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ARTICLE *in* GAMETE RESEARCH · JULY 1989

DOI: 10.1002/mrd.1120230309 · Source: PubMed

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Maintenance of Meiotic Arrest in Mouse Oocytes by Purines: Modulation of cAMP Levels and cAMP Phosphodiesterase Activity

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Hypoxanthine and adenosine are present in preparations of mouse ovarian follicular fluid, and these purines maintain mouse oocytes in meiotic arrest in vitro (Eppig et al.: *Biology of Reproduction* 33:1041–1049, 1985). The first hypothesis tested in this study is that purines which maintain meiotic arrest act by maintaining meiosis-arresting levels of cyclic adenosine monophosphate (cAMP) in the oocyte. Oocyte-cumulus cell complexes were incubated in control medium (no added purines), or medium containing 0.75 mM adenosine, 4 mM hypoxanthine, or both for 3 hr and the percentage of the oocytes that underwent germinal vesicle breakdown (GVB) and the cAMP content of the intact complexes and the oocytes were determined. Adenosine alone had little inhibitory effect on GVB at this time point but sustained higher levels of cAMP in the oocytes. Hypoxanthine maintained 80% of cumulus cell-enclosed oocytes in meiotic arrest and also sustained higher cAMP levels in the oocytes. The additon of adenosine to hypoxanthine-containing medium increased the percentage of oocytes maintained in meiotic arrest, and increased the amount of cAMP in the oocytes above that maintained by either hypoxanthine or adenosine alone. Neither hypoxanthine, adenosine, nor hypoxanthine plus adenosine altered the cAMP content of intact complexes when assayed after 3 hr culture. Microinjection of an inhibitor of the catalytic subunit of cAMP-dependent protein kinase induced GVB in denuded oocytes cultured in medium containing hypoxanthine. This purine, therefore, maintained meiotic arrest by sustaining elevated cAMP levels within the oocytes.

The second hypothesis tested in this study is that purines maintain meiosis-arresting levels of cAMP, at least in part, by inhibiting cAMP phosphodiesterase activity. In descending order of potency, 3-isobutyl-1-methylxanthine (IBMX), guanosine, hypoxanthine, adenosine, and xanthosine inhibited cAMP phosphodiesterase in oocyte lysates. Moreover, like the potent phosphodiesterase inhibitor IBMX, hypoxanthine augmented the

Received December 8, 1988; accepted January 31, 1989.

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meiotic arrest and cAMP accumulation mediated by follicle-stimulating hormone (FSH) in intact complexes. Therefore, inhibition of oocyte phosphodiesterase appears to be one mechanism by which the purines could maintain meiosis-arresting levels of cAMP.

Key words: oocyte maturation, hypoxanthine, adenosine, cAMP metabolism

INTRODUCTION

There is abundant evidence that cyclic adenosine monophosphate (cAMP) plays an important role in maintaining meiotic arrest in fully grown mammalian oocytes. Germinal vesicle breakdown (GVB) in mouse oocytes is preceded by a decline in oocyte cAMP [Schultz et al., 1983], and cAMP analogs or substances that promote cAMP production in the oocyte prevent the spontaneous maturation of oocytes in vitro [Cho et al., 1974; Wassarman et al., 1976; Magnusson and Hillensjo, 1977; Dekel and Beers, 1978; Urner et al., 1983; Bornslaeger and Schultz, 1985; Racowsky, 1985a,b]. Moreover, the adenylate cyclase produced by the bacterium *Bordetella pertussis,* which is invasive to mammalian cells, increases the cAMP levels in cumulus cell-denuded rat oocytes and maintains them in meiotic arrest [Aberdam et al., 1987]. In addition, cAMP phosphodiesterase (PDE) inhibitors such as IBMX and theophylline [Chasin and Harris, 1976] maintain oocytes in the immature germinal vesicle stage, and this has been interpreted to be due to an inhibitory action on oocyte cAMP PDE [Cho et al., 1974; Magnusson and Hillensjo, 1977]. Indeed, Bornslaeger et al. [1984] demonstrated that the ability of several PDE inhibitors to maintain mouse oocytes in meiotic arrest correlates with the drugs' ability to inhibit the cAMP PDE activity in oocyte lysates. Further convincing evidence that cAMP in the oocyte maintains meiotic arrest comes from the observation that microinjection of an inhibitor of the catalytic subunit of cAMP-dependent protein kinase induces the maturation of mouse oocytes cultured in medium containing phosphodiesterase inhibitors or membrane-permeable analogs of cAMP [Bornslaeger et al., 1986].

