Effects of Cytotoxins on Intracellular Na/K Balance in Mouse Embryonic Cell

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Cytoplasmic Na/K imbalance induced by cytotoxic substances (ethylene glycol and cytochalasin B) was studied. Incubation in Dulbecco's medium with these cytotoxins caused changes in K and Na concentrations in the mouse two-cell embryo blastomer. The effects of both substances resulted in a drop of Na content in the embryonic cell. Washing from cytochalasin B restored the initial Na/K balance in the cytoplasm. Possible adaptive mechanisms involved in the regulation of intracellular ionic homeostasis are discussed.

Key Words: *mouse embryonic cell; cytochalasin B; ethylene glycol; cytoplasmic Na/K balance; electron probe microanalysis*

Many cell technologies, *e.g.* cryopreservation or enucleation, involve addition of cytotoxic substances (DMSO, propanediol, cytochalasins, *etc.*) to the incubation medium. The first targets of these substances are membrane proteins responsible for ion transport and hence, cell ion homeostasis. The effect of DMSO on Na/K-ATPase is direct [10,14], while cytochalasin B modifies ionic channels via transformation of the cytoskeleton [22].

Changes in K^+ content in the cytoplasm characterize not only traumatic effect of the exogenous agent, but reflect transition of the cell into a new functional status. Intracellular microclimate established *de novo* as a result of the effects of cytotoxins, can be the key factor in the regulation of cell status [9]. It is known that changes in the concentrations of the main cytoplasmic ions (K^+, Na^+) induce gene expression [21], DNA recombination [15], or actin depolymerization [11].

We analyzed disorders in Na/K balance in the cytoplasm of embryonic cell induced by cytotoxic substances used in cell technologies.

MATERIALS AND METHODS

The study was carried out on F_1(CBA×C57Bl) mice. The animals were kept under standard conditions (20±2°C, PK-121-2 granulated fodder, and free access to water). Water, fodder, and flooring were replaced daily without preliminary sterilization. Two-cell embryos were obtained as described previously [4]. Washed embryos were incubated for 10 min in Dulbecco's medium with 10% cryoprotector or 5 μg/ml cytochalasin B, which was then washed out for 20 min.

Experimental samples were compared with untreated embryos, which were incubated for 40 min in Dulbecco's solution. Embryos fixed directly after isolation from the oviduct served as the control. We previously described the scheme of cytochalasin B treatment [1] and the cryoprotector protocol, for example, with ethylene glycol, for mouse two-cell embryos [17,18].

Cytoplasmic concentrations of K and Na were measured by electron probe microanalysis (EPMA);

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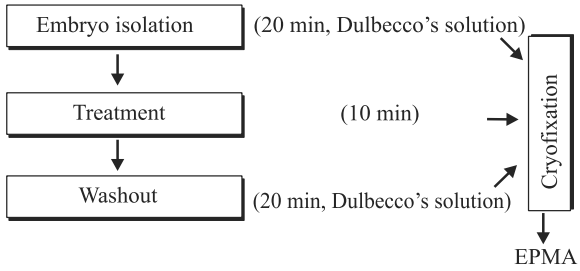

Fig. 1. Scheme of treatment of two-cell mouse embryo with cytotoxin and subsequent washing in Dulbecco's solution.

this method provides a unique possibility for measuring the concentrations of elements (K, Na) in a single morphologically identified cell [5] (Fig. 1).

The philosophy of the method based on cryofixation of biological tissue was described previously [5,12,16]. The initial stage is superquick freezing of the object in liquid propane, cooled with liquid nitrogen to -188°C. Frozen samples were lyophilized under vacuum (~10^{-5} Pa) at -90°C. After low-temperature dehydration, dry object was embedded in Epon 812 (medium on the base of epoxy resin). After polymerization, a section (diameter 2 μ) was prepared from the embryo plane with a glass blade on an ultramicrotome (Riechert) and mounted on a bland. Cryomethods for making the two-cell mouse embryo preparations were used in one's lifetime distribution of elements in the cell.

The preparation was examined under a JSM-U3 scanning electron microanalyzer microscope (JEOL) with an X-ray detector. The cytoplasmic concentrations of K and Na were calculated from the intensity of K_a line of X-ray radiation of the elements. Electron probe microanalysis was carried out at accelerating electron voltage of 25 kV (time of X-ray radiation recording 40 sec, probe current ~5 nA, which corresponds to probe diameter of ~0.1 μ). The morphology of unstained section was observed in the passed electron mode.

