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Induction of Maturation in Follicle-Enclosed Oocytes: The Response to Gonadotropins at Different Stages of Follicular Development[1]

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ABSTRACT

Antral follicles, isolated from either nontreated or pregnant mare's serum gonadotropin (PMSG)-primed 27-day-old rats, were incubated in the absence or the presence of either luteinizing hormone (LH), follicle-stimulating hormone (FSH), or forskolin. The effect of these agents on oocyte maturation and cyclic adenosine 3',5'-monophosphate (cAMP) accumulation was studied and compared. Both gonadotropins, LH and FSH, as well as forskolin, effectively induced maturation of oocytes enclosed by large antral follicles isolated from PMSG-primed rats. On the other hand, we found that maturation of oocytes enclosed by small antral follicles, isolated from both nonprimed and PMSG-primed rats, could be induced by either FSH or forskolin but not by LH. cAMP determinations revealed that, in spite of the inability of LH to induce oocyte maturation, elevated concentrations of the nucleotide were detectable in small antral follicles exposed to this gonadotropin. Since granulosa cells isolated from the large but not the small antral follicles were stimulated by LH to generate cAMP, the elevation of cAMP concentrations in the small antral follicle apparently represented the response of the theca cells to this gonadotropin. Since it is the ability of the granulosa cells to interact with the hormone that determines whether or not oocyte maturation will occur, we suggest that the granulosa, but not the theca cells, mediate LH action to induce oocyte maturation.

INTRODUCTION

Oocyte maturation in vivo occurs subsequent to the preovulatory luteinizing hormone (LH) surge (Ayalon et al., 1972; Dekel et al., 1979). While it is clearly evident that rat granulosa and cumulus cells in the maturing follicle are the target for LH, the oocyte does not appear to respond to this gonadotropin (Dekel and Beers, 1978, 1980). These observations suggest that LH action, which leads to oocyte maturation, is probably mediated by the somatic components of the follicle and does not represent a direct interaction of the female gamete with the hormone. Inability to detect specific binding sites for LH in preparations of denuded oocytes is in accord with this idea (Lawrence et al., 1980). In view of these observations, it can be predicted that maturation of the oocyte in response to LH should correlate with the presence of receptors for this hormone on the somatic-follicular cells.

It has been demonstrated that granulosa cells from follicles at early developmental stages are endowed with follicle-stimulating hormone (FSH) receptors, whereas LH receptors are acquired only at the final stages of follicular growth (Channing and Kammerman, 1973; Midgley, 1973; Zeleznik et al., 1974; Nimrod et al., 1976). Independently, it has also been

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shown that isolated large preovulatory follicles respond to both gonadotropins in terms of oocyte maturation (Lindner et al., 1974). Whether the response of the oocytes enclosed by small antral follicles is specific to follicle-stimulating hormone (FSH) has not yet been examined.

The objective of our present study was to investigate the specific effect of either gonadotropin, LH or FSH, on oocyte maturation at different stages of follicular development. Since many of the actions of gonadotropins are mediated by cyclic adenosine 3′,5′-monophosphate (cAMP), the effect of the diterpene forskolin, which activates adenylate cyclase in a receptor-independent manner, was also examined in this system. Finally, the responses of the follicle and isolated granulosa cells to the gonadotropins and forskolin, in terms of intracellular cAMP levels, were determined.

MATERIALS AND METHODS

Large antral follicles (>0.9 mm diameter) were isolated from immature, 27-day-old, pregnant mare's serum gonadotropin (PMSG)-primed Wistar rats. PMSG (Gestyl, Organon, Oss, The Netherlands) was administered s.c. on Day 25 of age at a dose of 6 IU in 0.1 ml of 0.9% NaCl. Small antral follicles (<0.4 mm diameter) were isolated from 27-day-old rats, either PMSG-primed or nontreated.

