Oocyte Maturation Involves Compartmentalization and Opposing Changes of cAMP Levels in Follicular Somatic and Germ Cells: Studies Using Selective Phosphodiesterase Inhibitors

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The second messenger cAMP has been implicated in the regulation of mammalian and amphibian oocyte maturation. Although a decrease in intracellular levels of cAMP precedes germinal vesicle breakdown (GVBD), the gonadotropin induction of ovulation and oocyte maturation is associated with major increases of cAMP in ovarian follicles. In the mammalian system, isolated oocytes undergo spontaneous maturation in vitro but this process is blocked by treatment with a phosphodiesterase (PDE) inhibitor, IBMX, which increases intracellular cAMP levels. In contrast, the same inhibitor, when added to cultured follicles for a brief time, increases follicle cAMP levels, followed by the induction of GVBD. To resolve the paradoxical actions of this PDE inhibitor on the maturation of isolated and follicle-enclosed oocytes, we hypothesized that meiotic maturation requires opposing fluctuations of cAMP levels in the somatic granulosa and germ cells. Such opposing fluctuations may result from selective expression and regulation of PDEs in the somatic and germ cell compartments of the follicle. To test this hypothesis, PDE activity was manipulated in different follicular cells using type-specific inhibitors. The impact of the ensuing changes in cAMP levels in the two compartments was monitored by the induction of GVBD. In isolated oocytes, spontaneous GVBD was blocked by two inhibitors of type 3 PDE (cGMP-inhibited: CGI-PDE), milrinone and cilostamide. In contrast, treatment with an inhibitor for type 4 PDE (cAMP-specific), rolipram, was ineffective. These findings suggest that the oocyte expresses type 3 but not type 4 PDE and that increases in intracellular cAMP suppress GVBD. This hypothesis was confirmed by in situ hybridization studies with PDE3 and PDE4 probes. PDE3B mRNA was concentrated in oocytes while PDE4D was mainly expressed in granulosa cells. In cultured follicles, LH treatment induced oocyte maturation but the gonadotropin action was blocked by inhibitors of type 3 but not the type 4 PDE inhibitors. Furthermore, treatment with the type 4, but not the type 3, PDE inhibitor mimics the action of LH and induces oocyte maturation, presumably by increasing cAMP levels in granulosa cells. Our findings indicate that PDE subtypes 4 and 3 are located in follicle somatic and germ cells, respectively. Preferential inhibition of PDE 3 in the oocyte may lead to a delay in oocyte maturation without affecting the cAMP-induced ovulatory process in the somatic cells. Conversely, selective suppression of granulosa cell cAMP-PDE may enhance the gonadotropin induction of ovulation and oocyte maturation. Thus, in addition to the well-recognized differential expression and regulation of adenylyl cyclase in the somatic and germ cell compartments of the follicle, we suggest that selective regulation and expression of PDEs may be involved in the regulation of cAMP levels and control of oocyte maturation in the preovulatory mammalian follicle.

INTRODUCTION

Two major in vitro models have been employed for investigating the control of meiotic maturation in mammalian oocytes. Pincus and Enzmann (1935) demonstrated that rabbit oocytes liberated from their follicles undergo spontaneous maturation in culture, without any need for hormonal stimulation. Such spontaneous maturation of oocytes cultured either denuded of their cumulus cells or within their cumulus complex was confirmed in all mammalian species examined (reviewed by Tsafriri, 1978) and has been widely employed for studying oocyte maturation. Spontaneous maturation of mouse oocytes in vitro when released from their follicles can be reversibly blocked by addition of a derivative of cAMP or a phosphodiesterase inhibitor.
inhibitor (Cho et al., 1974). These findings, confirmed in several mammalian species (Dekel and Beers, 1978; Eppig and Downs, 1984; Schultz, 1986), led to the hypothesis that this cyclic nucleotide could serve as the physiological inhibitor involved in the maintenance of meiotic arrest (Lindner et al., 1974).

