ISSN 0022-0930, Journal of Evolutionary Biochemistry and Physiology, 2012, Vol. 48, No. 2, pp. 147—151. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © M.Yu. Skorkina, 2012, published in Zhurnal Evolyutsionnoi Biokhimii i Fiziologii, 2012, Vol. 48, No. 2, pp. 126—129.

COMPARATIVE AND ONTOGENIC BIOCHEMISTRY

Osmoregulatory Reactions of Frog Erythrocytes under Conditions of Activation and Blockade of Ca^{2+}-Channels

M. Yu. Skorkina

Belgorod State University, Belgorod, Russia
E-mail: marina_skorkina@mail.ru

Received November 24, 2010

Abstract—The kinetics of cell osmoregulatory reactions under conditions of activation and blockade of Ca^{2+}-channels was studied on a model of frog polyfunctional nucleated erythrocytes. Both activation and blockade of Ca^{2+}-channels has been established to promote swelling of nuclei and an increase of the nucleocytoplasmic ratios under conditions of hypotonic exposure. The osmoregulatory cell reactions after activation of Ca^{2+}-channels are manifested as a decrease of the cell volume. The blocker of Ca^{2+}-channels verapamil produces a transitory increase and decrease of the erythrocyte volume with time intervals of 30 and 60 s. The clearly expressed functional activity of the nuclear membrane in response to the hypotonic action under conditions of activation and blockade of Ca^{2+}-channels indicates participation of Ca^{2+} ions in mechanisms of the nucleocytoplasmic transfer.

DOI: 10.1134/S002209301202003X

Key words: erythrocytes, osmoregulatory reactions, Ca^{2+}-channels, verapamil.

INTRODUCTION

The maintenance of cell volume represents a fundamental physiological mechanism controlled by universal regulatory systems—intracellular messengers, one of which is Ca^{2+} ions [1]. Adaptive cell response to osmotic swelling is realized with involvement of membrane ion-transporting systems [2]. In the animal cells the regulatory volume decrease is associated with the loss of sodium chloride due to activation of specific channels for sodium and chloride simultaneously. In several cases the regulatory volume decrease depends on Ca^{2+} inflow to the cell [3], whereas in other cell types the response is associated with instant Ca^{2+} efflux from the cellular depots [4]. In the same cell types the response to osmotic swelling includes an increase in glycogen synthesis and deceleration of glycolysis [5], an inhibition of proteolysis and intensification of protein synthesis [2, 6]. The regulatory volume increase is associated with uptake of sodium chloride and potassium chloride, which are accumulated in the cell due to activation of exchange channels of sodium for hydrogen and chloride for hydrocarbonate or activation of co-transporter for sodium, potassium, and chloride [7]. The morphological basis for the maintenance of volume homeostasis is the membrane reserve of cells. Its biological role, apart from activation of volume-sensitive ion transport pathways [8], is also realization of specific types of cell motility [9], activation and stimulation of intracellular metabolic reactions [10, 11]. In spite of wide spectrum of investigations focused on mechanisms of cellular osmoregulatory reactions performed by the present moment, participation of nucleus in these reactions has not been described. In the scientific literature, data on changes of nucleus volume and

reserve of caryolemma are practically absent. The goal of the performed work was study of kinetics of osmoregulatory reactions of nucleated erythrocytes—the whole cell and nucleus—at activation and blockade of Ca^{2+}-membrane transport systems under conditions of hypoosmotic swelling.

MATERIALS AND METHODS

The experiments were carried out on erythrocytes of frog *Rana ridibunda* Pall. The blood was taken by the heart puncture. The blood stabilization was performed by heparin (5 units/ml). The erythrocyte osmoregulatory reactions at hypoosmotic stress were studied under conditions of activation and blockade of membrane calcium channels in experiments *in vitro*. Activation of the Ca^{2+}-channels was performed in the Hank's medium containing 10–6 mmol/l Ca^{2+} for 15 min at room temperature. Incubation of erythrocytes in calcium medium results in an increase of its intracellular concentration [12] and induces their premature aging [13]. The Ca^{2+}-channels were blocked by incubation in the Hank's medium containing 10–6 mmol/l verapamil for 15 min at room temperature. After termination of incubation, two one-layer suspensions were prepared from each sample. One contained the cell samples in hypoosmotic medium (0.2% sodium chloride), the other—in isotonic medium (0.65% sodium chloride). The images on the preparations were recorded each 30 s for 10 min, then additionally each 10 min for 1 h of exposure a complex of apparatus-software of visualization of morphological preparations, analysis, and recording of optical and morphological parameters "VideoTest" (reg. license # 29/20010702/6102-04 from 16.02.2004). On the preparations the overall dimensions of all erythrocytes and nuclei were measured. From the measured overall cell dimensions there were calculated values of morphometric parameters (volume values, nucleocytoplasmic ratios, the surface area, coefficient of reserve membrane surface). The coefficient of reserve membrane surface at each time interval was calculated as the ratio of the cell surface area in 0.2% sodium chloride to the cell surface area in 0.65% sodium chloride. The statistical significance was evaluated by using Student's *t*-criterion.