The rats were killed by cervical dislocation, and the ovaries were removed and placed in Leibovitz's L-15 tissue culture medium (L-15, GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (Sera-Lab, Crawley Down, England), penicillin (100 units/ml) and streptomycin (100 μg/ml, GIBCO). Some of the ovaries were used for preparation of isolated intact follicles, as described previously (Dekel and Sherizly, 1983). Isolated granulosa cells were recovered from another group of ovaries, as described by Lawrence et al. (1980). After isolation, either granulosa cells or individual follicles were incubated in the above medium in the presence or absence of one of the following agents: rat LH (rLH) NIH LH I-5 (1 μg/ml), rat FSH (rFSH), NIH FSH I-3 (1 μg/ml) or forskolin (Calbiochem-Behring Co., La Jolla, CA) (100 μM). These concentrations have previously been determined to produce maximal responses in these species (Dekel et al., 1981; Dekel and Sherizly, 1983). Incubation of follicles was carried out in an N_2/O_2 (1:1) atmosphere in an oscillating bath at 37°C. Granulosa cells were incubated at 37°C in 35-mm Petri dishes at 100% humidity.

Oocyte maturation was examined morphologically with a Zeiss microscope equipped with Nomarski interference contrast optics. Preliminary experiments revealed that maximal rate of maturation is observed in the oocytes after 4 h exposure to either of the above agents. Thus, after 4 h of incubation the follicles were incised and the cumulus/oocyte complexes were recovered. Resumption of meiosis was indicated by the absence of the germinal vesicles (GV; germinal vesicle breakdown, GVB) in the oocytes. For each study, the data of several individual experiments were combined, and the results are reported as the fraction of GVB oocytes.

cAMP determinations were performed by using the competitive protein binding assay (Gilman, 1970), as modified by Lamprecht et al. (1973). Our assay detects between 0.5 and 50 pmol cAMP. The data represent accumulation of cAMP after 1 h of incubation as performed in tissue homogenates of intact follicles or granulosa cells. The values obtained for cAMP concentrations under the different treatments were statistically analyzed by using the a priori comparison of means and groups of means in single classification mode I ANOVA (Sokal and Rohlf, 1969).

RESULTS

Oocytes recovered from the large preovulatory follicles of PMSG-primed rats were stimulated to resume meiosis by both gonadotropins, LH and FSH. However, meiosis resumption in oocytes enclosed by small antral follicles isolated from both PMSG-primed and nonprimed rats was induced only by FSH (Fig. 1). Highly purified rat LH preparation failed to induce oocyte maturation in these follicles. In fact, the fraction of GV oocytes in small follicles incubated with LH was not significantly different from that in follicles incubated in the absence of gonadotropins. Forskolin was equally successful in inducing maturation in oocytes enclosed by large and small antral follicles (Fig. 1).

cAMP determinations revealed that, in spite of its inability to induce maturation of the oocytes, LH did

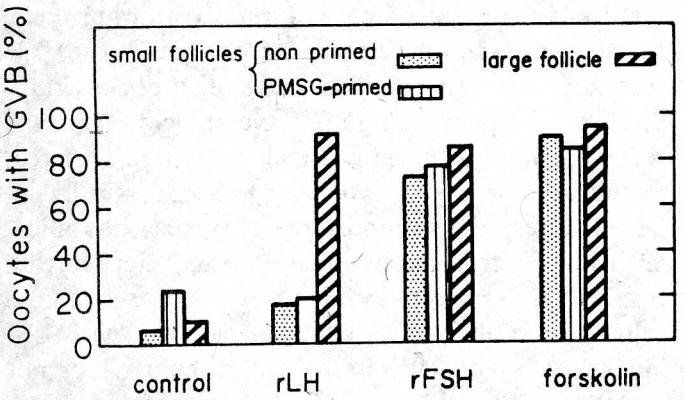

FIG. 1. Effect of gonadotropins and forskolin on maturation of follicle-enclosed oocytes isolated from either pregnant mare's serum gonadotropin (*PMSG*)-*primed* or *nonprimed* immature rats. Twenty-seven-day-old rats were killed and the ovaries were removed. After isolation, individual follicles were incubated, as described in *Materials and Methods*. After 4 h, cumulus-oocyte complexes were recovered, and the oocytes were examined for the presence or absence of the germinal vesicles (GV). The results represent the pooled data of three individual experiments. Sixty to one hundred oocytes were examined for each experimental group. *GVB* = germinal vesicle breakdown, *rLH* = rat luteinizing hormone, *rFSH* = rat follicle-stimulating hormone.

