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Original article

Difference in activation capacity between oocytes of *Pleurodeles waltl* matured *in vivo* and *in vitro*

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Summary — Oocytes of *Pleurodeles waltl* were activated after *in vivo* maturation by needle pricking or electric shock. After *in vitro* maturation, the oocytes were not activated by these stimuli. Coelomic oocytes and the oocytes which began their maturation *in vivo* could be activated by electric shock. During *in vivo* oocyte maturation, the activity of glucose-6-phosphate dehydrogenase (G6PDH), the key enzyme of the pentose phosphate cycle, increased while that of phosphofructokinase, the key enzyme of glycolysis, remained unchanged. During progesterone-induced *in vitro* oocyte maturation, the activity of both enzymes remained unchanged. Oocytes of *Misgurnus fossilis* matured *in vivo* and *in vitro* were activated spontaneously. No changes in the activity of G6PDH were observed during their maturation. These results suggest a relationship between G6PDH activity in the oocyte and oocyte capacity for activation by needle pricking or electric shock.

oocyte maturation / carbohydrate metabolism / activation / amphibian / fish

Résumé — Différences de capacités d'activation des ovocytes maturés *in vivo* et *in vitro*. *Des ovocytes de* Pleurodeles waltl *sont activés par piqûre ou choc électrique après maturation* in vivo. *Après maturation* in vitro, *les ovocytes ne sont pas activés par ces stimuli. Des ovocytes cœlomiques et des ovocytes ayant commencé leur maturation* in vivo *peuvent être activés par choc électrique. Pendant la maturation de l'ovocyte* in vivo, *l'activité de la glucose-6-phosphate déshydrogénase (G6PDH), l'enzyme clé du cycle pentose phosphate, augmente, tandis que celle de la phosphofructokinase, l'enzyme clé de la glycolyse, demeure inchangée. Pendant la maturation ovocytaire provoquée* in vitro *par la progestérone, l'activité des 2 enzymes ne change pas. Les ovocytes de* Misgurnus fossilis *maturés* in vivo *et* in vitro *sont activés spontanément; aucune modification de l'activité G6PDH n'est observée pendant la maturation. Ces résultats suggèrent un rapport entre l'intensité de l'activité G6PDH de l'ovocyte de* Pleurodeles waltl *et son aptitude à être activé par piqûre ou par choc électrique.*

maturation ovocytaire / métabolisme des sucres / activation / amphibien / poisson

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INTRODUCTION

The degree of oocyte maturation can be evaluated using morphological (metaphase of the 2nd meiotic division) or functional (acquisition of the capacity for activation) criteria. It was shown first in mammals that metaphase II oocytes are not always fertilizable (Thibault, 1977). Also unable to be activated by needle pricking were the oocytes of *Bufo arenarum* (Legname *et al*, 1972) and *Rana temporaria*, matured *in vivo* beyond the season of reproduction (Sharova, 1989), and the oocytes of *Pleurodeles waltl* matured *in vitro* (Bilinkis, 1990). It has been shown in lower vertebrates during gonadotropin-induced oocyte maturation that the carbohydrate metabolism changes (Legname *et al*, 1972; Yurovitzky and Milman, 1973). In the oocytes of *B arenarum* matured *in vivo* a correlation has been found between the acquisition of the capacity for activation and changes in carbohydrate metabolism (Legname and Buhler, 1978).

In order to test the hypothesis of a possible influence of the type of carbohydrate metabolism in the oocyte on oocyte capacity for activation, we studied the activity of glucose-6-phosphate dehydrogenase (G6PDH), the key enzyme of the pentose phosphate cycle, and phosphofructokinase (PFK), the key enzyme of glycolysis, in maturing oocytes of *P waltl* and *Misgurnus fossilis*. Special attention was paid to their capacity for activation.

MATERIALS AND METHODS

Experime nts were carried out on fully grown oocytes and mature eggs of *Pleurodeles waltl* and *Misgurnus fossilis*. In order to obtain eggs matured *in vivo* the females of *P waltl* were injected, 20 to 24 h before the experiment, with a synthetic analog of LH-RH (synthesized in the Laboratory of Peptide Synthesis, National Cardiology Research Center of the USSR Academy of Medical Sciences) at a dose of 4 μg/female. The oocytes of *M fossilis* matured *in vivo* were obtained after females were injected with 300 IU of human chorionic gonadotropin. For *in vitro* maturation, fragments of *P waltl* ovary were placed in a modified K^+-free Barth solution containing 1.5 g/l $NaHCO_3$ or in OR_2 (Wallace *et al*, 1973) solution; pieces of *M fossilis* ovary were placed in medium 199 containing 20% cattle serum. All media were supplemented with 5 μg/ml of progesterone. Oocytes were incubated and all steps were carried out at 18 °C. The completion of oocyte maturation in *P waltlii* was assayed for the appearance of a characteristic pigment pattern on the animal pole, a white spot with a small dark dot in the middle. This was shown in cytological studies (Skoblina *et al*, 1984; Taghy-Sadak and Vilain, 1985) to correspond to the metaphase of the 2nd meiotic division. Maturation of *M fossilis* oocytes *in vitro* was assayed for germinal vesicle breakdown after fixation with Bau-Kien-Tenig mixture. The mature eggs of *P waltl* were activated with needle pricking or electric shock as described elsewhere (Bilinkis, 1990). The eggs of *M fossilis* matured *in vivo* or *in vitro* underwent spontaneous activation within 20-30 min after ovulation.

