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Regulation of Oocyte Maturation

The Role of cAMP[a]

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INTRODUCTION

It is very well established that luteinizing hormone (LH) elicits ovarian functions by elevation of cAMP concentrations. However, the available data related to the possible involvement of cAMP in regulation of oocyte maturation are controversial, since they provide evidence for both inhibitory and stimulatory actions of the nucleotide. Tsafriri *et al.*[1] have reported that injection of a cAMP derivative into isolated rat follicles could mimic the effect of LH and induce oocyte maturation. In a later study, however, the presence of the cyclic nucleotide phosphodiesterase inhibitor, theophylline, was found to antagonize LH action on follicle-enclosed oocytes.[2] Moreover, other studies demonstrated that either membrane-permeable derivatives of cAMP or cyclic nucleotide phosphodiesterase inhibitors completely block the spontaneous maturation *in vitro* of isolated oocytes[3-5] as well as LH-induced maturation of follicle-enclosed oocytes.[6,7] These later reports demonstrate, however, that it is only the continuous presence of cAMP modulators that blocks LH-induced oocyte maturation, while a transient exposure to elevated levels of the nucleotide will, by itself, induce meiosis resumption. Is cAMP an inducer or an inhibitor of oocyte maturation? Considering that *in vivo,* following the preovulatory LH surge, both oocyte maturation and cAMP elevation are concomitantly stimulated, this question becomes even more relevant.

cAMP AS A MEDIATOR OF LH ACTION

To study the possible role of cAMP as a mediator of LH in the induction of oocyte maturation, we exposed follicle-enclosed rat oocytes to forskolin, which interacts with the catalytic subunit of the adenylate cyclase to stimulate cAMP generation.[8] In this study[9] we found that forskolin is a potent inducer of oocyte maturation. Induction of oocyte maturation by forskolin was associated with elevation of cAMP concentrations in the follicle and was potentiated by a phosphodiesterase inhibitor.[10] We sug-

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gested, therefore, that LH-induced oocyte maturation is a cAMP-mediated response. This conclusion seems to present an apparent paradox. If cAMP mediates LH action to induce oocyte maturation, since cAMP inhibits oocyte maturation,[3-5] what mechanism allows the oocyte to mature under the influence of LH?

DISSOCIATION BETWEEN THE STIMULATORY AND INHIBITORY ACTION OF cAMP

In an attempt to resolve the puzzle mentioned above we have tried, in a recent study, to dissociate the inhibitory from the stimulatory effect of cAMP on rat oocyte maturation. Specifically, experiments were designed to find out whether maturation is induced by cAMP concentrations that are different from that required to block this process.[11]

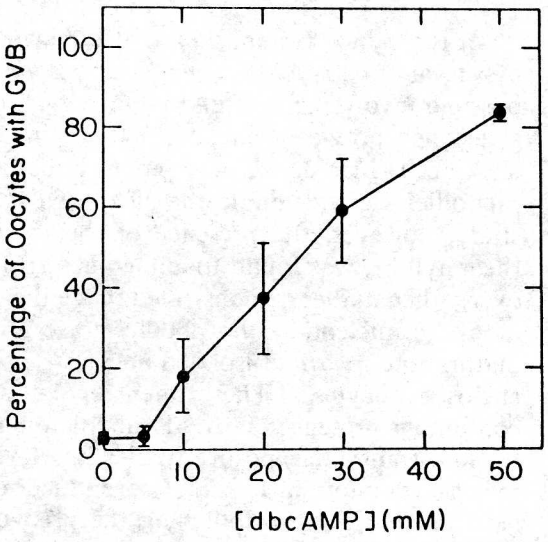

FIGURE 1. Dose-response of the induction of follicle-enclosed oocyte maturation by dbcAMP. Follicles were incubated in the presence of the indicated concentrations of dbcAMP. After 20 hours the follicles were rinsed (× 4) and further incubated for five hours in dbcAMP-free medium. The oocytes were recovered and analyzed for maturation by Nomarski interference contrast microscopy. Resumption of meiosis was indicated by the absence of the germinal vesicle (GV) in the individual oocytes. For each study, the data of several individual experiments were combined and the results are reported as the means ± SE of the fraction of oocytes with GV breakdown (GVB). (From Dekel *et al.*[11] Used with permission.)

To induce maturation, we employed the method described by us previously, by which oocytes enclosed by their follicles were transiently exposed to either dibutyryl cAMP (dbcAMP) or to the phosphodiesterase inhibitor methylisobutylxanthine (MIX).[7] Inhibition of maturation was obtained by the addition of these agents to either follicle-enclosed oocytes incubated in the presence of LH or isolated cumulus-free oocytes that mature spontaneously *in vitro*. Our results indeed demonstrated that the concentrations of either dbcAMP or MIX, which are needed for induction of meiosis resumption (FIGS. 1,2), are substantially higher than those required for inhibition of oocyte maturation (FIGS. 3,4). We therefore suggest that cAMP plays a dual role in regulation of oocyte maturation. Basal levels of cAMP result in maintenance of meiotic arrest, while elevated, LH-stimulated levels of the nucleotide mediate the induction of oocyte maturation.

