

# Induction of maturation in rat follicle-enclosed oocyte by forskolin

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The diterpene forskolin, which was found to be a potent and reversible activator of adenylate cyclase in intact tissues as well as in broken cell preparations, was employed to investigate the role of cAMP in the induction of oocyte maturation. We have found that forskolin can mimic the effect of LH on the ovarian follicle stimulating both cAMP accumulation and oocyte maturation. These findings suggest that LH-induced maturation in follicle-enclosed oocytes is a cAMP-mediated response.

*Oocyte maturation      cAMP      Forskolin      Ovarian follicle      Germinal vesicle*

## 1. INTRODUCTION

LH triggers the resumption of meiosis in rat oocytes both *in vivo* [1] and in *in vitro* cultures of intact follicles [2,3]. Either cAMP derivatives or cyclic nucleotide phosphodiesterase inhibitors block LH-induced maturation of follicle-enclosed oocytes [4,5] as well as the spontaneous maturation of isolated oocytes [6-8]. The apparent antagonism between the stimulatory action of LH and the inhibitory influence of these modulators of cAMP levels on the female gamete seems to be contradictory since a predominant effect of LH is to elevate cAMP levels in the ovary [9,10].

The question addressed in our study is whether LH-induced oocyte maturation is a cAMP-mediated response. For this investigation the diterpene forskolin, a potent and reversible activator of adenylate cyclase [11], was employed. As yet, forskolin has been effective in increasing cAMP levels in all mammalian cells tested, eliciting cellular responses which have been proposed to depend on cAMP as a second messenger. This, in addition to the fact that forskolin action is not limited to

broken cell preparations but also can be demonstrated in intact tissues, makes this agent a particularly suitable tool to investigate the possible relationship between the elevation of cAMP levels following the pre-ovulatory LH-surge and reinitiation of meiosis in the maturing oocyte.

## 2. MATERIALS AND METHODS

Sexually immature Wistar female rats (26 days old) from our departmental colony were injected subcutaneously with 15 IU of pregnant mare's serum gonadotropin (PMSG, Gestyl, Organon, Holland) in 0.1 ml 0.9% NaCl. The rats were killed by cervical dislocation 48 h after the injection. The ovaries were removed and placed in Leibovitz's L-15 medium (Gibco USA), supplemented with 10% fetal bovine serum (Sera-Lab, England), penicillin (100 units/ml) and streptomycin (100 g/ml) (Gibco). This composition is referred to as control medium throughout this paper.

The ovarian follicles were dissected under a stereoscopic microscope, rinsed and placed in control medium in 25 ml flasks, gassed with 50% O<sub>2</sub> and 50% N<sub>2</sub> [5]. Incubations were carried out at 37°C in an oscillating water bath in the presence or absence of forskolin (7 $\beta$ -acetoxy-8, 13-epoxy-1 $\alpha$ , 6 $\beta$ ,9 $\alpha$ -trihydroxy-labd-14en-11-one, Calbiochem-

*Abbreviations:* GV, germinal vesicle; GVB, GV breakdown

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Behring Corp. USA). At the end of the incubation times the follicles were incised and the oocytes were recovered and analyzed for maturation by Nomarski Interference Contrast microscopy [5]. Resumption of meiosis was indicated by the disappearance of the GV in the individual oocytes. For each study, the data of several individual experiments were combined and the results are reported as the fraction of oocytes with GVB.

cAMP determinations were performed by the competitive protein binding assay [12] as modified in [13]. The tissue and the medium were assayed separately and the data represent accumulation in the tissue as determined in samples of two follicles each.

### 3. RESULTS AND DISCUSSION

To characterize the response of the rat ovarian follicle to forskolin the timing and the concentration dependency of cAMP accumulation was analyzed. We found that cAMP levels in isolated follicles were elevated by 5 min after exposure to forskolin reaching a plateau at 15 min. The dose-dependent analysis, which was therefore assessed after 15 min incubation, showed that accumulation of cAMP by isolated follicles approached maximal values at concentrations of 60–100  $\mu\text{M}$

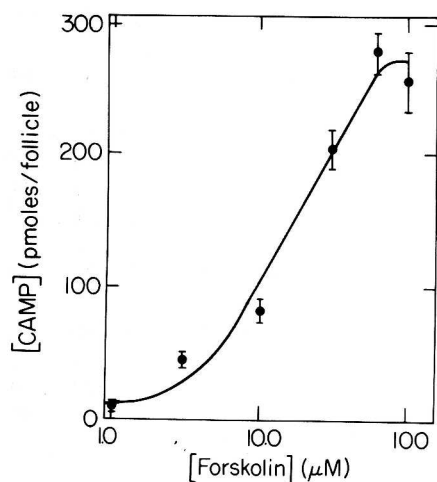


Fig.1. Concentration dependence of cAMP accumulation in isolated follicles stimulated by forskolin. Isolated follicles were incubated for 15 min in the presence of forskolin. The data of one representative experiment are presented.

with a half-maximal effect at 11  $\mu\text{M}$  of forskolin (fig.1).