In the second model, consisting of explanted preovulatory follicles, resumption of meiosis is dependent upon gonadotropin stimulation in vivo or in vitro (Tsafiriri et al., 1972) and this effect can be mimicked by other hormones (Tsafiriri and Dekel, 1994). In contrast to the isolated oocyte model, in follicle-enclosed oocytes treated with agents that increase cAMP also leads to the resumption of oocyte maturation (Hillensjö et al., 1978b; Lindner et al., 1974; Tsafiriri et al., 1972). Thus, GVBD is induced following: (i) injection of the cAMP derivative dibutyryl cAMP (dbcAMP) into the follicular antrum (Tsafiriri et al., 1972); (ii) transient exposure of follicles to 8-bromo-cAMP (Hillensjö et al., 1978b), dbcAMP or isobutylmethylyxanthine (IBMX) (Dekel et al., 1981), and addition of forskolin to cultured follicle-enclosed oocytes (Dekel, 1986; Dekel and Sherizly, 1983; Yoshimura et al., 1992a,b). Although these findings suggest that LH-induced oocyte maturation is a cAMP-mediated response, the continuous presence of membrane-permeable cAMP derivatives or inhibitors of phosphodiesterase prevents the LH-stimulated oocyte maturation in explanted follicles (Dekel et al., 1981; Hillensjö et al., 1978a; Lindner et al., 1974; Tsafiriri et al., 1972). The apparently opposing actions of cAMP in the two models present a paradox. A rise in follicular cAMP mediates LH action to induce oocyte maturation, while intraoocyte cAMP inhibits the process. Thus far several hypotheses were proposed to account for this apparent paradox by proposing compartmentalization and differential regulation of cAMP levels in the two major compartments of the follicle: the germ cell and the somatic cells.

Cellular steady-state levels of cAMP are the result of a balance among the rate of synthesis (activity of adenylate cyclase), degradation by phosphodiesterases (PDEs), and extrusion from the cell. Since the extrusion was estimated to account for, at most, 20% of cAMP disposal (Brunton and Mayer, 1979), PDEs may play a major role in the regulation of cellular cAMP levels. There is ample evidence for the hormonal regulation of cellular PDE (reviewed by Conti et al., 1991, 1995), including the Sertoli cells (Conti et al., 1982), the ovary (Schmidtke et al., 1980), and the granulosa cells (Conti et al., 1984). Furthermore, by transfecting a cDNA coding for a PDE into hormone responsive cells, it was demonstrated that even minor changes in the rate of PDE degradation elicited a major impact on intracellular cAMP levels (Swinnen et al., 1991). The complexity of the PDE system has become apparent only recently (Beavo and Relfsnyder, 1990; Conti et al., 1991), consisting of a large family of forms. The initial cloning of cAMP-specific PDEs (cAMP-PDE: type 4) was followed by identifications of at least 25 different PDE forms in mammals. The PDEs were classified into seven distinct families (types) on the basis of their kinetic characteristics, substrate specificity, and regulation (Bentley and Beavo, 1992; Conti et al., 1995; Michaeli et al., 1993). Furthermore, at least four type 4 PDE genes are present in the rat, mouse, and human. Since several mRNA variants were characterized, it seems that more than one protein is derived from each gene (Conti et al., 1995; Monaco et al., 1994). Likewise, cDNAs for two type 3 PDEs (cGMP-inhibited: CGI-PDE) have been cloned (Macci et al., 1992; Taira et al., 1993). PDE3A mRNA is highly expressed in rat adipocytes, whereas PDE3B is highly expressed in rat cardiac tissue.

As indicated above, the nonselective PDE inhibitor IBMX has been used to dissect the role of cAMP in the resumption of meiosis. Recently, a "second generation" of PDE inhibitors, selective for various families of PDEs, have been developed (Nicolson et al., 1991). For example, rolipram inhibits the type 4 PDE with an ED50 of 0.1-0.5 μM and has an effect on the calmodulin-stimulated PDEs only in the millimolar range. Likewise, milrinone and cilostamide are much more effective in suppressing the type 3 PDE than the type 4 PDE.

In amphibian oocytes progesterone-induced maturation involves the inhibition of adenylyl cyclase activity (reviewed by Muller, 1985; Smith, 1989). More recently, the meiosis-inducing actions of insulin, IGF-1, and transforming p21 ras in Xenopus oocytes were correlated with both inhibition of oocyte adenylyl cyclase and stimulation of PDE activity (Sadler and Muller, 1987). The effect of insulin, IGF-1 and p21 ras, but not of progesterone, on oocyte maturation was inhibited by specific inhibitors of type 3 PDE, whereas inhibitors of types 4 or 5 PDEs did not affect oocyte maturation (Sadar, 1991b).