Hypoxanthine is present in preparations of ovarian follicular fluid from both pigs [Downs et al., 1985] and mice [Eppig et al., 1985], and this purine maintains mouse oocytes in meiotic arrest in vitro. In fact, hypoxanthine and adenosine, which is also present in mouse follicular fluid, interact in a synergistic manner to prevent spontaneous maturation in culture [Eppig et al., 1985]. Other evidence indicates that purines are also of critical importance in maintaining mouse oocytes in the germinal vesicle stage in vivo [Downs and Eppig, 1987].

The evidence implicating cAMP in the maintenance of meiotic arrest, combined with the inhibitory action of purines on oocyte maturation, raises the possibility that purines act by modulating cAMP metabolism in the oocyte. The aim of this project was to determine if 1) hypoxanthine and/or adenosine affect the amount of cAMP in cultured oocyte-cumulus cell complexes and cumulus cell-enclosed oocytes, 2) cAMP mediates the action of hypoxanthine in maintaining meiotic arrest, and 3) meiosis-arresting purines can function as inhibitors of cAMP PDE activity in oocytes.

To address these questions, we have determined whether 1) maintenance of meiotic arrest in oocytes cultured in medium containing hypoxanthine and/or adenosine is correlated with higher levels of cAMP in oocyte-cumulus cell complexes and in cumulus cell-enclosed oocytes, 2) microinjection of an inhibitor of cAMP

dependent protein kinase induces GVB in oocytes cultured in medium containing hypoxanthine, 3) meiosis-arresting purines inhibit cAMP PDE activity in lysates of oocytes, and 4) hypoxanthine augments the action of follicle-stimulating hormone (FSH) on cAMP levels in oocyte-cumulus cell complexes. The results show that hypoxanthine and adenosine regulate the meiotic state of the oocyte in vitro by modulating cAMP metabolism, although the possiblity of additional modes of action of purines is not excluded.

MATERIALS AND METHODS

Culture System

(C57BL/6J × SJL/J)F_1 female mice were used for all experiments. Immature mice, 19–22 days old, received an intraperitoneal injection of 5 IU pregnant mare serum gonadotropin (Organon, Netherlands) and were killed 48 hr later by cervical dislocation. Ovaries were removed, placed in medium, and cumulus cell-enclosed oocytes were isolated by puncturing antral follicles with sterile 25 gauge needles. Denuded oocytes were obtained by gentle repeated pipetting with a sterile Pasteur pipet. The medium used for all experiments was Eagle's minimum essential medium (MEM) with Earle's salts, supplemented with 0.23 mM pyruvate and 3 mg/ml crystallized bovine serum albumin (Miles Scientific, Naperville, IL).

cAMP and Oocyte Maturation

The cAMP content of intact oocyte-cumulus cell complexes and oocytes derived from complexes (cumulus cell-enclosed oocytes that were intact during the treatment period but denuded before assay) was determined by radioimmunoassay. Complexes were collected after isolation from follicles and washed three times in MEM containing the appropriate addition for each treatment group. The complexes were then transferred in a volume of 10 μl to 1 ml of test medium in stoppered glass 12 × 75 mm tubes gassed with a humidified mixture of 90% N_2, 5% CO_2, and 5% O_2 and incubated for 3 hr in a water bath maintained at 37°C. After incubation, complexes were washed 4 times in MEM containing 0.2 mM 3-isobutyl-1-methylxanthine (IBMX), to prevent cAMP catabolism, and transferred in 10 μl of medium to 100 μl 0.1 N HCl and stored at −20°C until assayed. For some experiments, after complexes were cultured 3 hr, cumulus cells were removed, and the resulting denuded oocytes assayed for cAMP; hereafter these are referred to as cumulus cell-enclosed oocytes, but the cumulus cells were coupled to the oocyte only during the treatment period. At least 100 oocytes were assayed per treatment group. In experiments with complexes, 25 were assayed for groups without FSH and 5 for groups with FSH. Blanks were obtained from the last wash dish for each treatment. Samples were dried under reduced pressure and redissolved in 100 μl 0.05 M sodium acetate buffer, pH 6.2. cAMP was measured by radioimmunoassay according to the method of Brooker et al. [1979]. A highly specific antibody [Reddoch et al., 1986] was generously provided by Dr. D.T. Armstrong, MRC Group in Reproductive Biology, University of Western Ontario (London, Canada). Inter- and intra-assay coefficients of variation were 11.9% and 8.4%, respectively. Some experiments were designed to correlate complex and oocyte cAMP with meiotic maturation. At the end of the 3 hr incubation, oocytes were examined for the presence or absence of a GV. The presence

