Vol. 96, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1980 Pages 1274-1281

INCREASED ORNITHINE DECARBOXYLASE ACTIVITY DURING MEIOTIC MATURATION IN XENOPUS LAEVIS OOCYTES

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Received July 11,1980

SUMMARY. The activity of ornithine decarboxylase (ornithine carboxy-lyase E.C. 4.1.1.17) was studied during meiotic maturation induced in vitro by progesterone in follicle cell-free oocytes. Enzyme activity increased 4-6 fold during maturation, preceding germinal vesicle breakdown. The increase in ornithine decarboxylase activity was inhibited by cholera toxin, an agent that blocks meiotic maturation and increases cAMP levels within the cell. It was also prevented by cycloheximide but not by actinomycin D. Treatment of oocytes with D,L-α-difluoromethyl-ornithine, an irreversible inhibitor of ornithine decarboxylase and of putrescine synthesis, effectively abolished enzyme activity without preventing germinal vesicle breakdown. These observations show that the progesterone-induced increase in ornithine decarboxylase activity is not required for completion of meiotic division of the oocyte.

Ornithine decarboxylase (ODC) is the first enzyme in the polyamine biosynthetic pathway (1, 2). Increased levels of polyamines and of their biosynthetic enzymes have been found to be closely correlated with the growth of various eukaryotic cells under the effect of a variety of agents. Hormones (3), growth factors (4,5), mitogens (6), chemicals (7), viruses (8) all induce ODC activity and polyamine accumulation within target cells. When correlated with cell cycle, polyamine biosynthesis is increased primarily during the late G_1 phase (9) ; it has also been observed at high levels during the G_2 phase (10,11), and many reports have supported the view that polyamine accumulation might be required for DNA synthesis. Several authors have suggested a mechanism involving cAMP and protein kinase activation (12, 13). Some reports, however, present evidence that this model may not be of general significance (14).

Stage VI Xenopus laevis oocytes, free of follicule cells, are an homogenous cell population arrested in the G_2-M phase of the cell cycle (15). Upon stimulation in vitro by progesterone they synchronously re-enter cell cycle (16) and progress through a round of meiotic cell division without interphase and without DNA synthesis (S-phase absent) (17). They subsequently undergo a second arrest in the M phase of the second meiotic division. A possible involvement of cAMP in controlling the meiotic block of the oocytes is suggested by experiments with agents such as cholera toxin (18, 19) as well as by injec-

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0006-291X/80/191274-08$01.00/0

tion into oocytes of cAMP derivatives (19) and cAMP-dependent protein kinase subunits (20, 21).

A possible role of polyamine biosynthesis in oocyte meiotic maturation has not yet been studied. The studies reported here were carried out in order to investigate the relationship between the ODC activity and the phase of cell cycle. To our knowledge, this is the first report concerning ODC activity with reference to meiotic cell division.

MATERIALS AND METHODS

Chemicals. DL-1-^{14}C ornithine monohydrochloride (40-60 mCi/mmole) was obtained from the Radiochemical Centre (Amersham, England). Progesterone was a gift from Roussel-Uclaf, France. Other chemicals (Merck, Prolabo, Sigma) were of analytical grade. Cholera toxin was from Schwartz-Mann, USA. DL-α-difluoromethyl ornithine (α-DFMO) was a gift from Centre de Recherche Merrell International, Strasbourg, France. Methyl-glyoxal bis(guanylhydrazone) was from Aldrich. Buffer A : 1 mM $MgCl_2$, 1 mM dithioerythritol, 0.01 % NaN_3, 250 mM sucrose and 20 mM Tris-HCl (pH 7.8). Buffer B : 9 mM dithioerythritol, 0.4 mM pyridoxal phosphate, 2 mM L-ornithine, 0.2 μCi DL-1-^{14}C-ornithine monohydrochloride, 80 mM Tris-HCl (pH 7.0).