Using subtype-specific inhibitors of PDEs, we examined whether differential regulation of PDEs in the two major compartments of the mammalian follicle, i.e., the somatic cells and the oocyte, may participate in the control of meiosis. Such differential regulation of PDEs may underlie the paradoxical role of cAMP in the maintenance of meiotic arrest and/or stimulation of the resumption of meiosis during mammalian ovulation.

**MATERIALS AND METHODS**

**Animals.** Rats were from either the Department of Hormone Research Wistar-derived colony or Sprague–Dawley obtained from Simonsen Laboratories (Gilroy, CA). Animals were provided with water and rat chow ad libitum and housed in air-conditioned rooms illuminated for 14 hr/day. The results obtained using animals from both sources were indistinguishable and therefore pooled.

**Culture media and inhibitors.** Oocytes and follicles were cultured in Leibovitz's L-15 medium (L-15, Gibco, Grand Island, NY), supplemented with 5% fetal bovine serum (Gibco or Sera-Lab, Crawley Down, England), penicillin (100 units/ml), and streptomycin (100 μg/ml; Gibco). The PDE inhibitors IBMX (Sigma, St Louis, MO), rolipram, and milrinone were a gift from Syntex ( Palo Alto, CA) and cilostamide was a gift from Dr. H. Hidaka (Nagoya University, Japan). These compounds were kept in stock solution in dimethyl sulfoxide (10 mM), diluted as indicated in the medium and added at the start of the culture.

**Oocyte collection and culture.** Immature rats were injected with eCG (10 IU) between 0900 and 0930 hr on Day 25–26 of age.
in order to enhance multiple follicular development. The animals were killed 48–52 hr later by cervical dislocation. Oocytes were collected by puncturing the largest ovarian follicles with a 25-gauge needle under a dissecting microscope and exerting gentle pressure. Twenty-five to fifty oocytes, within their attached cumulus cell mass (“isolated oocytes”), were cultured in organ culture dishes (Falcon, Cockeysville, MD) for 6 hr in 1 ml of control or test media, as indicated under Results. Oocytes from three rats were pooled and distributed into, at least, three different dishes. The oocytes were collected into a medium containing IBMX (200 μM) and washed twice when transferred to the test medium. To confirm a direct action of cilostamide and milrinone on the oocyte, cumulus cells were removed by passage through a series of micropipettes, after preincubation with collagenase (“denuded oocytes”) (Piontkewitz and Dekel, 1993).

**Follicle cultures.** Immature Sprague–Dawley rats treated 2 days earlier with equine chorionic gonadotropin (eCG) (10 IU) or mature rats, which have shown two consecutive 4-day cycles immediately before the experiment, were sacrificed on the morning of the day of proestrus by cervical dislocation. Preovulatory follicles were excised under a dissection microscope as previously described (mature rats: Tsafri et al., 1972; immature rats: Dekel et al., 1983). The follicles, 7–15 per vial, were cultured for 24 hr in control medium, with LH (5 μg/ml) alone or in combination of LH and the indicated PDE inhibitors and doses. The vials were flushed at the start of the culture with O₂/N₂ (1/1), closed tightly, and gently shaken during the culture.

In order to test whether any of the PDE inhibitors is capable of inducing the resumption of meiosis in follicle-enclosed oocytes, we have employed a two-stage culture system. This consisted of an initial culture period of 18 hr with PDEs inhibitors, which was followed after a thorough washing in control medium by an additional 6-hr culture in control medium, without the inhibitors.