Enzyme activity was assayed on 10 portions of oocytes or eggs obtained from different females of *P waltl* in extracts prepared in Ringer solution and 6 portions of oocytes or eggs obtained from different females of *M fossilis*.

Activity of PFK was assayed according to Underwood and Newsholme (1965): the increase in fructose-1,6-diphosphate was determined after incubation of the extract obtained from 30-60 embryos in a mixture containing 0.0015 M ATP (Calbiochem, Switzerland), 0.003 $MgSO_4$, 0.0015 M fructose-6-phosphate (Serva, Germany), 0.005 M mercaptoethanol and 0.05 M Tris-HCl (pH 8.5). The extracts were prepared in 0.05 M phosphate buffer (pH 7.5) containing 0.005 M mercaptoethanol (5 min at 25 °C). The reaction was arrested by hydrochloric acid. Samples to which hydrochloric acid was added before the onset of incubation were used as controls. In the neutralized extracts, fructose-1,6-diphosphate was determined using reactions coupled with NAD-H oxidation. A mixture of aldolase, triosephosphate isomerase and alpha-glycerophosphate dehydrogenase was used.

Activity of G6PDH was assayed according to Glock and McLean (1953) for the reduction of NADP in a system containing 0.05 M Tris (pH 7.5), 0.003 M $MgSO_4$, 0.00015 M NADP (Serva, Germany) and 0.001 M substrate.

RESULTS AND DISCUSSION

In our previous study (Bilinkis, 1990) it was shown that after both *in vivo* and *in vitro* maturation the oocytes of *P waltl* reached metaphase II but differed in their capacity for activation. After *in vivo* maturation, the oocytes could be activated by needle pricking or electric shock, whereas after maturation *in vitro* they were not activated by these stimuli. Incubation of the oocytes *in vitro* in different media (Ringer, Barth, K^+-free Barth, Barth added with 1.5 g/l $NaHCO_3$, Merriam, OR_2) gave similar results. The oocytes underwent maturation in all media but could not be activated (Bilinkis, unpublished data).

We have also studied the capacity for activation in the oocytes of *P waltl* that matured and were ovulated under different conditions. Around 50% of the coelomic oocytes and those which matured *in vivo* during the first 8–10 h, but completed maturation in the presence of progesterone *in vitro*, also acquired the capacity for activation by electric shock. Ovulation did not affect this process (table I).

The activity of G6PDH and PFK was assayed in fully grown oocytes of *P waltl* following their maturation *in vivo* and *in vitro*. The activity of G6PDH in the fully grown oocytes or after maturation *in vitro* was markedly lower than after *in vivo* maturation, while that of PFK was similar in all three cases (fig 1). According to the ratio between the PFK and G6PDH activities, the pentose phosphate cycle was very active during *in vivo* oocyte maturation, while glycolysis was relatively more active in the fully grown oocytes and during *in vitro* oocyte maturation.

We also assayed the activity of G6PDH and PFK in fully grown oocytes of *M fossilis* and after their maturation *in vivo* and *in vitro*. Hormonal stimulation caused no changes in glucose metabolism: the activity of both enzymes remained unchanged during oocyte maturation (fig 2).

The data obtained in this study suggest a relationship between the prevailing type of carbohydrate metabolism in the oocyte of *P waltl* and the acquisition of a capacity for activation. Only mature eggs, in which carbohydrates were utilized mainly through the pentose phosphate cycle, were capable of activation and subsequent development. Similar data have also been ob-

Table I. Activation by electric shock of *Pleurodeles waltl* oocytes matured and ovulated *in vivo* and *in vitro*.