To test the ability of forskolin to induce oocyte maturation, intact follicles were incubated overnight in the presence of different concentrations of the diterpene. The dose-dependency curve (fig.2) revealed that 0.4  $\mu\text{M}$  forskolin was required to induce GVB in 50% of the oocytes while 3  $\mu\text{M}$  stimulated almost all the oocytes examined. Higher concentrations of forskolin (up to 100  $\mu\text{M}$ ) were equally effective in stimulating GVB in the follicular oocytes excluding a possible biphasic effect of cAMP. Timing analysis of forskolin-induced maturation, assessed by the maximal effective dose of the diterpene (3  $\mu\text{M}$ ) revealed that 50% of the follicle-enclosed oocytes completed their GVB by 3 h incubation (fig.3). After 8 h culture almost all the incubated oocytes lost their GV. The timing of forskolin-induced GVB in follicle enclosed oocytes is very similar to that obtained by LH [2].

Our data demonstrate that forskolin can mimic the effect of LH, stimulating both cAMP accumulation in rat ovarian follicles and resumption of meiosis in oocytes enclosed by these follicles. These findings suggest that rat oocyte maturation is coupled to activation of the adenylate cyclase sys-

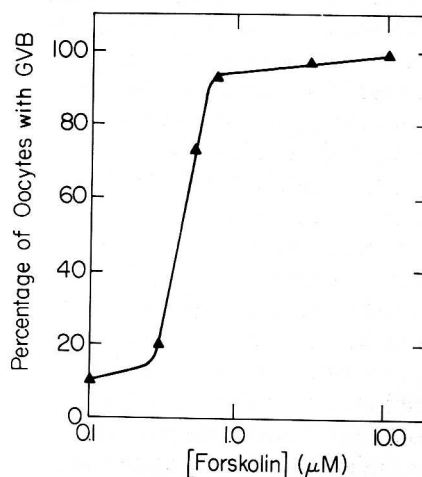


Fig.2. Concentration dependence of forskolin-induced GVB in follicle-enclosed oocytes. Isolated follicles were incubated in the presence of forskolin. After 17 h cumulus-oocyte complexes were recovered and the presence of GV in the oocyte was determined. The results represent data of 3 individual expt. Over 80 oocytes were examined for each experimental point.

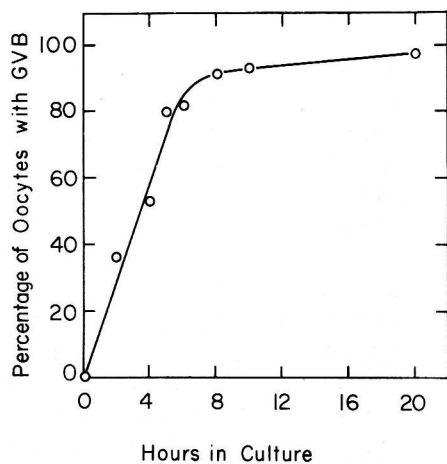


Fig.3. Time-course of forskolin-induced GV in follicle-enclosed oocyte. Isolated follicles were incubated in the presence of  $3 \mu\text{M}$  forskolin. At the indicated times cultures were terminated and cumulus-oocyte complexes were recovered and the presence of GV in the oocyte was determined. The results represent data of 4 individual experiments. Over 150 oocytes were examined for each experimental point.

tem in the follicle and that cAMP probably serves as a mediator in the induction of this process by LH. Maintenance of meiotic arrest obtained by either cAMP derivatives or cyclic nucleotide phosphodiesterase inhibitors [5,6] may derive from unphysiological effects of these cAMP modulators in this system. As it is likely that the potential of the rat oocyte to generate cAMP differs from that of the somatic cellular components of the follicle(s), external administration of the nucleotide does not necessarily represent physiological conditions. Moreover, if compartmentalization of cAMP within the cell has any role in hormonal regulation, neither administration of the nucleotide nor massive inhibition of its degradation can mimic hormonal action. Thus, as forskolin acts by activation of the cellular cAMP-generating system, it pro-

vides a more reliable tool for investigation of hormonal responses mediated by cAMP. The specific effects of forskolin on the different cellular components of the rat follicle are now being investigated.

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