**Examination of oocytes.** At the end of the 6-hr culture of isolated oocytes they were collected under the dissecting microscope and distributed into, at least, three different dishes. The oocytes were fixed in 4% paraformaldehyde, and stored at 70° C for up to 1 month. Hybridization and washing of cryosections of rat ovaries were adapted from previously described methods (Chun et al., 1993). The antisense and sense probes for the different PDEs were labeled with [³²P]UTP (1000 Ci/mmol; NEN DuPont, Boston MA). The sections were hybridized under coverslips overnight at 50°C in 50% formamide, 300 mM NaCl, 10 mM Tris–HCl (pH 8.0), 5 mM EDTA, 1× Denhardt’s solution, 1 mg/ml yeast tRNA, and 10% dextran sulfate. After RNase (25 μg/ml) treatment, slides were washed to a final stringency of 0.1 SSC at room temperature. After 2–3 weeks of exposure to NTB2 emulsion (Eastman Kodak, Rochester, NY), sections were developed, counterstained, and mounted with Permoun (Fisher Scientific, Fair Lawn, NJ) for observation with bright- and dark-field illumination using a Nikon Microphot-FXA.

**RESULTS**

**Inhibition of maturation of isolated oocytes by type-selective PDE inhibitors.** In this model the spontaneous maturation of rat oocytes (72.9% GVBD; n = 312) was blocked, in a dose-dependent manner, by IBMX, a nonselective inhibitor of PDEs, as previously demonstrated, and by milrinone and cilostamide— inhibitors of the type 3 PDEs (Fig. 1). Cilostamide was more effective, with an apparent ED₅₀ of <1 μM compared to milrinone (ED₅₀ 5 μM). To rule out nonspecific toxic effects of these inhibitors, a two-stage culture was employed. After initial 3-hr culture with IBMX
(200 μM), milrinone or cilostamide (each at 10 μM), washing, and transfer to a control medium for overnight culture resulted in complete reversal of inhibitory effects of these drugs on spontaneous GVBD [96 (n = 101), 95.2 (167), and 100% (108) GVBD, respectively, compared to 95.9% (73) in control medium]. In addition, direct action of the type 3 PDEs inhibitors on the oocyte was ascertained by using oocytes denuded from their cumulus cells. At 10 μM cilostamide reduced GVBD to 20.6 ± 3.9% (n = 107), whereas milrinone reduced GVBD to 19.4 ± 4.7 (67), compared to 81.7 ± 4.9% (60) GVBD in denuded oocytes cultured without any inhibitor. By contrast, rolipram, the specific inhibitor of type 4 PDEs, did not affect the spontaneous maturation which proceeded undisturbed in its presence, even at the high dose of 500 μM (Fig. 1).

Inhibition of maturation of follicle-enclosed oocytes by type-selective PDE inhibitors. In this model, the oocytes mature only in response to hormonal stimuli. LH/hCG is the physiological trigger of the resumption of meiosis and this response was blocked when the follicles were cultured in the presence of IBMX, milrinone or cilostamide (Fig. 2A). While IBMX, as previously reported by others, was able to induce the resumption of meiosis in a two-step model (consisting of initial 18-hr culture with the inhibitor, followed by an additional 6-hr culture in a control medium), milrinone and cilostamide did not elicit the resumption of maturation in this model (Fig. 2A).

Treatment with increasing doses of rolipram (up to 5 μM), a selective inhibitor of type 4 PDE, did not inhibit the maturation of follicle-enclosed oocytes exposed to LH/hCG. In the absence of LH/hCG, rolipram stimulated the resumption of meiosis in follicles cultured without LH at doses between 0.05 and 5 μM. At concentrations of 50 μM or higher it partially inhibited LH/hCG-stimulated oocyte maturation, while still inducing the maturation of follicle-enclosed oocytes cultured in hormone-free medium. At these high concentrations, this compound was less effective in stimulating GVBD (Fig. 2B). This may be due to the fact that at these higher doses rolipram inhibits PDE3 or that cAMP from the granulosa cell compartment is transmitted to the oocyte compartment.

Expression of type 3 and type 4 PDE in somatic and germ cell compartments of the follicle. A preliminary experiment using PCR demonstrated that PDE3 and PDE4 mRNAs are present in the rat follicle (data not shown). To determine the exact site of expression of the PDE3 and PDE4 in the somatic and germ cell compartments of the follicle, in situ hybridization was performed on section of ovaries of eCG±hCG-treated animals. A probe specific for PDE3B hybridized exclusively to the oocyte (Fig. 3). No specific signal was observed on granulosa cells (Fig. 3). When a probe specific for a different PDE3 isoform, PDE3A, was used, no specific signal was detected throughout the ovary (data not shown). Completely different patterns of expression were obtained with the PDE4-specific probes. A PDE4D probe hybridized in a region corresponding to mural granulosa cells (Fig. 4). Differences where also observed in the level of the signal during follicular maturation. While large antral follicle had the strongest signal, that in small immature follicles was only marginally above background (Fig. 4). A PDE4B probe hybridized mostly to theca and interstitial cells (data not shown). No signal on oocytes was observed with either PDE4 probe (Fig. 4).