Biological materials. Xenopus laevis females were obtained from South African Snake Farm, Fish Hoeck, Cape Province, South Africa. Ovarian fragments collected from females anaesthesized with tricaine (MS 222, Sandoz) were digested with 0.2 % collagenase (Sigma type I) for 2 h at 28°C. Barth's medium (22) and stage V-VI oocytes (23) were selected. In each experiment oocytes were obtained from the same animal. Maturation was induced by continuous exposure to progesterone (10 μM) in 3 ml medium at 20-22°C and was scored by the appearance of a white spot at the animal pole. Dissection of 2.5 % glutaraldehyde fixed oocytes confirmed the loss of the germinal vesicle. For enzyme studies 200-500 oocytes were incubated and at various time intervals 20-50 oocytes were removed and homogenized in buffer A (10 μl per oocyte) by three freezing and thawing cycles. The homogenate was centrifuged at 105,000 g for 1 h and the supernatant was assayed for enzyme activity. The enzyme assay, a modification of (24), was carried out in closed glass vials containing 0.1 ml of buffer B and a disposable polypropylene centre well with 0.2 ml Soluene (Packard). The reaction was started by adding 0.1 ml of the 105,000 g supernatant to the buffer B. After 1 h at 37°C the reaction was stopped by injecting 0.3 ml of 40 % trichloroacetic acid through rubber stopper. The incubation was continued for a further 30 min before the centre well was counted in 10 ml scintillation fluid (0.4 % Scintimix, 2 % ethanol in toluene). Blanks (80-100 cpm) were subtracted from the values obtained.

RESULTS

During incubation of oocytes with progesterone, oocytes started to display germinal vesicle breakdown (GVBD) after about 4 h. ODC activity was increased in progesterone-exposed oocytes already at 4 h, preceding appearance of the white spot at the animal pole of the oocytes, and reached a maximum of about 5 times the control level by the time of completion of GBVD, i.e. about 8 h in this experiment. The ODC activity subsequently declined and by 12 h after beginning of the incubation differed little from

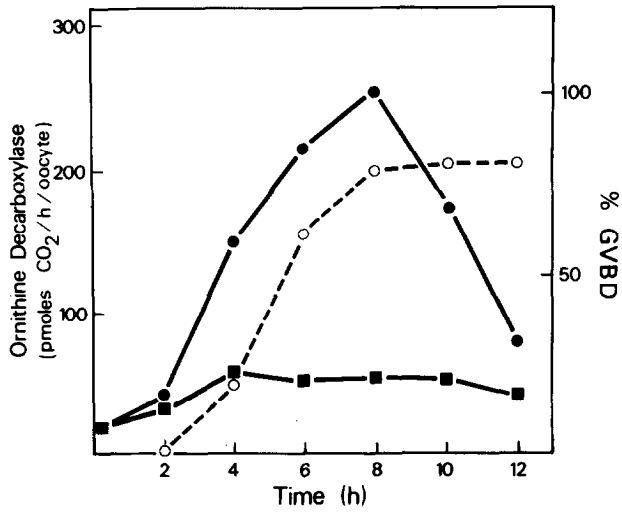


Figure 1. ODC activity during oocyte meiotic maturation in vitro. Oocytes (500 per dish) with (circles) or without (squares) progesterone (10 µM) ; samples of 50 oocytes were withdrawn at various time intervals for ODC determination (full symbols). Maturation induced by progesterone is represented by open circles.

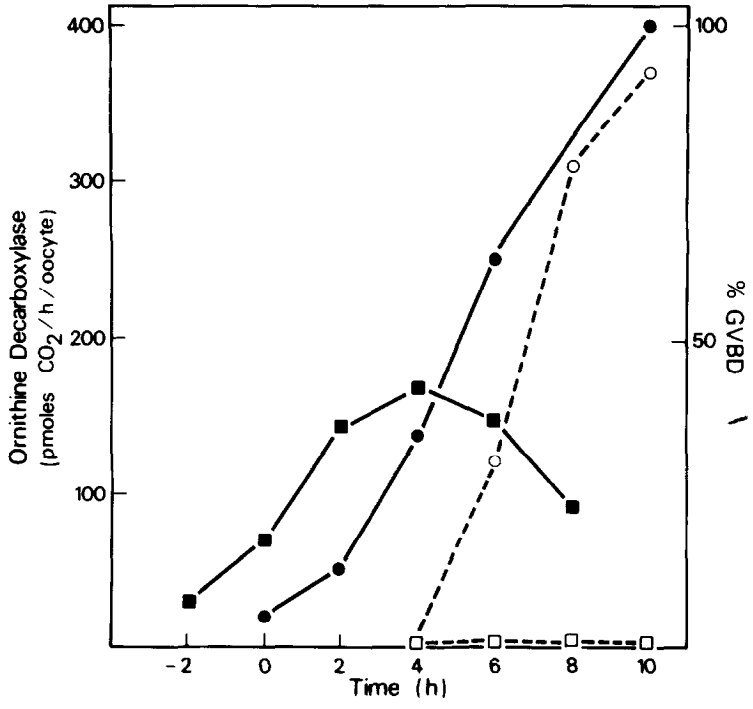
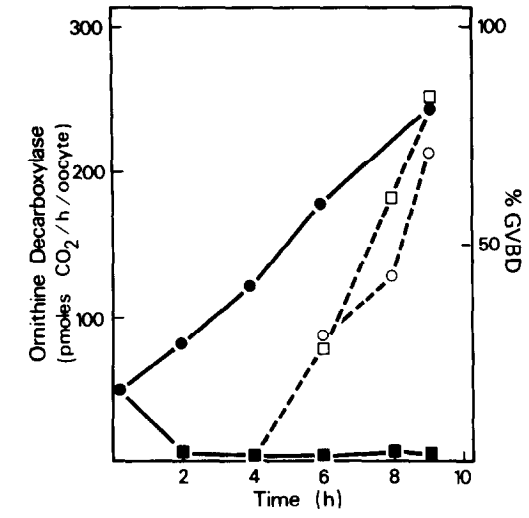
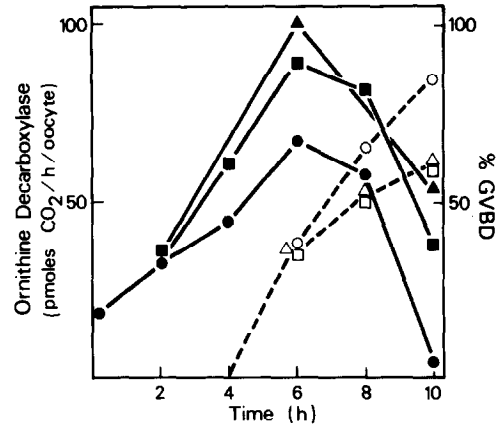


