incised and the oocytes were recovered for microscopic examination. Oocytes, enclosed by their cumulus cells, were isolated from ovaries of PMSG-primed rats and incubated in the above medium with or without 100 ng/ml EGF. Incubations took place in 35-mm Petri dishes at 37 C in 100% humidity in air.

Oocytes were examined for maturation by Nomarski Interference Contrast microscopy. Meiotically arrested oocytes were indicated by the presence of the nuclear structure or germinal vesicles (GV). The breakdown of the GV (GVB) served as the marker for resumption of meiosis or oocyte maturation. GVB was not the only parameter used to indicate oocyte maturation. Since, during the normal course of oocyte maturation, the formation of the first polar body occurs within 5 h after the disappearance of the GV (5), some cultures terminated at this later time point (13 h) were analyzed for the presence of the polar body. The polar body is a very labile structure, which

degenerates shortly after its formation. GVB, rather than polar body formation, served therefore, as a more convenient and more accurate marker to indicate maturation of the oocyte. For each study the data of several individual experiments were combined and the results are reported as the fraction of oocytes with GVB.

In the absence of cumulus cells, oocytes incorporate negligible amounts of uridine (6, 7). Thus, analysis of the level of coupling in the cumulus oocyte complex is based on the assumption that the amount of uridine present in the cumulus-enclosed oocyte is a function of the extent of communication between the oocyte and the cumulus cells. Communication between the oocyte and the cumulus cells was studied after incubation of ovarian fragments with or without EGF (100 ng/ml) for the indicated times. At the end of the incubation period cumulus-oocyte complexes were recovered and further incubated with 10 μCi/ml [5,6-^{3}H]uridine (SA, 43 Ci/mmol, International, Amersham, England). After 1 h the complexes were rinsed and the oocytes were mechanically treated to remove the cumulus cells (8). The denuded, cumulus-free oocytes, were then transferred to scintillation vials, dissolved in 1 N NaOH, acidified with 1 N HCl, and counted. The level of communication is presented as the fraction of incorporated radioactivity into oocytes after incubation with EGF out of incorporation into oocytes after incubation in control medium. The results represent mean values ± SE of three individual experiments.

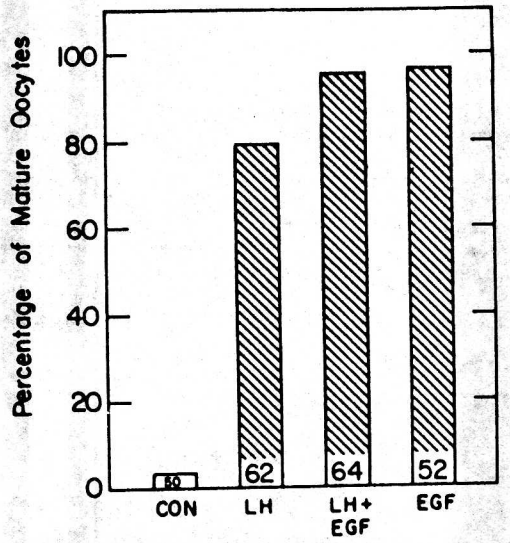

FIG. 1. Effect of either LH, EGF, or their combination on follicle-enclosed oocytes. Maturation was indicated by the breakdown of the GV in oocytes isolated from ovarian fragments after 20 h of incubation with or without either ovine LH (1 μg/ml), EGF (100 ng/ml), or their combination. The total number of oocytes examined is indicated.

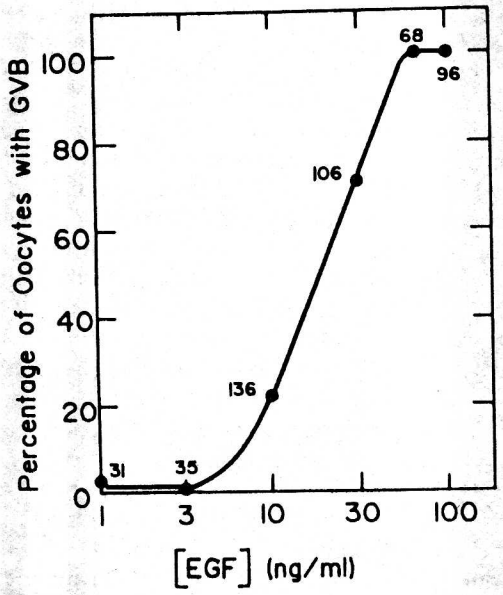

FIG. 2. Concentration dependence of EGF-induced maturation of follicle-enclosed oocytes. GVB was analyzed in oocytes isolated from ovarian fragments incubated for 20 h in the presence of the indicated concentrations of EGF. The total number of oocytes examined is indicated.