**DISCUSSION**

The use of selective inhibitors of PDEs allowed us to investigate the opposing roles of cAMP in the somatic and germ cell compartments of the mammalian follicle. Furthermore, they demonstrate that distinct types of phosphodiesterases may selectively control cAMP levels in the granulosa cells and in the oocyte and thus participate in the regulation of oocyte maturation.

Two inhibitors of type 3 PDEs, cilostamide and milrinone, consistently blocked the resumption of meiosis in both models. Rolipram, an inhibitor of type 4 PDEs, did not affect the spontaneous matura-
Phosphodiesterases and Oocyte Maturation

FIG. 3. Ovarian and follicular expression of PDE3B. In situ hybridization with the antisense probe reveals expression only in the oocytes (A, B, E) while the sense controls (C, D, F) are negative.

tion of isolated oocytes nor the maturation of follicle-enclosed oocytes triggered by hCG/LH. However, treatment with rolipram by itself effectively stimulated the maturation of follicle-enclosed oocytes cultured without hCG/LH. Thus, the effects of these selective inhibitors differed markedly from the previously published (Dekel et al., 1988, 1981) actions of the nonselective PDE inhibitor, IBMX. While IBMX consistently inhibited the maturation of isolated and follicle-enclosed oocytes as long as it was present in the culture medium, removal of IBMX from follicle-enclosed oocyte cultures revealed its ability to trigger the resumption of meiosis, like rolipram. Conversely, removal of milrinone or cilostamide, did not result in GVBD (Table 1).

These studies are consistent with the idea that the granulosa-cumulus cell compartment is endowed with the rolipram-sensitive type 4 PDEs, as already suggested in a previous study (Conti et al., 1984). The oocyte, by contrast, seems to contain the type 3 PDEs since milrinone and cilos-
tamoxifen inhibit both the spontaneous maturation of isolated oocytes and the LH–hCG-induced maturation of follicle-enclosed oocytes presumably by inhibiting oocyte PDE. The nonselective inhibitor IBMX, by increasing granulosa cell cAMP levels which is transmitted to the oocyte (Tsafiriri and Dekel, 1994) and by blocking oocyte PDE, prevents the resumption of oocyte maturation. Removal of IBMX in the two-step incubation model allows oocyte maturation to progress due to the initial rise in cAMP in the granulosa cell compartment followed by a decline in oocyte cAMP levels by local PDEs. Finally, rolipram, which inhibits only type 4 of PDEs which are localized primarily in the somatic compartment of the follicle, does not inhibit spontaneous and gonadotropin-induced maturation of oocytes because it does not affect degradation of oocyte cAMP by its type 3 PDE. Conversely, rolipram can induce the maturation of follicle-enclosed oocytes by increasing cAMP levels in the somatic cell compartment. This conclusion is supported by

FIG. 4. Ovarian and follicular expression of PDE4D. In situ hybridization with the antisense probe reveals expression mainly in the granulosa cells of small and large antral follicles (A, B, C, D) while the sense controls (E, F) are negative.
Phosphodiesterases and Oocyte Maturation

TABLE 1
Summary of the Effect of PDE Inhibitors on Spontaneous and Hormone-Induced Oocyte Maturation

<table>
<thead>
<tr>
<th>Inhibitor / Type PDE</th>
<th>Oocyte cumulus complex or demuded oocyte</th>
<th>Follicle-enclosed oocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>hCG</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>ND</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MIX / Unspecific</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Rolipram / 4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Milrinone / 3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cilostamide / 3</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- : Immature oocytes, germinal vesicle intact;
+ : Germinal vesicle breakdown; GVBD

our in situ hybridization studies showing PDE3B mRNA expressed in oocytes, while PDE4 mRNAs were expressed in somatic cells of the ovary. The localization of PDE3 to the oocyte is in agreement with the recent detection of the PDE3B mRNA in immature rat oocytes (Reinhardt et al., 1995).