Variant	n	*Activated*	%
Oocytes matured and ovulated *in vitro*	83	0	0
Oocytes matured *in vivo* + *in vitro* but not ovulated	820	414	50.5
Coelomic oocytes	138	73	53

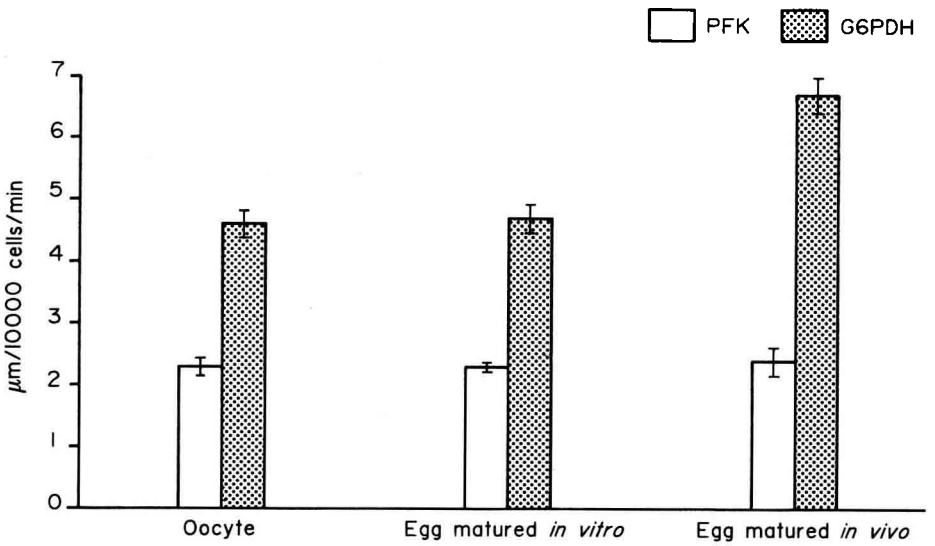

Fig 1. Activity of phosphofructokinase (PFK) and glucose-6-phosphate dehydrogenase (G6PDH) in fully grown oocytes and eggs matured *in vitro* and *in vivo* in *Pleurodeles waltl.* Ordinate: enzyme activity.

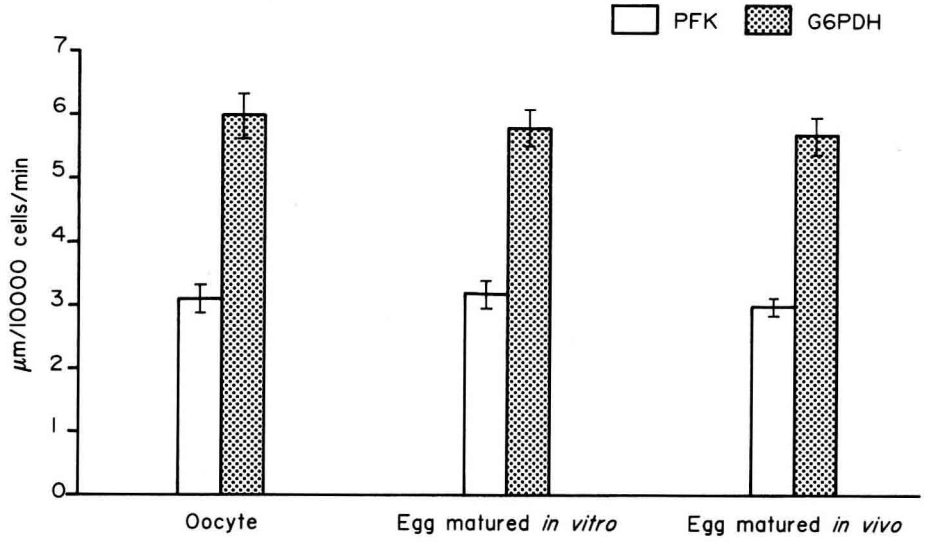

Fig 2. Activity of phosphofructokinase (PFK) and glucose-6-phosphate dehydrogenase (G6PDH) in fully grown oocytes and eggs matured *in vitro* and *in vivo* in *Misgurnus fossilis.* Ordinate: enzyme activity.

tained with the oocytes of *B arenarum*: the mature coelomic eggs obtained in winter could not be fertilized or artificially activated. In these eggs glucose was used predominantly by the glycolytic pathway, whereas in the mature eggs obtained during the normal reproduction season the carbohydrates were mainly used through the pentose phosphate cycle (Legname *et al,* 1972). Glucose metabolism normally undergoes rearrangement in the fully grown oocyte during the reproduction season (Legname *et al,* 1976). In *P waltl* oocytes the glycolytic processes prevailed and the system of carbohydrate metabolism was rearranged only during *in vivo* maturation. In the oocytes of *M fossilis* the necessary level of G6PDH activity was already achieved at the time of growth completion and remained unchanged throughout later development. This appears to explain the similar capacity for activation in the oocytes matured *in vivo* and *in vitro*. This agrees with results obtained on the oocytes of *Xenopus laevis* (Dworkin, Dworkin-Rastl, 1989).

It is widely accepted that activation, as one of the main components, comprises a transient increase in free extracellular Ca^{2+} (Epel, 1990). This mechanism is being studied intensively (Busa *et al,* 1985; Rusinko and Lee, 1989; Han and Nuccitelli, 1990). It remains to be seen whether the changes in carbohydrate metabolism in *in vitro* matured oocytes of *P waltl* eventually affect the systems providing for the mobilization of intracellular Ca.

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