of the GV was used as an indicator that meiotic arrest had been maintained by the experimental protocol.

Effect of Microinjection of Protein Kinase Inhibitor (PKI)

Oocyte-cumulus cell complexes were isolated in medium containing 4 mM hypoxanthine, denuded of their cumulus cells, and cultured for 2–4 hr in medium containing hypoxanthine. GV-stage oocytes were then microinjected, in medium containing hypoxanthine, with approximately 10 pl of either the PKI solution or buffer. The PKI, obtained from James Maller, was purified from rabbit skeletal muscle and dissolved at a concentration of 0.65 mg/ml in 5 mM MES buffer, pH 7.0. The injected oocytes were cultured for 2.5 hr in 0.5 ml of hypoxanthine-containing medium under paraffin oil, and then assessed for GVB.

Oocyte cAMP PDE Activity

Cumulus cell-enclosed oocytes were collected and denuded in MEM containing 0.2 mM IBMX (Aldrich Chemical Co., Milwaukee, WI) to prevent GVB. Oocytes were washed three times in this medium and transferred to a final wash in isotonic saline containing 1.2 mM KH_2PO_4, 4.9 mM KCl, and 25 mM HEPES (pH 7.3; Sigma, St. Louis, MO). Oocyte extracts were prepared by repeated freezing and thawing on dry ice and incubated for 1 hr at 30°C with various concentrations of test compounds in 50 μl of assay buffer containing 40 mM Tris-HCl (pH 7.2), 1 mM mercaptoethanol, 5 mM $MgCl_2$, 1 mg/ml BSA, and (2,3-^{3}H)cAMP (41.7 Ci/mmol; Amersham International, Amersham, UK). Each incubation contained the extracts of 10 oocytes. PDE activity was assayed by the two-step method of Thompson et al. [1979] modified for oocytes by Bornslaeger et al. [1984].

Chemicals

Hypoxanthine, adenosine, guanosine, and xanthosine were purchased from Sigma Chemical Co. (St. Louis, MO). Ovine FSH (NIADDK-oFSH-16; with a potency approximately 20 × NIH-FSH-S1 and luteinizing hormone (LH) contamination 0.04 × NIH-LH-S1) was generously provided by the National Institute of Arthritic, Diabetes, and Digestive and Kidney Diseases and the National Hormone and Pituitary Program (Bethesda, MD). A heat-stable inhibitor of cAMP-dependent protein kinase inhibitor (PKI) purified from rabbit skeletal muscle was generously provided by Dr. James L. Maller, University of Colorado Health Science Center.

Analysis of Data

Effects of treatments on oocyte and oocyte-cumulus cell complex cAMP content were assessed by analysis of variance (ANOVA) and when the ANOVA indicated significant differences, the treatments were compared by paired t-tests. Effects of treatments on oocyte maturation were determined using chi-square analysis. All statistical tests were performed as described by Steel and Torrie (1980) and significance inferred at $P < 0.05$.

RESULTS

Effect of Hypoxanthine and Adenosine on Oocyte and Oocyte-Cumulus Complex cAMP Content and Oocyte Maturation In Vitro

In analyzing the effects of hypoxanthine and adenosine on cAMP levels and GVB, two different groups were assayed after 3 hr of incubation: intact oocyte-cumulus cell complexes and cumulus cell-enclosed oocytes (cultured with an intact cumulus oophorus but assayed for cAMP content after removing the cumulus cells). Hypoxanthine and adenosine were used at 4 and 0.75 mM, respectively, because they were estimated to be present in preparations of mouse follicular fluid at these concentrations [Eppig et al., 1985]. Moreover, this concentration of hypoxanthine maintains mouse oocytes in meiotic arrest, and adenosine at this concentration augments the action of hypoxanthine on meiotic arrest [Eppig et al., 1985].