Type 4 PDEs are the mammalian homologues of the Drosophila melanogaster dunce PDE. Mutations of this Drosophila PDE produce sterility of the female fly, as well as dysfunction of the central nervous system (Dudai, 1986). A causative link between mutations in the dunce gene and sterility is further demonstrated by the transgenic expression of a wild-type PDE in the dunce flies which causes a recovery of the reproductive function (Dauwalder and Davis, 1995). Careful dissection of this dunce phenotype has indicated that the reproductive failure is due to defects both in the supporting cells and in the eggs (Bellen et al., 1987). Our demonstration that PDE4 is expressed and active in mammalian granulosa cells reiterates the important role of these enzymes in follicle function and resumption of meiosis. While the mutation data suggest that the dunce PDE plays a role in the Drosophila egg, our data indicate that type 4 PDEs are not expressed, or are mostly inactive, in mammalian oocytes. Since Drosophila does not express a PDE with the properties of a PDE3, it is possible that PDE3 has appeared later during evolution and has taken on some of the functions of the dunce PDE. It is also intriguing that PDE3 appears to be a major form also expressed during meiosis in male germ cells (Reinhardt et al., 1995; our unpublished observation). In mammalian adipocytes, PDE3 is regulated by insulin, IGF-1, and adrenergic agonists via kinase-dependent phosphorylations (Manganiello et al., 1990). Whether similar signals regulate the PDE3 expressed in the mammalian oocytes remains to be determined.

In amphibian oocytes progesterone or progesterone-like steroids serve as the meiosis-inducing hormone produced by follicle cells and act through receptors on the oocyte membrane (Maller, 1985; Smith, 1989). The action of these steroids involves the inhibition of oocyte adenylyl cyclase activity. In more recent studies the meiosis-inducing actions of insulin, IGF-1, and transforming p21 ras in Xenopus oocytes were correlated with both inhibition of oocyte adenylyl cyclase and stimulation of its PDE activity (Sadler and Maller, 1987). The effect of insulin, IGF-1, and p21 ras, but not of progesterone, on oocyte maturation was inhibited by type-specific inhibitors of PDE3, whereas inhibitors of types 4 or 5 did not affect oocyte maturation (Sadler, 1991a). Progesterone appears to exert its meiosis-inducing action in Xenopus oocytes through another isoform of PDE (Sadler and Maller, 1989). Thus, in mammalian oocytes, as in these of amphibians, selective regulation of the activities of specific PDEs in the ovarian follicle by gonadotropins and other factors inducing resumption of meiosis (reviewed by Tsafirri and Dekel, 1994) may lead to the reduction in oocyte cAMP levels in face of its rise in the adjacent somatic cells.

There are notable differences between the regulation of meiosis in amphibian and mammalian oocytes: (i) In amphibians, steroids serve as the signal molecule transmitted from follicle cells and the oocyte to induce maturation. In mammals, inhibition of steroidogenesis did not affect the meiosis-inducing action of gonadotropins (Lindner et al., 1974). Nevertheless, indirect evidence for such a positive signal has been accrued (reviewed by Downs, 1995) but its molecular nature in mammals remains to be identified. (ii) Adenylate cyclase is active in amphibian oocytes and in most cases produces sufficient cAMP to suppress the resumption of meiosis. By contrast, in isolated mammalian oocytes cAMP levels are insufficient to prevent the spontaneous resumption of meiosis, due to the low activity of adenylate cyclase and the rapid degradation of cAMP by

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PDE. It was suggested, therefore, that in follicle-enclosed oocytes inhibitory levels of cAMP are maintained due to gap junctional transport from the somatic compartment. In view of the preferential compartmentalization of adenylate cyclase within the mammalian follicle to the somatic compartment (Sherizly et al., 1988; Tsafriri and Dekel, 1994) it is possible that the PDEs present in the amphibian oocyte became partitioned between the germinal and somatic compartments of the mammalian follicle.