RESULTS AND DISCUSSION

Upon exposure of erythrocytes in the hypotonic medium after calcium loading, swelling of nuclei and a regulatory decrease of cell volume were established. The volume of nuclei rose throughout the entire time of exposure. The most significant considerable differences in comparison to isotonic medium have been established at 150, 420, and 1800 s of incubation. By the end of incubation time (3600 s) a rise of volume was 170% ($p < 0.05$) as compared with control. The absence of recovery of nucleus volume by the end of incubation indicates development of disregulatory reactions in the cell. In particular, it was shown that the calcium influx to the cell was a potent pro-apoptotic signal activating the system of intracellular caspases and triggering apoptotic mechanisms [14]. At the morphological level, the beginning of apoptotic reactions was expressed in cell shrinkage and alterations of their morphology [13]. In the performed experiments there was noted a regulatory decrease of cell volume in hypotonic medium after calcium loading at 180 s (62.17%, $p < 0.05$), 360 s (64.88%, $p < 0.05$), and 540 s (70.46%, $p < 0.05$) of incubation.

Reactivity of plasmalemma to hypotonic action was revealed with interval of 180 s, while that of caryolemma—with interval of 150 s. An important morphological characteristics for evaluation of developing compensatory reactions in the cell is a parameter of the nucleocytoplasmic ratio (NCR). A pronounced NCR increase in response to hypotonic medium was observed at 30 s (46.15%, $p < 0.05$), 150 s (84.61%, $p < 0.05$), by the end of incubation—3600 s (73.33%, $p < 0.05$) as compared with isotonic medium. The functional activity of the nuclear membrane in response to hypotonic treatment is associated with involvement of Ca^{2+} in mechanisms of the nucleocytoplasmic transport. According to contemporary ideas, the subunits of nuclear pores function in the caryolemma as ion channels. The investigations performed on a PC identified the central granule that can function as ion channel and simultaneously as the route for proteins/RNA [15]. Besides, it was shown that protein of subunit composing nuclear pore was mobile and changed its configuration depending on surrounding milieu [16].

Several works have established that hypotonic shock (50% tonicity) stimulates an increase of free calcium in the cytoplasm. The literature reviews present examples of various cells whose level of intracellular calcium increases in response to acute osmotic swelling [17, 18]. Therefore, in the next series of experiments we used a blocker of slow calcium channels (L-channels) verapamil. Calcium antagonists in micro molar amounts modulate receptor-operating channels suppressing their permeability to calcium and intensifying permeability to sodium [19]. As a results of the performed experiments, there was established a decrease in the cell volume to 90 s of their incubation by 32.18% ($p < 0.05$), which was replaced by a regulatory volume increase to 150 s of exposure to the isotonic values, then to 180 s—by a volume decrease by 15.43% ($p < 0.05$).

After action of the Ca^{2+}-channel blocker the volume of nucleus sharply increased as soon as after 30 s of incubation by 172% ($p < 0.05$) and was significantly higher than values in the isotonic medium throughout the entire exposure. The recovery of nuclear volume by the end of exposure was not observed. Under conditions of calcium channel blockade throughout the entire period of hypotonic loading, NCR was elevated. The greatest differences with isotonic medium were noted at 30 s (0.43 ± 0.06 rel. units) and 150 s (0.26 ± 0.01 rel. units) of incubation. It cannot be ruled out that the observed reactions from caryolemma at the blockage of Ca^{2+}-channels are due to an increase of intracellular concentration of Ca^{2+} ions in the hypotonic medium [17], which, in turn, activates the processes in the nucleus.