The cAMP content of intact oocyte-cumulus cell complexes was the same whether they were incubated in control medium, or medium containing either hypoxanthine or adenosine alone, or together (Fig. 1A). In contrast, when compared to control levels, the cAMP content of the cumulus cell-enclosed oocytes was higher after incubation in medium containing either adenosine or hypoxanthine alone, and still higher after incubation in these two purines together (Fig. 1B). Although the cAMP content of cumulus cell-enclosed oocytes was higher after incubation in medium containing adenosine than in control medium, adenosine alone failed to maintain the oocytes in meiotic arrest when assesed after 3 hr incubation. Hypoxanthine, on the other hand, permitted only 20% of the oocytes to undergo GVB. Only 2% of the cumulus cell-enclosed oocytes underwent GVB when cultured in medium containing both adenosine and hypoxanthine (Fig. 1B).

Effect of Microinjection of an Inhibitor of the Catalytic Subunit of cAMP-Dependent Protein Kinase (PKI) Into Denuded Oocytes in Medium Containing Hypoxanthine

Although the results described above suggest a correlation between elevated cAMP levels in oocytes and the maintenance of meiotic arrest by hypoxanthine, the results do not clearly establish that hypoxanthine maintains meiotic arrest by sustaining meiosis-arresting levels of cAMP in the oocyte. To address this question, denuded oocytes cultured in medium containing 4 mM hypoxanthine were microinjected with PKI. If the PKI induced maturation of oocytes cultured in medium containing hypoxanthine, this would mean that hypoxanthine maintains meiotic arrest by a mechanism that is mediated by the cAMP-dependent protein kinase and, therefore, cAMP. Controls were microinjected with an equal volume of MES buffer. Sixty-four percent ($n = 64$) of the oocytes injected with PKI and cultured in the presence of hypoxanthine underwent GVB by 2.5 hr after injection and continued culture in medium containing hypoxanthine. Only 8% ($n = 52$) of the buffer-injected oocytes underwent GVB.

Inhibition of Oocyte PDE Activity by Purines and IBMX

The experiments shown above indicate that purines that maintain meiotic arrest in vitro function, at least in part, by maintaining meiosis-arresting levels of cAMP within the oocytes. Since some purines have been shown to inhibit cAMP PDE in other cell systems [Chasin and Harris, 1976], we assessed the effectiveness of purines