It is probably not only the changes in concentrations of cAMP but also the different target cells for the nucleotide that generate the opposite responses. The follicle represents a heterologous system composed of two entirely different cell populations: the somatic follicular cells and the oocyte. It is of course possible that responses to

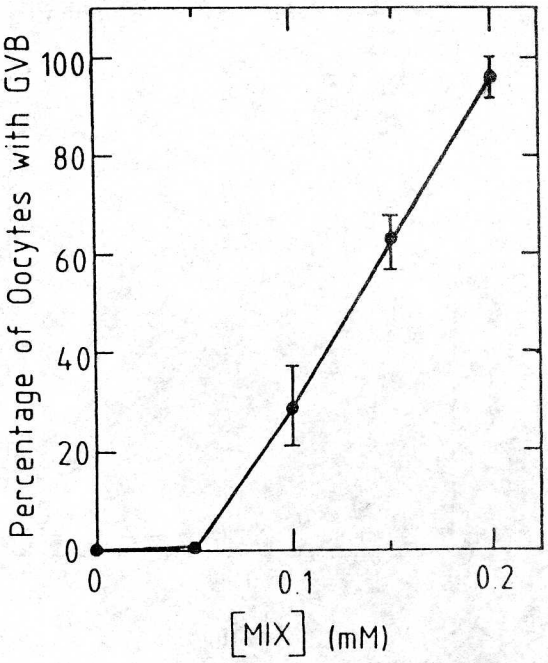

FIGURE 2. Dose-response of the induction of follicle-enclosed oocyte maturation by MIX. Follicles were incubated in the presence of the indicated concentrations of MIX. After 20 hours the follicles were rinsed and further incubated for five hours in MIX-free medium. The oocytes were recovered and examined for maturation as described in legend to FIGURE 1. (From Dekel *et al.*[11] Used with permission.)

hormones and other agents are limited to one cell type and absent in the other. Indeed, even though dbcAMP and MIX as well as forskolin induce cAMP accumulation in the intact follicle,[9] they differ in their action on the isolated cumulus-free oocyte. While both dbcAMP and MIX block maturation of isolated cumulus-free oocytes,[5] forskolin failed to elicit any inhibitory response.[12] The failure of forskolin to inhibit maturation of isolated, denuded oocytes suggests that, in terms of cAMP accumulation, the oocyte is probably not a target cell for forskolin action. Forskolin-induced cAMP elevation, in the follicle,[9] reflects therefore the specific response of the somatic cells. Thus, induction of follicle-enclosed oocyte maturation by forskolin is probably mediated through the somatic cells of the follicle. The absence of LH receptors on the oocyte[13] and the lack of response of the isolated cumulus-free oocyte to this

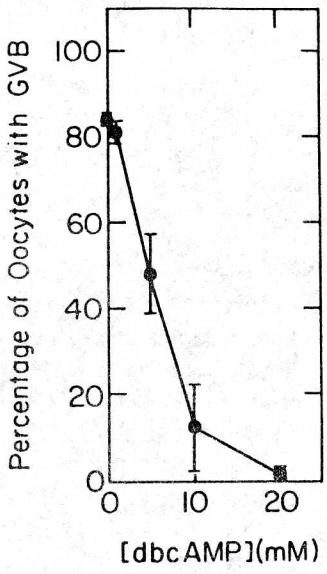

FIGURE 3. Dose-response of the inhibitory action of dbcAMP on LH-induced maturation in follicle-enclosed oocytes. Follicles were incubated in the presence of 5 μg/ml of oLH with or without the indicated concentrations of dbcAMP. After 20 hours of incubation, oocytes were recovered and analyzed for maturation as described in legend to FIGURE 1. (From Dekel *et al.*[11] Used with permission.)

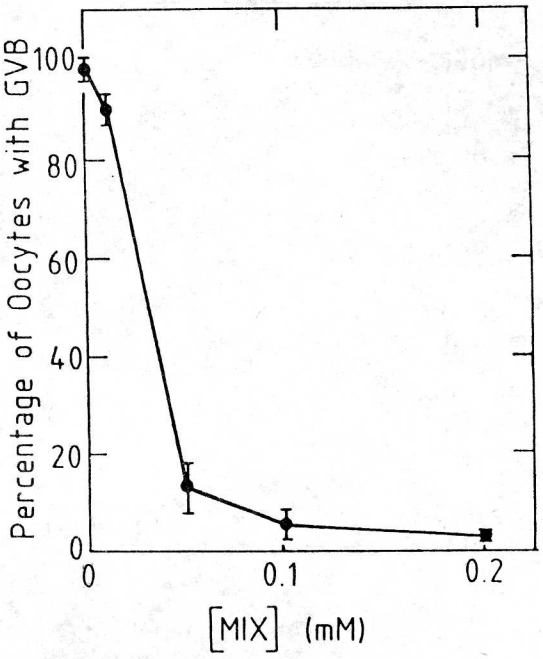

FIGURE 4. Dose-response of the inhibitory action of MIX on LH-induced maturation in follicle-enclosed oocytes. Follicles were incubated in the presence of 5 μg/ml of oLH with or without the indicated concentrations of MIX. After 20 hours of incubation, oocytes were recovered and examined for maturation as described in legend to FIGURE 1. (From Dekel *et al.*[11] Used with permission.)

