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BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

The Inhibitors of Arp2/3 Complex and WASP Proteins Modulate the Effect of Glutoxim on Na^+ Transport in Frog Skin

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Abstract—Using voltage-clamp technique, the involvement of WASP proteins and Arp2/3 complex in the effect of immunomodulator drug glutoxim on Na^+ transport in frog skin was investigated. It was shown for the first time that preincubation of the skin with the N-WASP inhibitor wiskostatin or the Arp2/3 complex inhibitor CK-0944666 significantly decreases the stimulatory effect of glutoxim on Na^+ transport. The data suggest the involvement of actin filament polymerization and branching in the glutoxim effect on Na^+ transport in frog skin.

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The skin of amphibians and other isolated epithelial systems are classic model objects for studying the mechanisms of ion transport through biological membranes. By the ability to transport electrolytes and by the response to certain hormones, the skin and bladder of amphibians are similar to distal parts of renal tubules [1], which makes it possible to use the data obtained on these objects for clarifying the mechanisms of water and ion transport in kidney cells.

It is known that the key Na^+-transporting proteins, such as amiloride-sensitive epithelial Na^+-channels (ENaC), Na^+/K^+-ATPase, and Na^+/H^+-exchanger, are targets for oxidizing and reducing agents [2]. However, the molecular mechanisms of action of oxidants and reductants on the components of the transepithelial Na^+ transport system remain practically unstudied.

Earlier, we were the first to show that Na^+ transport in frog skin is modulated by oxidants, such as cysteamine, cystine, oxidized glutathione (GSSG), and its synthetic analogue drug Glutoxim® (PHARMA-VAM, Russia) [3]. In the cited paper, it was shown for the first time that, when applied on the basolateral surface of frog skin, GSSG and glutoxim mimic the effect of insulin and stimulate transepithelial Na^+ transport. With the use of pharmacological agents that affect the structural elements and signaling system components in cells, we also first showed that the regulation of Na^+ transport in frog skin by glutoxim is mediated by tyrosine kinases and phosphatidylinositol kinases [4], protein kinase C [5], serine/threonine protein phosphatases PP1/PP2, microtubules and microfilaments [6], the products of the cyclooxygenase pathway of arachidonic acid oxidation [7], and vesicular transport processes [8].

It is known that actin cytoskeleton is involved in the modulation of activity of many Na^+-transporting proteins colocalized to actin filaments and actin-binding proteins (ankyrin and spectrin) as well as participates in the regulation of transepithelial Na^+ transport by some hormones [9]. The Arp2/3 complex (actin-related proteins) plays a key role in the formation of microfilaments consisting of G-actin monomers. The nucleation sites also comprise WASP proteins (Wiskott–Aldrich syndrome family proteins), which activate the Arp2/3 complexes, ensure their interaction with actin monomers, and trigger actin polymerization and formation of branched actin filaments [10].

In view of above, it was of interest to investigate the possible involvement of actin filament growth and branching in the mechanisms of action of glutoxim on Na^+ transport in frog skin. This was the subject of this communication.

Experiments were performed on male frogs *Rana temporaria* in the period from November to March. Abdominal frog skin was cut and placed in an Ussing chamber (World Precision Instruments, Inc., Germany) with a diameter of the inner orifice of 12 mm. Experiments were performed at room temperature (22–23°C). The current-voltage characteristics (I-V relations) of frog skin were recorded using an automated voltage-clamp device [3]. This allowed us to determine the following electrical parameters of the skin: the short-circuit current I_{SC} ($I_{SC} = I_T$ at $V_T = 0$, where I_T is the transepithelial current), the open-circuit potential V_{OC} ($V_{OC} = V_T$ at $I_T = 0$, where V_T is the transepithelial potential), and the transepithelial conductance g_T. Na^+ transport was estimated as amiloride-sensitive I_{SC}. The reagents used in experi-

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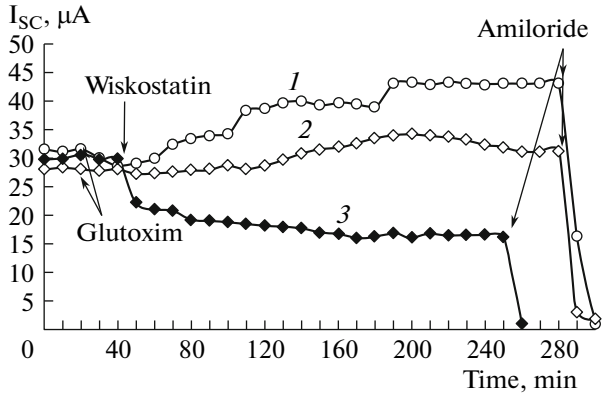