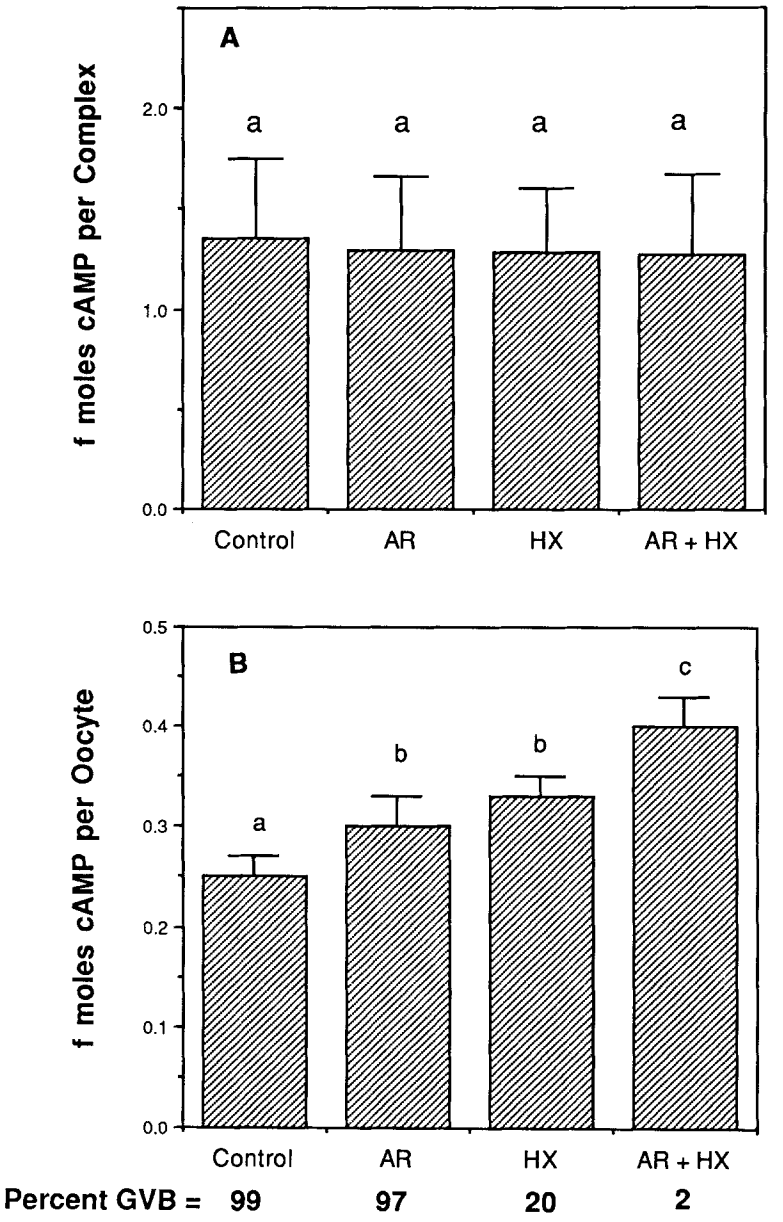

Fig. 1. Effect of adenosine and hypoxanthine on cAMP and meiotic maturation in intact oocyte-cumulus cell complexes and cumulus cell-enclosed oocytes. Complexes were incubated in medium containing 0.75 mM adenosine (AR), 4 mM hypoxanthine (HX), both purines, or in control medium without purines for 3 hr. Groups marked with different letters over the bars are significantly different from the other groups. **A:** The cAMP content of intact oocyte-cumulus cell complexes. **B:** The cAMP content of cumulus cell-enclosed oocytes when the cumulus cells were removed after the culture period and the percentage of the oocytes that had undergone GVB is indicated below the group designation. Values are the means and the bars are the standard errors of the means of 4 and 3 experiments, respectively, for A and B.

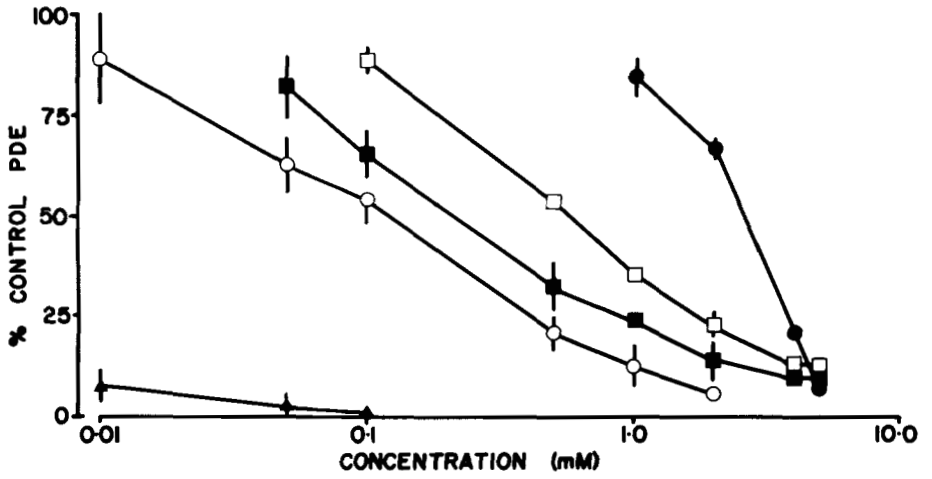

Fig. 2. Effects of IBMX (▲), guanosine (○), hypoxanthine (■), adenosine (□), and xanthosine (●) on cAMP PDE activity in oocyte lysates.

to inhibit PDE activity in lysates of mouse oocytes. Figure 2 shows that all compounds tested inhibited oocyte PDE activity in a dose-dependent manner. IBMX was the most potent inhibitor and xanthosine, which is not an effective inhibitor of oocyte maturation [Downs et al., 1985], was the least effective inhibitor of PDE activity. Guanosine was slightly more potent that hypoxanthine, and adenosine was less effective than hypoxanthine.

