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.

Abbreviations: GV, germinal vesicle; GVB, GV breakdown

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_2 and 50% N_2 [5]. Incubations were carried out at 37°C in an oscillating water bath in the presence or absence of forskolin (7β-acetoxy-8, 13-epoxy-1α, 6β,9α-trihydroxy-labd-14en-11-one, Calbiochem-

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