An important peculiarity of cell structure is their ability to maintain volume homeostasis under conditions of hypoosmotic loading. The basis of adaptation morphogenesis is the membrane reserve of cells, which was estimated quantitatively on the basis of the reserve surface coefficient. During hypotonic loading, plasmalemma and caryolemma use as an additional reserve the surface containing membrane folds and providing their morphological integrity. Most probably, this process occurs due to structural rearrangements of sub membranous layers of the cite- and caryolemma and formation of cutovers [20]. The used membrane reserve of plasmalemma after calcium and verapamil loadings under conditions of hypotonic medium was increased for the first 90 s of exposure. Starting from 120 s of exposure, the kinetics of reactions had the opposite-directed character. Under effect of calcium loading the used membrane reserve decreased, whereas upon blockage of calcium channels with verapamil it increased (Fig. 1). An effect of verapamil on calcium channels at decreased medium similarity induced functioning of calcium channels of the L-type [21], thus activating Na^+/K^+-co transport and influx of water into the cell, which leads to an increase of volume and maximal use of membrane reserve structures for compensatory response. The equilibrium redistribution of membrane structures in response to hypotonic effect to activation and blockade of calcium channels was observed at 150, 300, and 3600 s of exposure.

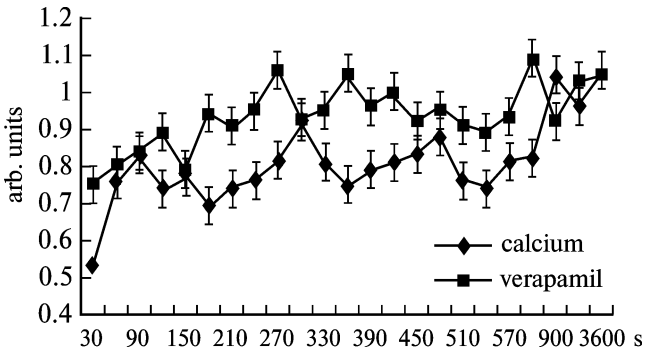

Fig. 1. Dynamics of the plasmalemma membrane reserve in hypotonic medium. *Abscissa*: time of incubation (s), *ordinate*: coefficient of the plasmalemma reserve surface, in rel. units.

In caryolemma, there was observed a tendency for an increase of use of membrane reserve under conditions of calcium loading and for its decrease at blockage of calcium channels for the first 270 s of incubation. The dynamics of response of the caryolemma reserve surface coefficient to the hypotonic action was similar within the time intervals from 300 to 360 s and from 390 s to the end of incubation (Fig. 2).

The greater use of the caryolemma membrane reserve at activation of calcium channels might be due to excitation of P_2 receptor on erythrocyte membrane under conditions of hypotonic loading, which results in an increase of cryptozoic Ca^{2+} that triggers intracellular endonucleases damaging the nucleus structure [10].

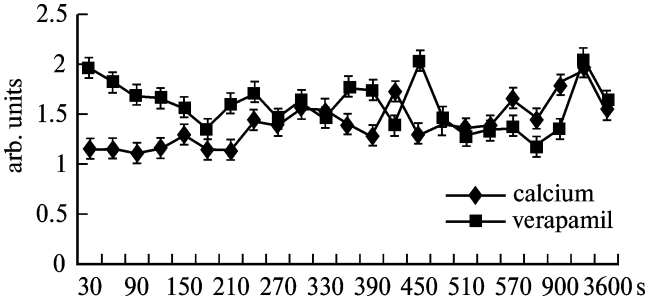

Fig. 2. Dynamics of membrane reserve of caryolemma in hypotonic medium. *Abscissa*: time of incubation (s), *ordinate*: coefficient of the caryolemma reserve surface, in rel. units.

Thus, the cell response to hypotonic action represents an integral reaction whose morphofunctional basis is the membrane reserve of plasmalemma and caryolemma. The objective quantitative criterion reflecting kinetics of osmoregulatory reaction is the reserve surface coefficient. After the Ca^{2+}-loading there is observed swelling of nuclei in the hypotonic medium. The volume of cells decreases and its increase to the values of isotonic medium is noted only by the end of incubation. The absence of the nuclear volume restoration on the background of cell shrinkage is a parameter of development of disregulatory reactions. Application of Ca^{2+}-channel inhibitor verapamil leads to a regulatory increase and a decrease of cell volume with time intervals of 30 and 60 s. The volume of nucleus sharply increases at 30 s of incubation and remains increased for the entire period of exposure. The stimulation and blockage of Ca^{2+}-channels initiates an increase of NCR toward the increase of the nucleus volume.

ACKNOWLEDGMENTS

The work was performed with financial support of grants within realization of Federal Central Program "Scientific and scientific-pedagogic staff of the innovative Russia" for 2009-2013.

