Changes in Intracellular Potassium Concentration in a One-Cell Mouse Embryo after Enucleation

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Comparative analysis of potassium concentrations in the cytoplasm of intact and enucleated one-cell mouse embryos of showed that microsurgical manipulations during collection of pronuclei disordered potassium homeostasis in the embryonic cell.

Key Words: *enucleation; zygote; cytoplasmatic potassium; electron probe microanalysis*

Embryonic development starts from oocyte fertilization. The first division plays the key role in early embryogenesis [1,2]. Processes running in the embryonic cell at this stage determine the realization of the genetic program [4]. Induced modification of the status of a one-cell embryo can have unpredictable effects on its development. Effects of enucleation on the zygote should be taken into consideration when developing the methods for microsurgical removal of the nuclei [3]. Intracellular potassium content is the most sensitive parameter reflecting impairment of cell homeostasis. Therefore, the aim of our study was to measure potassium content in a one-cell mouse embryo and evaluate the effect of enucleation on this parameter.

MATERIALS AND METHODS

One-cell embryos of NMRI mice were examined. The procedure of zygote enucleation was described previously [3]. Isolation of the zygote and microsurgical manipulations were carried out using modified Vitten's medium. Experiments were carried out at 20-22°C. Morphological control of the cell cycle phase was realized under a light microscope. Three zygote types were analyzed: intact cell, cell after re-injection of own nuclear material, and cell after removal of its own nuclear material and introduction of somatic cell nucleus.

Potassium concentration in the cytoplasm was evaluated in a cell section prepared as described previously [5,6]. The zygote in the morphologically identified cell cycle phase (G_1/S) was frozen in liquid propane and lyophilized at 200°K. Lyophilized sample was then embedded into resin polymerized at 330°K. The sections (2 μ) of the cell were analyzed under a JSM-U3 scanning electron microscope (JEOL). Electron probe microanalysis of intracellular potassium concentration was carried out at accelerating voltage of 25 kV (probe electron current about 5 nA, duration of analysis 20 sec).

RESULTS

Electron probe analysis indicated that microsurgical enucleation appreciably decreased potassium concentration in the zygote. Potassium concentration in the cytoplasm of a one-cell mouse embryo in the G_1/S phase was 119±6 mM for intact zygote (20 cells analyzed), 29±7 mM after re-transfection of own nuclear material into zygote (5 cells analyzed), 21±3 mM after transfection of somatic cell nucleus (13 cells analyzed), and 35±3 mM for intact zygote in the mitosis prophase (9 cells analyzed). It seems that disorders in potassium homeostasis were caused by collection of pronuclei, because the observed changes virtually did not depend on the type of manipulations with the nu-

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clear material. Judging from the level of cytoplasmic potassium concentration, microsurgical manipulations transferred the cell into the initial stage of mitosis. This shift of potassium homeostasis can appreciably modify further development of the one-cell embryo [4].

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