stimulate accumulation of cAMP in the intact, small antral follicles. Of the three agents tested, forskolin produced the largest response; however, LH was a more potent stimulator of cAMP generation than FSH in this system (Fig. 2). On the other hand, no response to LH was detected in the granulosa cells recovered from the small antral follicles (Fig. 3). Although concentrations of cAMP in granulosa cells from small follicles of PMSG-primed rats were somewhat higher than that found in cells from control follicles they do not represent a significant elevation ($p>0.01$), and probably result from contamination of granulosa cells from the large follicles. These follicles are present in the ovaries of rats treated with PMSG but absent in ovaries of nonprimed rats. Granulosa cells recovered from small follicles of both nonprimed and PMSG-primed rats did respond to FSH and forskolin (Fig. 3). In the large follicles—unlike in the small follicles—a significant ($p<0.01$) LH-stimulated elevation of cAMP could be demonstrated in both preparations of intact follicles as well as in the recovered

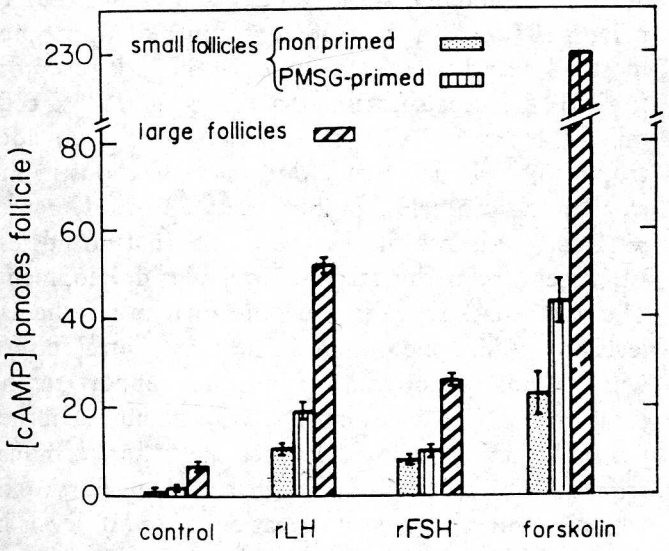

FIG. 2. Effect of gonadotropins and forskolin on cyclic adenosine 3′,5′-monophosphate (*cAMP*) accumulation in intact follicles isolated from either pregnant mare's serum gonadotropin (*PMSG*)-*primed* or *nonprimed* immature rats. Follicles were isolated and incubated as described in the *legend to Figure 1*. After 1 h, the follicles were homogenized and assayed for cAMP, as described in *Materials and Methods*. The results represent the mean values ± SEM of duplicates of samples collected from 4 to 6 individual experiments. *rLH* = rat luteinizing hormone, *rFSH* = rat follicle-stimulating hormone.

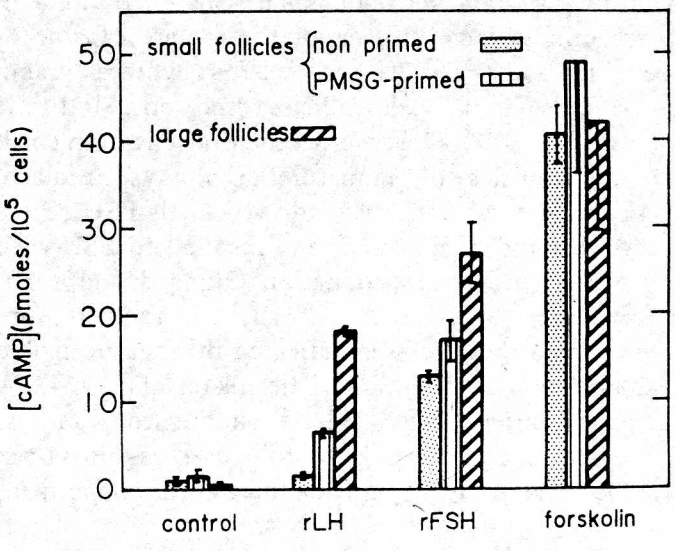

FIG. 3. Effect of gonadotropins and forskolin on cyclic adenosine 3′,5′-monophosphate (*cAMP*) accumulation in granulosa cells isolated from antral follicles of either pregnant mare's serum gonadotropin (*PMSG*)-*primed* or *nonprimed* immature rats. Granulosa cells were isolated and incubated, as described in *Materials and Methods*. After 1 h, granulosa cells were recovered, homogenized, and assayed for cAMP. The results represent the mean values ± SEM of duplicates of samples collected from 3 to 5 individual experiments. *rLH* = rat luteinizing hormone, *rFSH* = rat follicle-stimulating hormone.

granulosa cells (Figs. 2 and 3). These follicles and their granulosa cells were also responsive to FSH and to forskolin.