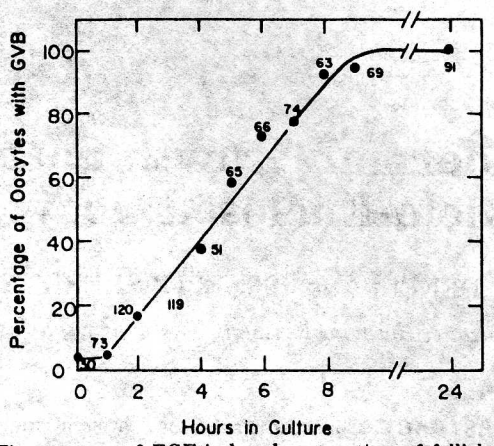

FIG. 3. Time course of EGF-induced maturation of follicle-enclosed oocytes. GVB was analyzed in oocytes isolated from ovarian fragments incubated in the presence of 100 ng/ml EGF for the indicated times. The total number of oocytes examined is indicated.

TABLE 1. Effect of either EGF, NGF, or insulin on follicle-enclosed oocytes

Hormone added	Concentration of hormone (ng/ml)	Fraction of GVB oocytes	% of GVB oocytes
EGF	100	91/91	100
NGF	200	1/83	1
Insulin	200	0/82	0

GVB was analyzed in oocytes isolated from ovarian fragments incubated for 20 h in the presence of the indicated concentrations of either EGF, NGF, or insulin as described in *Materials and Methods*.

Results

Rat follicle-enclosed oocytes are induced by LH to mature *in vitro*, (9). As seen in Fig. 1, EGF not only failed to inhibit LH action, but served by itself as a potent inducer of oocyte maturation. Induction of oocyte maturation by EGF is dose dependent (Fig. 2). The maximal effective dose is 50 ng/ml with an ED_{50} at 15

ng/ml. Ninety-five % of the oocytes lost their GV by 9 h of culture although 5 h were required for 50% of them to respond (Fig. 3). Induction of oocyte maturation is specific to EGF since neither NGF nor insulin could elicit this type of response (Table 1). The response to EGF was not limited to the large (>0.5 mm diameter) antral follicles. Oocytes enclosed by small antral follicles (<0.4 mm diameter), isolated from rats that were not treated by PMSG, were also induced to mature after treatment with EGF (Fig. 4).

It is known that LH-induced oocyte maturation is blocked when high levels of cAMP are introduced to the oocyte (10, 11). As seen in Fig. 5 the addition of $(Bu)_2$cAMP to ovarian fragments incubated in the presence of EGF clearly inhibited its stimulatory action. The fraction of maturing oocytes was reduced from 94% in the absence of $(Bu)_2$cAMP to 36% in its presence. The addition of the phosphodiesterase inhibitor MIX on top of these two agents, in a concentration which by itself

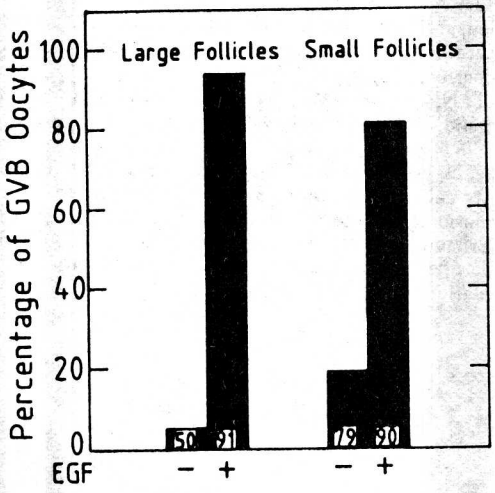

FIG. 4. Effect of EGF on oocytes enclosed by small antral follicles as compared to its effect on oocytes enclosed by large antral follicles. The large antral follicles were isolated from immature PMSG-primed rats. Small antral follicles were isolated from immature untreated rats. For incubation and examination procedure see legend to Fig. 1.