Figure 2. Effects of cholera toxin on ODC activity during progesterone-induced maturation. Oocytes (300 per dish) were preincubated with cholera toxin (1 mM ; squares) or with medium alone (circles) for 4 h before addition of progesterone at time 0 ; samples of 20 oocytes were withdrawn for ODC determination (full symbols). Maturation is represented by open symbols.



(3)



(4)

Figure 3. Effects of α-DFMO on progesterone induced maturation. Oocytes (300 per dish) were incubated with (squares) or without (circles) α-DFMO neutralized 5 mM solution. Progesterone (10 μM) was present in both dishes. Samples for ODC determination (50 oocytes) were withdrawn at various time intervals (full symbols) and maturation scored (open symbols).

Figure 4. Effects of actinomycin D (10 μg/ml ; triangles ; and 100 μg/ml ; squares) on progesterone (circles) induced maturation. Oocytes (150 per dish) were incubated with or without actinomycin and progesterone. Other symbols same as for Figure 3.

the control (Fig. 1). In oocytes treated with progesterone after a 4 h pretreatment with cholera toxin, ODC activity showed a rise during the preincubation and continued to increase during the early period following addition of progesterone. This rise was stopped by 4 h and the subsequent decrease in ODC activity contrasted with its continued augmentation in oocytes exposed to progesterone alone (Fig. 2). Cholera toxin completely abolished maturation (cf. 18). We have observed that 1 h preincubation with cholera toxin led to similar pattern of ODC activity variations as that shown in Figure 2 ; however, 10 h preincubation prevented any further increase of ODC activity under the effect of progesterone (not shown).

In order to establish whether ODC activity was required for maturation, experiments using α-DFMO, an irreversible inhibitor of this enzyme (25), were performed. The results demonstrated that while a 5 mM concentration of the drug was sufficient to abolish completely the activity of ODC, maturation could still be induced by progesterone as in the absence of α-DFMO (Fig. 3). To test the possibility that α-DFMO might not have inhibited the ODC activity within the oocytes and that the observed effect resulted from contamination of the cytosol by the drug after homogenization, whole oocytes were incubated with progesterone

TABLE I

Effects of α-DFMO on ODC activity in whole oocytes during progesterone induced maturation. 15 oocytes per vial were incubated in 0.2 ml of Buffer B per incubation. Incubations were terminated by injecting 0.3 ml of trichloracetic acid and $^{14}CO_2$ liberated was detected as described in Materials and Methods. By 3 h, 90 % of oocytes had developed the white spot at the animal pole, indicating GVBD.

Treatment	Time (h)	Cumulative ODC activity pmoles CO_2
Progesterone (10 μM)	4	30.5
	8	52.9
Progesterone (10 μM) + α-DFMO (5 mM)	4	8.6
	8	7.2

and/or α-DFMO in the presence of buffer B and the released $^{14}CO_2$ was determined as in the enzyme assay. As shown in Table I, α-DFMO did inhibit ODC activity under these conditions. In another experiment (not shown) preincubation of oocytes with 5 mM α-DFMO for as long as 10 h still failed to affect the induction of GVBD by progesterone ; the hormone could exert its action even on oocytes preincubated for 10 h with a combination of α-DFMO and methylglyoxal-bis-guanine hydrazone (10 mM), an inhibitor of S-adenosylmethionine

TABLE II

Effects of cycloheximide (10 μM) on progesterone (10 μM) induced germinal vesicle breakdown (GBVD) and ODC activity. 150 oocytes per incubation ; 20 oocytes were withdrawn for each determination of ODC activity. 50 oocytes were counted for scoring GBVD.