DISCUSSION

We have demonstrated in this study that oocytes enclosed by the small antral follicles will resume meiosis in response to FSH, whereas LH fails to induce oocyte maturation at this stage of follicular development. On the other hand, the large preovulatory follicles do respond to both gonadotropins in terms of oocyte maturation, as shown in the present and earlier studies (Tsafriri et al., 1972; Hillensjö, 1976; Dekel et al., 1981).

In the absence of detectable receptors for LH on the oocyte (Lawrence et al., 1980), we have previously suggested that induction of oocyte maturation does not represent a direct interaction of the hormone with the female gamete, but rather is mediated by the somatic follicular cells (Dekel and Beers, 1980). The somatic cellular components of the follicle are the cells of the theca and the granulosa. Unlike the theca cells, which possess LH receptors at early stages of follicular development (Erickson and Magoffin, 1983), granulosa cells in small antral follicles lack receptors for LH. Studies employing in vitro autoradiographic techniques have shown that granulosa cells in large follicles of the rat are capable of binding both ^{125}I-hCG and ^{125}I-FSH whereas granulosa cells from small follicles bind only ^{125}I-FSH (Midgley, 1973). Moreover, granulosa cells from small antral follicles of immature hypophysectomized, estrogen-treated rats are endowed with FSH receptors (Nimrod et al., 1976) and respond to FSH with increased progestin production (Nimrod and Lindner, 1976), whereas few if any LH receptors are present on these cells, as reflected by their negligible ability to bind ^{125}I-hCG (Zeleznik et al., 1974). A greater capacity for ^{125}I-hCG binding to granulosa cells harvested from large follicles has also been demonstrated in the pig (Channing and Kammerman, 1973).

Our study presents a clear correlation between the responsiveness of the granulosa cells to a specific gonadotropin and the capacity of the oocyte to resume meiosis following exposure to this hormone. In the small antral follicles, in the absence of LH receptors on the granulosa cells, the oocyte stays dictyate. At a later stage of development, when the receptors to this specific gonadotropin are acquired, maturation of the oocyte by LH is successfully induced. These data support the idea that the follicular cells control oocyte maturation. They further suggest that, since it is the ability of the granulosa cells to interact with LH that correlates with LH-induced oocyte maturation, the granulosa, but not the theca cells, mediate LH action to induce oocyte maturation.

The role of cAMP as a mediator of LH in the induction of oocyte maturation has been suggested by us earlier (Dekel et al., 1981; Dekel and Sherizly, 1983). This notion is supported by our present demonstration that forskolin, which interacts with the catalytic subunit of the adenylate cyclase system to stimulate cAMP generation, induces both oocyte maturation and cAMP elevation in large as well as in small antral follicles. Our findings that in small antral follicles LH-stimulated elevation of cAMP concentrations is not associated with oocyte maturation seem to disagree with this idea. Our further experiments, in which the follicle has been dissected and the isolated granulosa cells analyzed, seem to offer a solution to this apparent contradiction. Since in small antral follicles granulosa cells lack receptors for LH, it is not surprising that these granulosa cells fail to generate cAMP in response to this gonadotropin. The elevation of cAMP concentrations in the small antral follicles, in our study, probably represents the response of the theca cells that do possess LH receptors at this stage of follicular development. The fact that, regardless of the high intrafollicular levels of cAMP, the oocyte in the small antral follicle will not mature provided additional support to the idea that it is the response of the granulosa rather than the theca cells that mediates the hormonal stimulus to induce oocyte maturation. Forskolin, which presumably causes elevation of cAMP levels in the granulosa cells independently of the presence of any hormonal receptors, induces meiosis resumption in follicles of all the developmental stages tested.

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