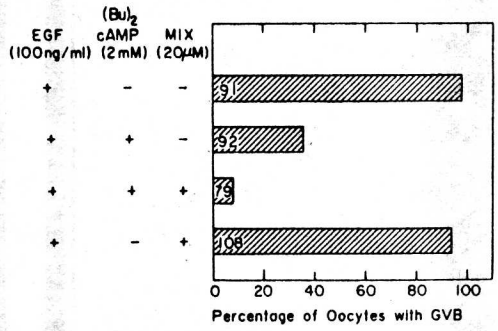

FIG. 5. Effect of $(Bu)_2$cAMP and MIX on EGF-induced maturation of follicle-enclosed oocytes. GVB was analyzed in oocytes isolated from ovarian fragments incubated with either EGF, $(Bu)_2$cAMP, MIX, or their combination. The total number of oocytes examined is indicated.

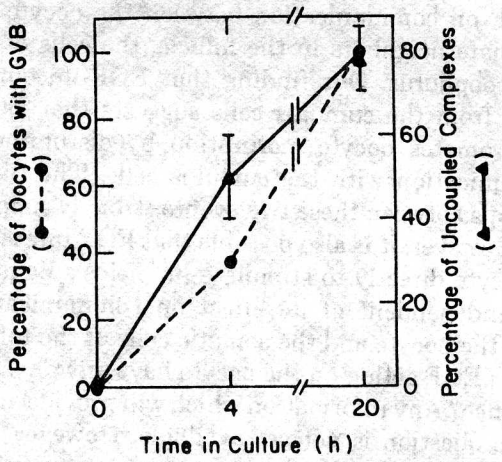

FIG. 6. Time course of EGF-induced uncoupling in the cumulus-oocyte complexes as compared to the time course of maturation of follicle-enclosed oocytes. Coupling was assessed by the transfer of labeled uridine from the cumulus cells to the oocyte as described in *Materials and Methods*.

had no effect on EGF action, significantly potentiated the inhibitory influence of $(Bu)_2$cAMP.

EGF action on the oocyte is apparently not elicited by a trigger type mechanism. Thus, a short exposure to the hormone will not result in oocyte maturation. In fact, even 2 h of exposure to EGF failed to induce its biological effect in this system (data not shown).

In addition to the induction of oocyte maturation EGF also interrupted the communication in the cumulus-oocyte complex (Fig. 6). The time relationship between these two events cannot exclude a possible correlation between them.

Isolated cumulus-enclosed oocytes mature spontaneously *in vitro* (10). EGF had no inhibitory effect on maturation of these oocytes (data not shown).

Discussion

The present study has demonstrated that: 1) EGF induces maturation in rat follicle-enclosed oocytes, 2) EGF-induced oocyte maturation is inhibited by cAMP, and 3) EGF interrupts communication in the cumulus-oocyte complex.

The mechanism by which EGF acts to induce oocyte maturation is unknown. However, our and other studies (12, 13) provide some evidence to support the following hypothesis for LH action. cAMP generated by the follicular cells but not by the oocyte, is transferred via junctional communication with the cumulus cells to the oocyte to keep it meiotically arrested. As a result of the surge of LH, which terminates communication in the cumulus-oocyte complex, the transfer of cAMP is stopped, inhibition is relieved, and the oocyte is allowed to resume meiosis. Is this model valid for EGF action in this system? To test this possibility we studied the effect

of EGF on communication between the oocyte and its immediate neighbors in the follicle, the cells of the cumulus oophorus. Our finding that EGF uncouples the oocyte from the cumulus cells suggests that EGF, like LH, promotes oocyte maturation by disruption of its communication with the cumulus cells. The time relationships between these two events strongly support this idea. However, it is also possible that EGF interacts with the oocyte directly to stimulate its meiosis by a mechanism independent of its effect on communication between the oocyte and the somatic cells of the follicle.

Does EGF action on the oocyte have any physiological relevance? Any information which will provide an answer to this question is not yet available. However, the fact that EGF receptor levels in the rat ovary varies during the estrous cycle being modulated by gonadotropins, recently reported by St.-Arnaud *et al.* (14), suggests that EGF could well play a physiological role in the regulation of ovarian functions.

Acknowledgments

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