Evidence for differential production of cAMP in mammalian follicular compartments as well as divergent sensitivity to cAMP and transport from the somatic cells to the oocyte has been obtained (see Tsafriri and Dekel, 1994). Oocyte maturation was induced in follicle-enclosed oocytes transiently exposed to either dibutyryl cAMP or IBMX. Inhibition of maturation was obtained by addition of the above agents to either follicle-enclosed oocytes incubated in the presence of LH or isolated cumulus-free oocytes that mature spontaneously in vitro. Incubation of isolated oocytes in medium containing IBMX prevents both the spontaneous maturation and the decrease in intraoocyte cAMP levels (Vivarelli et al., 1983). These findings indicate that, regardless of its limited potential to generate cAMP, the follicular oocyte does contain inhibitory levels of this nucleotide. Based on the close anatomical relationships between the oocyte and the cumulus cells, it was suggested that cumulus cell projections can serve as channels for communication of the follicular cAMP which maintains the oocyte in meiotic arrest (Dekel and Beers, 1978; Dekel et al., 1981; Lindner et al., 1974). Because of its size, cAMP can be transferred via the gap junctions that are known to be present in the regions of contact between cumulus cell projections and oocyte surface and demonstrated to be diminished following the stimulation of ovulation (Larsen et al., 1987, 1986; Racowsky et al., 1989). Furthermore, almost threefold higher levels of cAMP were found in oocytes that were derived from forskolin-stimulated cumulus–oocyte complexes compared to similarly stimulated cumulus-free oocytes (Dekel, 1987; Sherizly et al., 1988). Likewise, activators of adenylate cyclase delayed the spontaneous maturation of oocytes cultured within their cumuli but failed to affect the oocytes in the absence of the attached cumulus cells (Dekel et al., 1984). Taken together, the results of these experiments suggest that cAMP, generated by the cumulus cells, is transferred to the oocyte to maintain its meiotic arrest. From the data presented above it appears that elevated levels of cAMP in the oocyte are associated with arrest of meiosis. Therefore, induction of oocyte maturation by LH should lead to a drop in the supply of cAMP to the oocyte. When the supply of cAMP from the somatic compartment is blocked, oocyte levels of cAMP level off through the action of PDE. Indeed, heptanol, a seven-carbon alcohol that blocks cell to cell communication in various experimental systems, including the cumulus–oocyte complex, reduces intraoocyte concentrations of cAMP and promotes maturation of rat follicle-enclosed oocytes (Dekel and Piontkewitz, 1991; Piontkewitz and Dekel, 1993). These findings suggest that breakdown of communication between the somatic cell compartments and the oocyte can be sufficient for the resumption of oocyte maturation. Most probably, this action of heptanol is exerted through the constitutive activity of oocyte PDE. It remains to be determined whether hormonal induction of ovulation and oocyte maturation includes upregulation of oocyte PDE expression and activity.

Recently, differential regulation of cAMP-dependent protein kinase (PKA) isoforms was reported in mouse oocytes and granulosa cells. Only the type I regulatory subunit was present in oocytes while both type I and II regulatory subunits were detected in the cumulus mass (Downs and Hunzicker-Dunn, 1995). Furthermore, using site-selective cAMP analogs activation of type I PKA led to the inhibition of meiosis, while activation of type II resulted in cumulus expansion and GVBD. Thus, differential activation of PKA in the somatic compartment of the follicle or in the oocyte may allow regulation of meiosis. It is likely that inhibition of PDE3 in oocytes activates type I PKA and thus maintains meiotic arrest, while inhibition of PDE4 in granulosa cells causes activation of type II PKA, resulting in the inhibition or stimulation of the resumption of meiosis, depending on the intraoocyte levels of cAMP, a positive signal from the somatic compartment (Downs, 1995), or another, yet unidentified, factor(s).

In conclusion, we present here evidence for differential actions of subtype-specific inhibitors of PDEs in the somatic and germ cell compartments of the rat preovulatory follicle. Selective regulation of these PDEs by gonadotropins may account for the differences in the response and sensitivity of the oocyte and the granulosa cells to the ovulatory stimulus. Thus, in addition to the differential distribution of adenylate cyclase between the somatic and germ cell compartments, selective activation of PKA isoforms, and the regulation of oocyte cAMP levels through gap junctions, selective activation of oocyte PDE may participate in the control of the resumption of meiosis.

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REFERENCES


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