Fig. 1. Kinetics of changes in the short-circuit current I_{SC} through frog skin in response to glutoxim and wiskostatin. Designations here and in Fig. 2: *1*—I_{SC} after the application of 100 μg/mL glutoxim on the basolateral surface of intact skin; *2*—I_{SC} after the application of glutoxim on frog skin pretreated (30 min) from the apical surface with 10 μM wiskostatin; *3*—I_{SC} after the addition of 10 μM wiskostatin from the apical skin surface. Here and in the experiments, the results of which are shown in Fig. 2, 20 μM amiloride (ENaC blocker) was added to the solution bathing the apical surface of the skin at the end of each experiment.

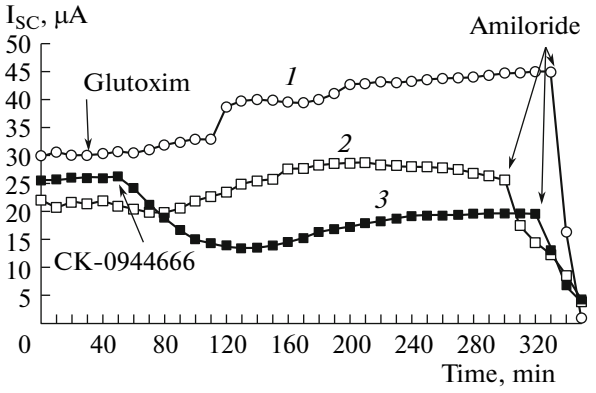

Fig. 2. *1*—Kinetics of changes in the short-circuit current I_{SC} through frog skin in response to glutoxim and compound CK-0944666; *2*—I_{SC} after the application of glutoxim on frog skin pretreated (30 min) with 100 μM CK-0944666 from the apical surface; *3*—I_{SC} after the application of 100 μM CK-0944666 from the apical skin surface.

ments were from Sigma (United States). In addition, we used wiskostatin, a selective inhibitor of the N-WASP protein [11], and compound CK-0944666, an inhibitor of the Arp2/3 complex [12]. Wiskostatin and CK-0944666 were added 30–40 min before the addition of glutoxim to the solution.

Statistical analysis was performed using Student's *t* test. The data were represented as $M \pm m$. Differences were regarded significant at $p \le 0.05$. The figures show the results of typical experiments.

The mean electrical characteristics of frog skin in the control (according to the results of ten experiments) were as follows: $I_{SC} = 30.31 \pm 3.14$ μA, $V_{OC} = -52.28 \pm 6.25$ mV, and $g_T = 0.57 \pm 0.14$ mS. We found that glutoxim (100 μg/mL) applied on the basolateral surface of intact frog skin, similarly to insulin, stimulated Na^+ transport (Figs. 1, 2, curve *1*). After glutoxim application, I_{SC} and V_{OC} increased, on average, by 31.24 ± 8.32 and 38.04 ± 5.15%, respectively, and g_T did not change (according to the results of ten experiments).

We also found that wiskostatin (Fig. 1, curve *3*) and CK-0944666 (Fig. 2, curve *3*) inhibited Na^+ transport in frog skin. For example, after the treatment of the apical skin surface with 10 μM wiskostatin or 100 μM CK-0944666 (according to the results of ten experiments), I_{SC} decreased by 32.34 ± 5.32 and 25.79 ± 3.5%, respectively; V_{OC} decreased by 28.01 ± 5.15 and 21.59 ± 8.34%, respectively; and g_T decreased by 10.77 ± 1.12 and 3.31 ± 0.91%, respectively. It was also found that the pretreatment of skin with wiskostatin (Fig. 1, curve *2*) or CK-0944666 (Fig. 2, curve *2*) reduced the stimulatory effect of glutoxim on Na^+ transport in frog skin. On average (according to the results of ten experiments), the electrical characteristics of frog skin after application of 100 μg/mL glutoxim on the basolateral surface of the skin that was pretreated for 30 min with 10 μM wiskostatin or 100 μM CK-0944666 on the apical surface, were as follows: I_{SC} increased by 14.34 ± 3.12 and 18.75 ± 4.01%, respectively; V_{OC} increased by 16.09 ± 5.11 and 5.98 ± 0.34%, respectively; and g_T increased by 1.58 ± 0.32 and 13.47 ± 2.85%, respectively.