gonadotropin[5] suggest that LH action, like forskolin action that induces oocyte maturation, is also mediated through the somatic follicular cells. As both dbcAMP and MIX elicit a direct inhibitory effect on the isolated oocyte,[4,5] it seems quite obvious that in the presence of these cAMP modulators maturation does not occur. The positive trigger that these agents provide by elevating cAMP levels in the follicular cells could therefore be demonstrated only upon their removal. Thus, induction of oocyte maturation by dbcAMP and MIX could be obtained only upon a transient exposure to these agents,[7] while continuous presence of forskolin did not interfere with its stimulatory action.[9]

Collectively, our earlier findings and later results suggest that when the oocyte is the target for cAMP, lower levels of the nucleotide are sufficient to induce inhibitory action. At higher cAMP concentration it is the response of the somatic cells to the nucleotide that leads to oocyte maturation.

MECHANISM OF cAMP ACTION

What is the mechanism by which the follicular cells mediate cAMP action to induce oocyte maturation? Our earlier studies indicate that maintenance of meiotic arrest depends on the integrity of the cumulus-oocyte complex that probably permits continuous transfer of the inhibitory cAMP to the oocyte.[12,14] It is therefore possible that uncoupling of the oocyte from the cumulus cells will decrease the flow of the nucleotide under the threshold level required to maintain meiotic arrest and lead to meiosis resumption. We have earlier demonstrated that cAMP indeed interrupts communication in the cumulus-oocyte complex.[7] In a more recent study the timing and dose of MIX that uncouples the oocyte from the cumulus cells was analyzed.[11] We found that follicle-enclosed oocytes remained fully coupled to their cumulus cells

following 20 hours of incubation in control medium (FIG. 5). The dose of 0.05 m*M* of MIX that is too low to induce resumption of meiosis failed to affect the coupling in the cumulus oocyte complex. However, in the presence of a concentration of MIX that is stimulatory for oocyte maturation (0.2 m*M*), coupling of the oocyte to the cumulus cells was reduced to 10% of its initial level (FIG. 5). These results indicate that the concentrations of cAMP that interfere with coupling in the cumulus-oocyte complex correspond with those required to induce, but not to inhibit maturation of the oocyte.

SUMMARY

We suggest that cAMP is a key molecule in regulation of oocyte maturation. In the small follicle, tonic levels of cAMP are continuously transferred to the oocytes to maintain meiotic arrest. In the preovulatory follicle, in response to LH, cAMP levels are elevated. These increased concentrations of the nucleotide affect the cumulus cells and interrupt communication in the cumulus-oocyte complex. Under these conditions the flow of cAMP to the oocyte declines, inhibition is relieved, and meiosis is resumed.

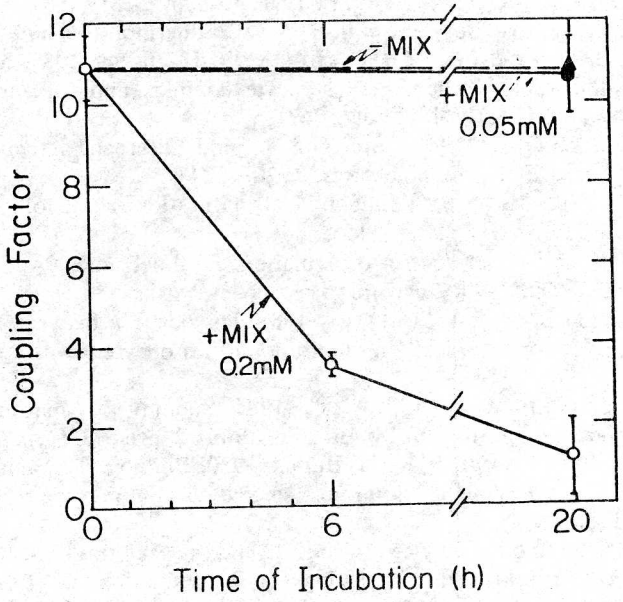

FIGURE 5. Uncoupling of the cumulus-oocyte complex by MIX. Follicles were incubated in the absence or the presence of the indicated MIX concentrations. At the indicated times cumulus-oocyte complexes were recovered and transferred to [^{3}H]uridine-containing control medium for further incubation of one hour followed by an extensive rinse of the labeled marker. Some of the cumulus-oocyte complexes were transferred directly to scintillation vials, dissolved, and counted to obtain a value for total uptake of radioactivity by the cumulus-oocyte complexes. Another group of complexes was mechanically treated to remove the cumulus cells. The denuded, cumulus-free oocytes were then transferred to scintillation vials, dissolved, and counted to obtain a value for the amount of radioactive marker transferred to the oocytes. The coupling factor is expressed as the amount of uridine transferred to cumulus-enclosed oocytes as a fraction of total cumulus uptake. (From Dekel *et al.* Used with permission.)

ACKNOWLEDGMENTS

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