Comparison of Hypoxanthine and IBMX for Their Ability to Augment cAMP Accumulation Stimulated by FSH

While the experiments described above indicate the capacity of meiosis-arresting purines to inhibit PDE activity in oocyte lysates, they do not show whether they function as PDE inhibitors in intact cells. IBMX, a potent PDE inhibitor, often augments the ability of adenylate cyclase stimulators to elevate intracellular cAMP levels. Therefore, abilities of hypoxanthine and IBMX to augment cAMP accumulation in intact complexes treated with the adenylate cyclase stimulator FSH were compared. The cAMP level in oocyte-cumulus cell complexes declined from 1.88 fmol to 1.35 fmol per complex after a 3 hr culture in control medium (Fig. 3). Although hypoxanthine alone did not prevent this decline in the cAMP content of complexes, IBMX alone mantained the cAMP content at the same level as in the freshly isolated complexes (Fig. 3). FSH alone produced a 5.5-fold increase in cAMP over the freshly isolated level, and the addition of hypoxanthine or IBMX to FSH-containing culture medium produced 1.8–2.9-fold increases, respectively, in cAMP levels in oocyte-cumulus cell complexes compared to FSH alone.

DISCUSSION

The concentrations of purines used in the present study were those reported in mouse follicular fluid [Eppig et al., 1985]. Although care was taken to prevent artifactual production of purines during the assay procedure [Eppig et al., 1985],

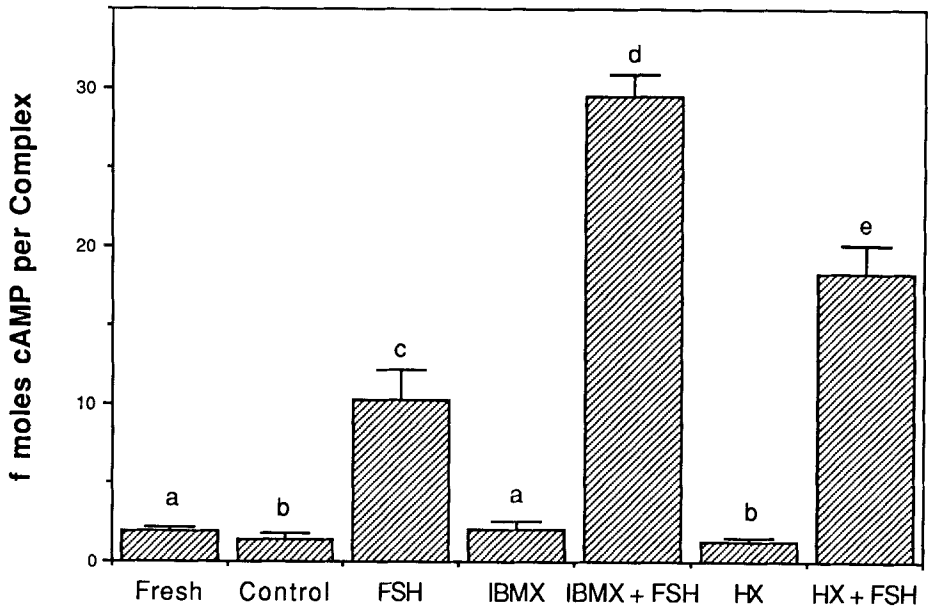

Fig. 3. Effect of IBMX and hypoxanthine on FSH-stimulated cAMP levels in intact oocyte-cumulus cell complexes. Complexes were incubated for 3 hr before assay. The concentrations of the various test substances was as follows: FSH, 100 ng/ml; IBMX, 200 nM; hypoxanthine, 4 mM. Values are the means and the bars are the standard errors of the means of 4 experiments. Groups with different letters above the bars are significantly different.

these concentrations are higher than those reported in most tissues. It is therefore possible that estimates of purine levels in mouse follicular fluid exceed those the oocyte normally encounters within the follicle. However, this possibility does not lessen the potential physiological importance of purines in the maintenance of meiotic arrest. Purines are indeed present in follicular fluid and maintain meiotic arrest in vitro [Downs et al., 1985; Eppig et al., 1985]. In addition, perturbants of purine metabolic pathways induce the resumption of meiosis both in vitro [Downs et al., 1986] and in vivo [Downs and Eppig, 1987], which implicates purines in the control of meiotic arrest. The goal of the present study was to evaluate the potential role of cAMP in mediating purine-maintained meiotic arrest.