RESULTS

Electron probe microanalysis of cytoplasmic concentrations of K, Na in a blastomer was carried out on a section of mouse two-cell embryo, lyophilized and embedded in Epon 812 (Fig. 2). The results of measurements of intracellular K and Na in experiment with embryo treatment with ethylene glycol cryoprotector were summed up (Fig. 3).

The content of Na in the cytoplasm of control embryonic cell was close to that of K (~120 mM). Incubation of the embryo in Dulbecco's medium did not change the intracellular Na/K balance. Incubation with ethylene glycol resulted in an almost 2-fold drop of the cytoplasmic levels of both elements (Fig. 3). Hence, Na was released from the cell against the concentration gradient, for which the mechanisms of active ion transport had to be involved. Presumably, "sodium" effect was caused by activation of Na^+/K^+-ATPase directed to compensation of potassium deficit. Sodium transport from the blastomer by Na^+ pump, which was detected for many specialized cells [7,8], is also probable.

The concentration of K in control blastomer (Fig. 3) corresponds to, and for Na is somewhat

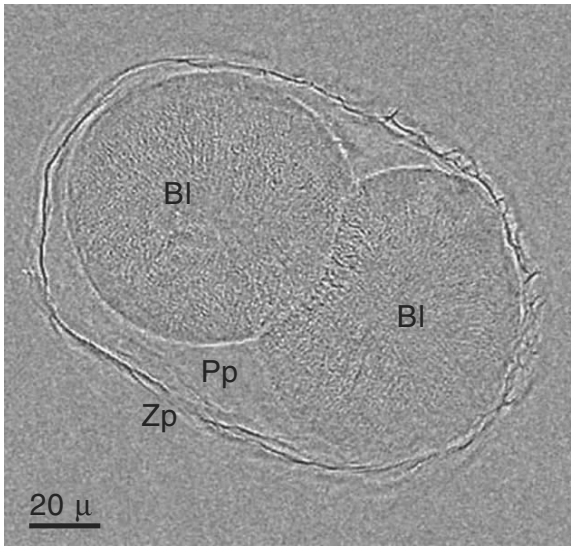

Fig. 2. Two-cell mouse embryo: unstained section (2 μ) of an embryo lyophilized and embedded in Epon 812 epoxy resin. Bl: blastomer; Pp: perivitelline space; Zp: zona pellucida.

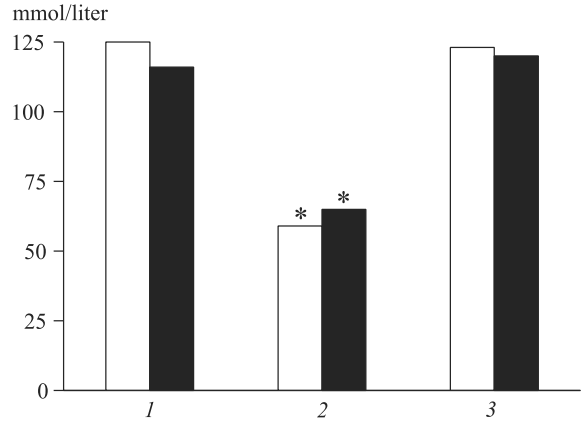

Fig. 3. Cytoplasmic concentrations of K (light bars) and Na (dark bars) in a two-cell blastomer of mouse embryo after incubation in Dulbecco's solution. Mean values for groups of at least 20 embryos. *1*) control (embryos fixed directly after isolation from the oviduct); *2*) ethylene glycol washing; *3*) incubation without ethylene glycol. Here and in Fig. 5: **p*<0.05 compared to the control. Data scatter did not exceed 10% for K and 15% for Na.

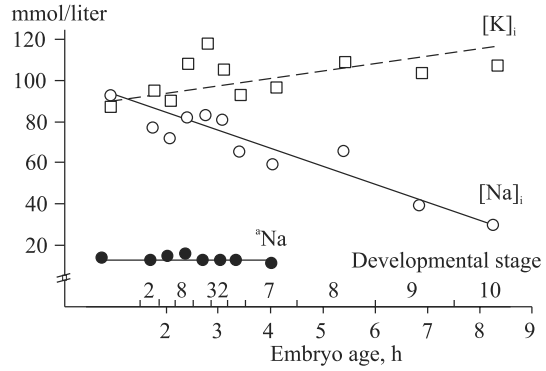

Fig. 4. Alteration of summary intracellular concentration of K and Na in early embryogenesis in frogs. Cytoplasmic concentrations of Na^+ cation presented for developmental stages were calculated from the results of measurements by ion-selective electrodes. Reproduced from C. Slack *et al.* [20].

higher than the value characteristic of somatic cells [6,19]. The same proportion was registered in early embryonic cells in frogs [20] (Fig. 4).