It is known that many Na^+-transporting proteins contain numerous cysteine residues, which are targets for intra- and extracellular oxidizing and reducing agents [2]. The addition of the ENaC blocker amiloride (20 μM) to the solution bathing the apical surface of frog skin completely inhibited Na^+ transport (Figs. 1, 2). This indicates that the effect of glutoxim on Na^+ transport is determined primarily by the modulation of ENaC activity.

Thus, we were the first to show that wiskostatin, an inhibitor of WASP proteins, and compound CK-0944666, an inhibitor of the Arp2/3 complex, inhibited Na^+ transport and suppressed the stimulatory effect of glutoxim on Na^+ transport in frog skin cells. The results are substantially complementary to the published data. Using various types of epithelial cells, it was found that actin-associated proteins are involved in the modulation of the activity of ENaC proteins. For example, using transfected ovarian cells of Chinese hamster (CHO), it was shown that the Arp2/3 complex and the actin-binding protein cortactin modulate the gating characteristics of ENaC, reducing the probability of the open channel state [13]. In addition, coexpression of ENaC and N-WASP protein significantly increased the ENaC activity in CHO cells [14]. At the same time, in other types of epithelial cells, such as freshly isolated cells of rat kidney collecting ducts (CCD cells), the inhibitor of N-

WASP proteins wiskostatin had no effect on Na^+ reabsorption and ENaC activity [13].

We have previously shown that Na^+ transport in frog skin depends on the structural and functional organization of the actin and tubulin cytoskeleton components [15]. It was also found that any changes in the structure of microtubules and microfilaments lead to a decrease in the stimulatory effect of glutoxim on Na^+ transport [6]. The results of this study also indicate that the inhibition of growth and branching of actin filaments inhibit Na^+ transport and suppress the effect of glutoxim on Na^+ transport in frog skin.

Thus, our results and published data allow the Arp2/3 protein complex and WASP proteins to be considered as an important component involved in the signaling cascades triggered by glutoxim in frog skin epithelial cells.

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REFERENCES

1. Natochin, Yu.V., *Osnovy fiziologii pochki* (Fundamentals of Kidney Physiology), Leningrad: Nauka, 1982.
2. Firsov, D., Robert-Nicoud, M., Gruender, S., et al., *J. Biol. Chem.,* 1999, vol. 274, pp. 2743–2749.
3. Krutetskaya, Z.I., Lebedev, O.E., Mel'nitskaya, A.V., et al., *Dokl. Biol. Sci.,* 2008, vol. 421, no. 5, pp. 235–238.
4. Melnitskaya, A.V., Krutetskaya, Z.I., Lebedev, O.E., et al., *Cell Tissue Biol,* 2010, vol. 4, no. 3, pp. 273–279.
5. Melnitskaya, A.V., Krutetskaya, Z.I., Lebedev, O.E., et al., *Biol. Membr.*, 2009, vol. 26, no. 4, pp. 320–321.
6. Melnitskaya, A.V., Krutetskaya, Z.I., Lebedev, O.E., et al., *Cell Tissue Biol.,* 2012, vol. 6, no. 3, pp. 248–253.
7. Krutetskaya, Z.I., Mel'nitskaya, A.V., Antonov, V.G., et al., *Dokl. Biol. Sci.*, 2013, vol. 451, pp. 193–195.
8. Melnitskaya, A.V., Krutetskaya, Z.I., Butov, S.N., et al., *Biophysics* (Moscow), 2014, vol. 59, no. 5, pp. 718–720.
9. Els, W.J. and Chou, K.Y., *J. Physiol.,* 1993, vol. 462, pp. 447–464.
10. Bouma, G., Burns, S.O., and Thrasher, A.J., *Immunobiology,* 2009, vol. 214, pp. 778–90.
11. Peterson, J.R., Bickford, L.C., Morgan, D., et al., *Nat. Struct. Mol. Biol.,* 2004, vol. 11, pp. 747–755.
12. Nolen, B.J., Tomasevic, N., Russell, A., et al., *Nature*, 2009, vol. 20, pp. 1031–1034.
13. Karpushev, A.V., Levchenko, V., Ilatovskaya, D.V., et al., *Hypertension*, 2011, vol. 57, pp. 996–1002.
14. Ilatovskaya, D.V., Pavlov, T.S., Levchenko, V., et al., *FASEB J.*, 2011, vol. 25, pp. 2688–2699.
15. Melnitskaya, A.V., Krutetskaya, Z.I., and Lebedev, O.E., *Tsitologiya*, 2006, vol. 48, no. 10, pp. 817–840.

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