Hypoxanthine maintained meiotic arrest in mouse oocytes in vitro and sustained higher levels of cAMP in the oocytes than did medium without purines. When oocytes were cultured in hypoxanthine-containing medium, microinjection of oocytes with an inhibitor of cAMP-dependent protein kinase induced GVB. These data strongly support the hypothesis that hypoxanthine maintains meiotic arrest by maintaining meiosis-arresting concentrations of cAMP within the oocytes. Hypoxanthine, adenosine, and guanosine inhibited cAMP phosphodiesterase activity in mouse oocyte lysates. In addition, hypoxanthine, like the potent phosphodiesterase inhibitor IBMX, augmented the cAMP-elevating action of FSH in intact oocyte-cumulus cell complexes. These data suggest that one way in which hypoxanthine could function to maintain meiosis-arresting levels of cAMP in oocytes is by preventing the hydrolysis of cAMP.

That meiotic arrest is maintained by cAMP-dependent protein phosphorylation

was demonstrated by microinjecting mouse oocytes, maintained in meiotic arrest by either dibutyryl cAMP or IBMX, with an inhibitor of cAMP-dependent protein kinase (PKI) [Bornslaeger et al., 1986]. This inhibitor induced GVB and specific changes in oocyte phosphoproteins that are associated with commitment to undergo GVB [Bornslaeger et al., 1986]. In the present study, the PKI was injected into mouse oocytes cultured in medium containing hypoxanthine to determine whether the action of this purine is mediated by a cAMP-dependent process. The PKI induced GVB and thereby clearly established that hypoxanthine maintains meiotic arrest, at least in part, by a cAMP-dependent mechanism.

To determine whether hypoxanthine, or other purines that maintain mouse oocytes in meiotic arrest in vitro, could act by inhibition of oocyte PDE, the ability of these purines to inhibit PDE activity in lysates of oocytes was determined. Consistent with previous studies [Bornslaeger et al., 1984], IBMX had a marked inhibitory effect on oocyte cAMP PDE activity and was the most potent compound tested. The purines tested were also inhibitory, and the potency of each was directly correlated with their relative abilities to maintain mouse oocytes in meiotic arrest in vitro; e.g., in descending order of effectiveness, guanosine > hypoxanthine > adenosine [cf. Downs et al., 1985]. These data are consistent with previous reports demonstrating inhibitory actions of purines on PDE from other tissues [Chasin and Harris, 1976; Oleshansky, 1980]. The PDE-inhibiting activity of xanthosine was assessed here because it is ineffective in maintaining meiotic arrest [Downs et al., 1985]; nevertheless, if added in high enough concentration, this pyrimidine nucleoside also inhibited cAMP PDE activity. The inhibitory action of guanosine, hypoxanthine and adenosine on oocyte cAMP PDE supports the idea that these compounds could maintain meiotic arrest in vitro by preventing the catabolism of cAMP, although it is likely that the purines maintain cAMP levels by other mechanisms as well.

Compounds that inhibit cAMP PDE often potentiate the action of cAMP analogs or adenylate cyclase-stimulating agents and increase the percentage of oocytes maintained in meiotic arrest. For example, the cAMP PDE inhibitors IBMX, papaverine, and dipyridamole all interact in a synergistc manner with a low concentration of the cAMP analog, dibutyryl cAMP, to maintain mouse oocytes in meiotic arrest (Downs and Eppig, unpublished data). It has also been demonstrated that hypoxanthine synergizes with FSH to maintain cumulus cell-enclosed oocytes in meiotic arrest [Downs et al., 1985]. We have shown herein that, in accord with the action of the established cAMP PDE inhibitor, IBMX, the stimulation of cAMP synthesis by FSH in oocyte-cumulus cell complexes is significantly augmented by hypoxanthine.

Neither hypoxanthine, adenosine, nor hypoxanthine plus adenosine had any detectable effect on cAMP in oocyte-cumulus cell complexes (Fig. 1A), though cAMP was elevated in oocytes from complexes cultured in medium containing either hypoxanthine or adenosine, or both. The highest cAMP levels in oocytes were usually associated with a low percentage of oocytes undergoing GVB. This suggests that the purines sustain oocyte cAMP levels and maintain meiotic arrest by acting either directly on the cumulus cell-enclosed oocyte or indirectly by a mechanism that is mediated by the cumulus cells without a detectable elevation of cumulus cell cAMP levels, or both.

