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Amino Acid Correction of Regulatory Volume Decrease Evoked by Hypotonic Stress in Mouse Oocytes *In Vitro*

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Regulatory volume decrease in response to hypotonic stress is typical of the oocytes and early mouse embryos. Changes in the kinetics of osmotic reaction can be used as a marker of the modulating effect of the incubation medium on transmembrane transport in embryonic cells. Quantitative laser scanning microtomography (QLSM) was used to measure oocyte volume. In this paper, it is shown that addition of 5 μM glycine, taurine, or GABA, as well as ATP to Dulbecco's medium abolished the regulatory volume decrease in mature mouse oocytes.

Key Words: *mature mouse oocyte; hypo-osmotic shock; cell volume regulation; ATP and amino acids; laser microtomography*

Given the ratio of the volumes of mammalian spermatozoon and mature oocyte (~1:10^6), fertilization causes no significant mass transfer. In other words, the fusion of male and female germ cell does not significantly change the composition of oocyte membrane proteins in comparison with zygote or two-cell embryo. So, in some cases the egg cell can be considered as an experimental model in the study of traumatic action of the incubation medium on the early embryo. Such experiments is of practical value when studying the effects of cryopreservation procedures on mature oocyte [15].

Improvement of the protocol of *in vitro* manipulations with early embryo is a requirement of clinical practice. However, a problem arises in the modification of the method due to ethical and moral norms that do not support research on embryos. The problem can be solved by using mature oocyte as the immediate precursor of early embryogenesis, for example, when testing the response of embryonic cells on altered physical and chemical properties of the incubation medium.

The history of the developing physiological solution for early human embryo is described in the literature. Such a medium was originally based on Krebs–Ringer or Tyrode's solution. M16 medium or MEM used for cultures of differentiated cell was applied at the stage of *in vitro* fertilization. Further research led to creation of a specialized SOM medium (Simplex Optimized Medium) as well as its modification by K^+/Na^+-balance, KSOM [6], and mKSOM [8]. In the latter case, the concentration of glucose and BSA was increased by several times. Addition of some amino acids into the medium ($mKSOM^{AA}$) improves embryo development [9].

A phenomenon is known in the experimental biology, when *in vitro* development of an early embryo is arrested, for example, at the stage of two-cell mouse embryos. This blockade is largely determined by osmotic stress, and one of the ways to overcome it is to add amino acid such as glycine in the incubation medium or to decrease its osmolality [3]. Noteworthy, the

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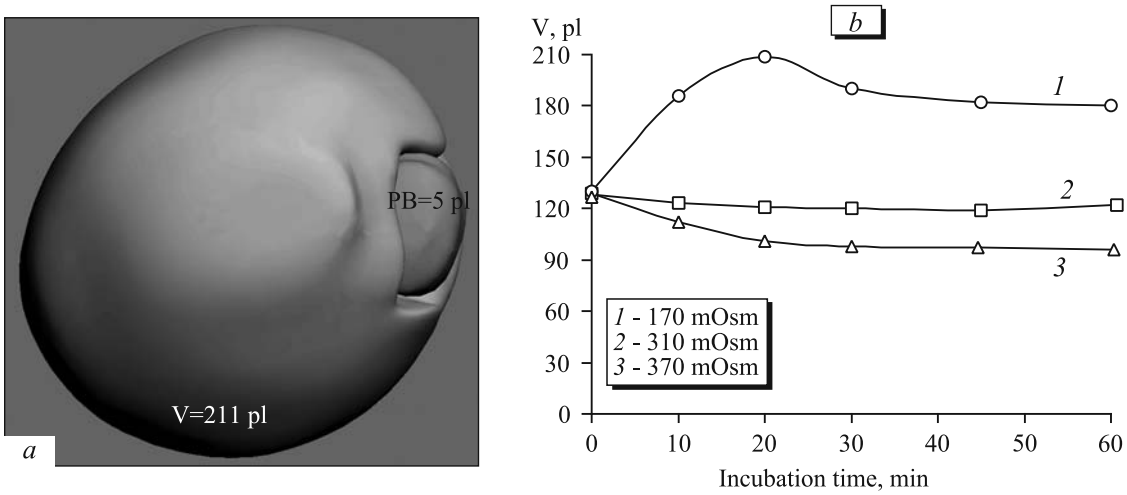

Fig. 1. Mature oocyte (QLSM) after 20-min incubation of the cell in hypotonic (170 mOsm) Dulbecco's solution (*a*) and kinetics of changes in the volume of a mature oocyte in Dulbecco's medium of different osmolality (*b*). PB, polar body; V, cell volume (pl).

presence of amino acids is similar to *in vivo* situation in the oviduct and affects the amino acid composition of the cytoplasm of early embryo cells [12].

Amino acids not only have osmoprotective properties, but are also directly involved in the compensatory reaction of oocyte as osmolytes [5,10], probably because the complex of the mechanisms of amino acid transmembrane transport [3,4,11,13,14]. Thus, participation of organic osmolytes in adaptation to osmotic stress is a physiological factor that should be considered at developing the incubation media.