Treatment	Time (h)	ODC pmoles CO_2/h/oocyte	GVBD (%)
Progesterone	0-2	32.1	0
	2-4	44.9	0
	4-6	67.6	38
Cycloheximide + Progesterone	0-4	1.3	0
Progesterone + Cycloheximide	0-6 / 4-6	8.3	35

decarboxylase, another enzyme known to play an important role in polyamine synthesis (1, 2).

The question as to whether ODC activity was under transcriptional or translational control was also examined. Actinomycin D did not impair the induction of ODC activity by progesterone (in fact, the levels observed in the presence of this inhibitor of RNA synthesis were higher than those measured in the oocytes treated with progesterone alone) and, as described previously, GVBD induction was also observed (Fig. 4). On the other hand, cycloheximide severely inhibited ODC activity and, when added at the same time as progesterone, prevented oocyte maturation (Table II). The apparent half-life of the enzyme was approximately 40 min as calculated from the data in Table II.

DISCUSSION

ODC activity is demonstrated in the soluble fraction as well as in whole defolliculated Xenopus laevis oocytes. This activity is increased by progesterone treatment of the oocytes about 5 times compared to the controls ; the increase in ODC activity is observed before GBVD, and is followed by a sharp decline once all oocytes have completed maturation. As in mammalian cells, the ODC of Xenopus oocytes has a rapid turnover (half-life of about 40 min at 20°C). The formation of new enzyme molecules require protein synthesis but cannot be blocked by actinomycin D suggesting translation of preexisting mRNA, like for several other oocyte proteins (26). The amplitude of the stimulation of ODC activity could not be accounted for by the stimulation of total protein synthesis which is only of the order of about 50 % (15, 16). This suggests a specific mechanism for the control of ODC activity. Inhibition by cholera toxin of the progesterone-induced increase in ODC activity suggested that this increase might be a necessary event in the sequence leading to GVBD. The failure to prevent the progesterone-induced oocyte maturation when ODC activity was inhibited by α-DFMO for as long as 6 h prior to hormone exposure excluded this possibility. ODC stimulation and presumably increased polyamine synthesis appear therefore to be a part of the program turned on by progesterone but not essential for germinal vesicle breakdown. One may speculate that the observed increase in ODC activity in the course of oocyte maturation leads to accumulation of polyamines needed for subsequent developmental events. The reported very low levels of putrescine in unfertilized sea urchin eggs (27), and the fact that α-hydrazinoornithine, a competitive inhibitor of ODC, blocks cleavage divisions in developing embryos (28), are compatible with this hypothesis. Similarly, inhibition of ODC activity in dividing cultured cells led to an arrest of cell division only after 2-3 unaffected cycles (29).

Amphibian oocytes are arrested in late G_2-M phase of cell cycle leading to meiotic division (15, 16). During the next cycle, following GVBD, there is no interphase and nuclear DNA replication does not take place; however, during the transition from oocyte to unfertilized egg, the cell acquires the ability to replicate DNA in the cytoplasm (17). It is not excluded that ODC stimulation relates to the acquisition of this competence. A peak of ODC activity in the late G_2-M phase was recently reported to occur in synchronized CHO cells (10) suggesting that this phenomenon may be of more general validity.

The presence of cholera toxin in the culture mediums caused in some experiments a small early increase of ODC activity. This effect of the toxin, if confirmed, may be of interest as it cannot be due to overall augmentation of cAMP content of the oocyte which becomes apparent only after about 4 h. At time intervals of 4 h and longer (i.e. coincident with increased cAMP concentrations), cholera toxin inhibited severely the progesterone-induced rise in ODC activity. Similar correlation between increased cAMP levels and inhibition of ODC activity was recently reported by Insel and Fenno in S_{49} cells (14).

ACKNOWLEDGEMENTS

This work was supported by Dgrst (contract n° 78.7.0356) and Inserm (contract ATP 58.78.90). We thank J.C. Lambert and C. Barrier for illustrations and M. Rossillon for dactylography. α-DFMO was a generous gift of Centre de recherche Merrell International, Strasbourg, France".

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