Adenosine is known to be an important regulator of adenylate cyclase in many

cell systems [Arch and Newsholme, 1978; Fain and Malbon, 1979; Londos et al., 1981]. Adenosine may act via an external cell receptor to stimulate adenylate cyclase activity. In support of this possibility, poorly-metabolized analogs of adenosine maintain meiotic arrest as effectively as native adenosine [Downs et al., 1986; Miller and Behrman, 1986; Salustri et al., 1988]; e.g. metabolism of adenosine may not be required to maintain oocytes in meiotic arrest. Nevertheless, when mouse oocytes and oocyte-cumulus cell complexes are cultured in medium containing adenosine, the nucleoside is rapidly metabolized to phosphorylated derivatives [Heller and Schultz, 1980; Downs et al., 1986]. In addition, ATP levels in granulosa and luteal cells are elevated in response to adenosine, presumably via phosphorylation of adenosine [Brennan et al., 1983; Ohkawa et al., 1985; Billig and Rosberg, 1986]. These latter data suggest that adenosine could also serve as a precursor for cAMP synthesis.

In the present study, adenosine alone had no apparent stimulatory effect on cAMP levels in intact oocyte-cumulus cell complexes, yet this purine nucleoside increased the inhibitory effects of hypoxanthine on oocyte maturation. The lack of effect of adenosine on cAMP levels in intact complexes is unexpected in light of recent studies that demonstrate stimulation by adenosine of cAMP synthesis in rat luteal cells [Hall et al., 1981; Polan et al., 1983], granulosa cells [Ohkawa et al., 1985; Billig and Rosberg, 1986] and oocyte-cumulus cell complexes [Preston et al., 1987]. It is possible that, in the present study, adenosine alone produced a transient increase in cAMP in the intact complexes that was not observed at the 3 hr time point examined. Adenosine transiently maintains both cumulus cell-enclosed and denuded oocytes in meiotic arrest in vitro [Eppig et al., 1985]; this would be expected if a temporary rise in cAMP resulted from adenosine treatment. Although adenosine alone maintained elevated cAMP levels in cumulus cell-enclosed oocytes, this was not reflected in a decrease in the frequency of GVB when assessed after 3 hr culture.

These results appear inconsistent with the view that elevated oocyte cAMP levels maintain meiotic arrest, especially when hypoxanthine produced a level of cAMP that was not significantly different yet effectively maintained meiotic arrest. It is possible that, in adenosine-treated oocytes, the level of cAMP after 3 hr may be just below the threshold value required for meiotic arrest, while that in hypoxanthine-treated oocytes may be at or just above this value. There was a consistent trend toward higher cAMP levels in hypoxanthine-treated oocytes; small differences in cAMP near the threshold value, while not statistically significant, might produce different frequencies of GVB. Alternatively, hypoxanthine, a more potent inhibitor of cAMP PDE in oocyte lysates, may produce more stable levels of the cyclic nucleotide and therby maintain meiotic arrest more effectively. Indeed, the addition of 4 mM hypoxanthine to medium containing 0.75 mM adenosine results in the maintenance of meiotic arrest in over 95% of cumulus cell-enclosed oocytes for up to 24 hr [Eppig et al., 1985]. This effect of the two purines could be explained by a synergistic interaction between activation of adenylate cyclase by adenosine and inhibition of cAMP PDE by hypoxanthine [Eppig et al., 1985]. Also, factors other than the generation of cAMP, or maintenance of elevated levels thereof, may be required to maintain meiotic arrest.

ACKNOWLEDGMENTS

This project was supported by grants HD20575 (J.J.E.), the Medical Research Council of Canada (S.A.J.D.), and HD10381 (P.C.H.) from the National Institute of

Child Health and Human Development (NICHHD). The NICHHD is not responsible for its contents, nor do they necessarily represent the official views of that agency. The authors thank Avis Silva for technical assistance, the National Hormone and Pituitary Program for the gift of FSH, and Dr. James L. Maller for the gift of the PKI. The Jackson Laboratory is fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

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