The purpose of this work was to establish changes in the kinetics of osmotic response of mouse oocyte on the hypotonic stress after addition of several amino acids into the incubation medium.

MATERIALS AND METHODS

The study was carried out on SHK mice from the nursery of the Institute of Theoretical and Experimental Biophysics. Oocytes were prepared according to the procedure described previously [1,2,7]. Groups of oocytes were incubated for 10, 20, 30, 45, or 60 min. Hypotonic conditions were created by decreasing NaCl concentration in Dulbecco's medium from 140 to 70 mM. The oocytes taken immediately before the action of osmotic stress were used as a starting point (controls). An amino acid (glycine, taurine, glutamate, and GABA) or ATP was added to Dulbecco's medium, equimolarly replacing NaCl.

Laser microtomography followed by quantitative 3D reconstruction of the oocyte (QLST) was used to analyze the kinetics of the changes in cell volume (Fig. 1, *a*) [1]. This technology saves cell shape in lifetime conditions and allows to measure its volume. The preparations were examined under a Leica TCS SPE confocal microscope in light transmission mode.

RESULTS

Under hypertonic conditions (370 mOsm), oocyte behaves like a classic osmometer, that is, cell volume is reduced to a fixed level, the value of which does not change in time (Fig. 1, *b*). Under isotonic conditions for differentiated cell (310 mOsm), the volume of mature oocyte decreases with time. Thus, physiological

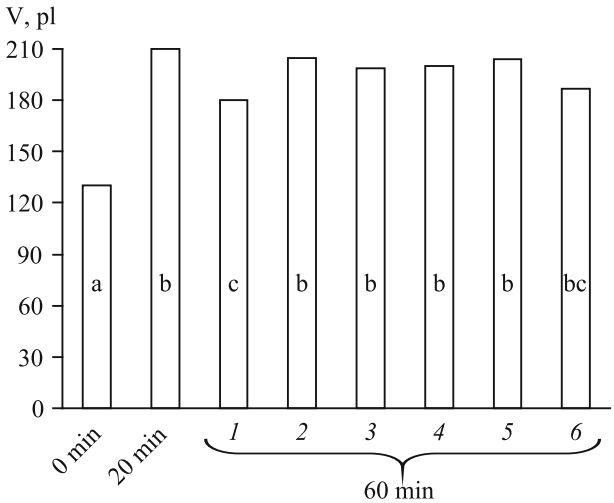

Fig. 2. Dynamics of cell volume of mature mouse oocyte in the characteristic points of the osmotic response in conventional Dulbecco's medium (*1*) and after equimolar replacement of NaCl with 5 mM ATP (*2*) or 5 mM amino acid taurine (*3*), glycine (*4*), GABA (*5*), and glutamate (*6*). The presence of ATP or amino acids does not affect significantly the indicators at the beginning (0 min) of hypo-osmotic stress and at the time of maximum swelling (20 min). Data in columns with different marks differ at the significance level of $p<0.05$. Significance of differences was assessed by Student's *t* test with the number of oocytes of at least 20 cells in each group.

medium of normal osmolality causes hypertonic stress in egg cell. Noteworthy, the oocyte responds like a two-cell mouse embryo [2].

Kinetics of changes in the volume of mouse oocytes in hypotonic conditions exhibits a compensatory phase following the initial swelling (Fig. 1, *b*). During this period, the cell volume tends to the value recorded at the start of hypnotonic stress. This "anomalous" osmotic behavior is also characteristic of cells of the early mouse embryo. This confirms the possibility of using oocyte as an experimental model in the study of the osmotic effect of the physiological medium on the embryonic cell.

In our study, the degree of the adaptive response was used as a criterion for testing the modulating effects of amino acids added to the incubation medium. We compared the data obtained on minute 60 of the regulatory volume decrease in the mature oocyte, when the value of cell volume reached a plateau (Fig. 1, *b*).

Reaching a maximum value by minute 20, oocyte does not recover its full size by minute 60 of regulatory volume decrease (Fig. 2). The inhibition of the adaptive response by millimolar concentrations of extracellular ATP is typical of hypoosmotic medium. Indeed, addition of 5 mM ATP to Dulbecco's solution abolished the compensatory decrease in the egg volume (Fig. 2). The same result was observed in the presence of all examined amino acids except glutamate. In the latter case, the effect was less pronounced, that did not allow distinguishing it from ordinary regulatory volume decrease of mature mouse oocytes.

Thus, we can conclude that addition of the examined amino acids (taurine, glycine, GABA) to the incubation medium alters the kinetics of oocyte response to the hypo-osmotic stress. The effect is probably due to the involvement of several mechanisms of transmembrane transport of organic osmolytes into the cell that hinder its compensatory compression. Quantitative laser microtomography is suitable for testing the modulating effects on physiological medium on mature oocyte by changes in the kinetics of its reaction on hypo-osmotic stress.

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