The concentrations of Na and K in early frog embryonic cell are shifted towards the values characteristic of a specialized cell. Presumably, this trend should be regarded as a sign of initial differentiation, determining the formation of blastocell.

The mechanism of the effect of cytochalasin on the complex of potassium channels and pumps *in vivo* is not quite clear. It is known that this chemical causes destruction of cell cytoskeleton and hypothetically can initiate cascade modifications in the membrane ion transport system, for example, ionic channels [22] or Na^+/K^+-ATPase [3].

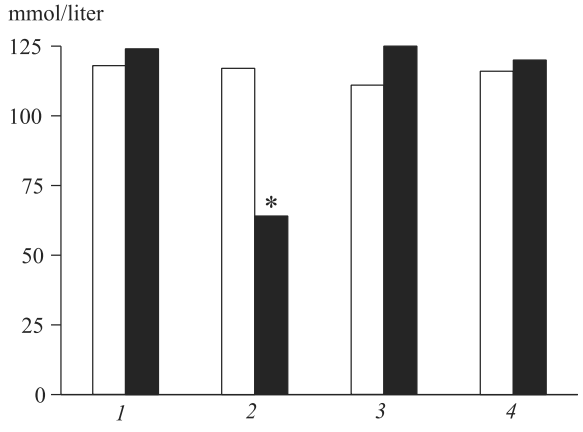

Fig. 5. Cytoplasmic concentrations of K (light bars) and Na (dark bars) in the mouse two-cell embryo blastomer after incubation in Dulbecco's solution with and without cytochalasin B (5 μg/ml). *1*) control (embryos fixed directly after isolation from oviduct); *2*) effect of cytochalasin B; *3*) cytochalasin B washing; *4*) incubation without cytochalasin B. Mean values for groups of at least 20 embryos.

Potassium deficit was registered in the early embryonic cell after transfer of the nucleus from somatic cell into the early embryo cell [2]. It is noteworthy that the intracellular K^+ concentration is an integrative result of countercurrent flows of passive and active transport.

The presence of cytochalasin B in the saline at all stages of the procedure is an obligatory condition for enucleation. Hence, changed cellular homeostasis is a result of combined effects of the substance and specific conditions characteristic of each stage of the protocol. The effect of cytochalasin B manifests only at the initial stage of embryo treatment with this substance.

Similarly as in the experiment with ethylene glycol, the level of cytoplasmic sodium in the control was much higher than in a somatic cell (Fig. 5). The "sodium" phenomenon is in line with the data obtained by other authors [20]. Comparison of the results of flame photometry and ion-selective electrodes suggested the presence of a pool of bound and free Na in the embryonic cell cytoplasm.

Cytochalasin B did not cause the expected deficit of intracellular potassium [1]: even if the chemical induced opening of potassium channels, this effect was compensated for by other mechanisms, for example, by activation of Na^+/K^+-ATPase. This conclusion is in good agreement with the fact that treatment with the chemical is associated with a drop of Na concentration in the embryonic cell (Fig. 5), directed against its concentration gradient. Na^+/K^+-ATPase can also be involved in the blastomer adaptation to the traumatic effect of cytochalasin B.

Washing of the cell from the substance restored abnormally high level of cytoplasmic Na characteristic of the early embryo. The mechanism of repeated accumulation of Na^+ needs further studies, as the known Na^+/H^+ exchange was not demonstrated in the mouse embryo at the two-cell stage [2]. It is noteworthy that washing from the cryoprotector in experiment with ethylene glycol did not lead to restoration of the Na/K balance in the blastomer (Fig. 3). Presumably, the difference in the biological activity of these substances is explained by different nature of their direct and(or) indirect effects on the system of ion transport through the embryonic cell membrane. It seems that we should evaluate the combined effect of chemical and physical "toxicities", for example, the osmos effect factor [13].

Hence, our findings demonstrate effective use of electron probe microanalysis for testing the traumatic effects of cell technology, for example, cryopreservation or enucleation. By using the proposed

approaches, we can see to what measure the *in vitro* cell status corresponds to the *in vivo* situation, in other words, evaluate the effects of biotechnological manipulations on cell homeostasis and cell biology in culture. The effect of cytotoxic substance on Na/K balance in embryonic cell cytoplasm is determined by specific modification of the K^+, Na^+ transport system or by its osmotic activity.

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