REFERENCES

1. Gusev, N.B., Intracellular Ca-Binding Proteins, *Sorosovskii Obrazovatel'nyi Zhurnal*, 1998, no. 5, pp. 2–9.
2. Haussinger, D. and Lang, F., Cell Volume in Regulation of Hepatic Function: a Mechanism for Metabolic Control, *Biochim. Biophys. Acta*, 1991, vol. 1974, no. 4, pp. 331–350.
3. Montrose-Rafizadeh, C. and Guggio, W.B., Role of Intracellular Calcium in Volume Regulation by Rabbit Medullary Thick Ascending Limb Cells, *Am. J. Physiol.*, 1991, vol. 260, pp. F402–F409.
4. Terreros, D.A. and Kanli, H., Role of Intracellular Calcium in Renal Proximal Tubule Cell Volume Regulation, *Am. J. Physiol.*, 1992, vol. 263, pp. R1086–R1092.
5. Baquet, A., Lavoinne, A., and Hue, L., Comparison of the Effects of Various Amino Acids on Glycogen Synthesis, Lipogenesis and Ketogenesis in Isolated Rat Hepatocytes, *Biochem. J.*, 1991, vol. 273, pp. 57–62.
6. Stoll, B., Grem, K.W., Lang, F., and Housinger, D., Liver Cell Volume and Protein Synthesis, *Biochem. J.*, 1992, vol. 287, pp. 217–222.
7. Strange, K., Cellular Volume Homeostasis, *Adv. Physiol. Educ.*, 2004, vol. 28, pp. 155–159.
8. Chebotareva, N.A., Effect of Molecular Crowding of Glycolytic Enzymes, *Usp. Biol. Khim.*, 2007, vol. 47, pp. 233–258.
9. Fedorova, M.Z., Nadezhdin, S.V., Golovko, S.N., and Zubareva, E.V., Comparative Evaluation of "Membrane Reserve" of Blood Cells of Amphibians and Mammals, *Zh. Evol. Biokhim. Fiziol.*, 2007, vol. 43, pp. 419–422.
10. Minton, A.O., Influence of Macromolecular Crowding upon the Stability and State of Association of Proteins: Predictions and Observation, *J. Pharm. Sci.*, 2005, vol. 94, pp. 1668–1675.
11. Al-Habori, M., Macromolecular Crowding and Its Role as Intracellular Signaling of Cell Volume Regulation, *Int. J. Biochem. Cell. Biol.*, 2001, vol. 33, pp. 844–864.
12. Light, D.B., Attwood, A.J., Siegel, C., and Baumann, L., Cell Swelling Increases Intracellular Calcium in *Necturus* Erythrocytes, *J. Cell. Sci.*, 2002, vol. 116, pp. 101–109.
13. Bratosin, D., Estraquier, J., Petit, F., and Arnoult, D., Programmed Cell Death in Mature Erythrocytes: a Model for Investigating Death Effectors Pathways Operating in the Absence of Mitochondria, *Cell Death Differ.*, 2001, vol. 8, pp. 1143–1156.
14. Romero, P.J. and Romero, E.A., Effect of Cell Ageing on Ca^{2+} Influx into Human Red Cells, *Cell Calcium*, 1999, vol. 26, pp. 131–137.
15. Folprecht, G.S. and Oberleithner, H., Aldosterone Activates the Nuclear Pore Transporter in Cultured Kidney Cells Image with Atomic Force Microscopy, *Pflugers Arch.*, 1996, vol. 432, pp. 831–838.
16. Mazzanti, M., Ion Permeability of the Nucle-

ar Envelope, *News Physiol. Sci.*, 1998, vol. 113, pp. 44–50.
17. Foskett, J.K., The Role of Calcium in the Control of Volume Regulatory Transport Pathways, *Cellular and Molecular Physiology of Cell Volume Regulation*, 1994, pp. 259–277.
18. McCarty, N.A. and O'Neil, R., Calcium Signaling in Cell Volume Regulation, *Physiol. Rev.*, 1992, vol. 72, pp. 1037–1061.
19. Avdonin, P.V. and Cheglakov, I.B., Modulation of Receptor-Controlled Channels in Platelets by Calcium Antagonists, *Farmakologiya*, 1999, no. 4, pp. 25–28.
20. Fedchenko, N.N., Fedchenko, N.P., and Garets, V.I., Cytometers—a Novel Type of Informogenic Cell Elements in Organism, *Voprosy morfologii XXI veka* (Problems of Morphology of the XXI Century), St. Petersburg, 2008, pp. 298–306.
21. Nohanty, M.J., Ye, M., Li, X., and Rossi, N.F., Hypotonic Swelling-Induced Ca^{2+} Release by an IP^3-Insensitive Ca^{2+} Store, *Am. J. Physiol. Cell. Physiol.*, 2001, vol. 281, pp. C555–C562.