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PHYSIOLOGY

Involvement of Microtubules in the Glutoxim Regulation of Na^+ Transport in the Frog Skin

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The amphibian skin and other isolated epithelial systems are classical model objects for studying the mechanisms of transepithelial ion transport. In their ability to transport electrolytes and in response to some hormones, the amphibian skin and bladder are similar to the distal renal tubules [1]; this allows the data for these model objects to be used for clarifying the mechanisms of water and ion transport in renal cells. The Na^+ transport in osmoregulatory epithelia is a complex multicomponent system providing the establishment and maintenance of electrolytic and water homeostasis. The key sodium transport proteins, such as amiloride-sensitive epithelial Na^+ channels (ENaCs), Na^+/K^+ ATPases, and Na^+/H^+ exchangers, are targets for oxidative stress [2]. However, the mechanisms underlying the effect of oxidants and reducing agents on individual components of the Na^+ transepithelial transport are still unknown.

Recently, new disulfide-containing agents with *d*-metals as nanoadditives, altering cell redox state, have been widely used. In particular, the drug Glutoxim® (disodium salt of oxidized gluthathione, GSSG, with platinum nanoaddition; FARMA-VAM, Moscow, Russia) has been introduced into clinical practice as an immunomodulator and a hemostimulant in the integrated therapy of bacterial and viral diseases, psoriasis, as well as radio- and chemotherapies of cancer [3].

We earlier demonstrated that the Na^+ transport in the frog skin was modulated by various oxidants. This was the first finding to demonstrate that GSSG and glutoxim applied to the basolateral frog skin surface imitate the effect of insulin and stimulate Na^+ transepithelial transport [4]. Later, it was shown that tyrosine kinases, phosphatidylinositol kinases [5, 6], protein kinase C [7], serine/threonine protein phosphatases PP1/PP2, and actin filaments [8] are involved in the glutoxim regulation of Na^+ transport in the frog skin. It is known that microtubules, similar to actin microfilaments, have a high redox sensitivity and are easily glutathionylated [9]. In addition, destruction of microtubules inhibits a stimulatory effect of several hormones on the Na^+ transport and prevents development of adaptive responses in osmoregulatory epithelial cells [10]. Correspondingly, it was reasonable to study the possible role of microtubules in the glutoxim regulation of Na^+ transport in the frog skin. Two structurally different microtubule depolymerizing agents, nocodazole and colcemid, were used in the experiments, as well as taxol, a microtubule-stabilizing agent.

The experiments were performed with male frogs *Rana temporaria* from October to March. The abdominal skin was cut off and placed into an Ussing chamber (World Precision Instruments, Germany) with an inner orifice 12 mm in diameter. The experiments were conducted at a room temperature (22–23°C). The current–voltage characteristics of the frog skin were recorded using an automated device for voltage-clamp [8]. The current–voltage characteristics were used to determine the skin electrical parameters, namely, short-circuit current, I_{SC} ($I_{SC} = I_T$ at $V_T = 0$, where I_T is transepithelial current); open-circuit potential, V_{OC} ($V_{OC} = V_T$ at $I_T = 0$, where V_T is transepithelial potential); and transepithelial conductance, g_T. The Na^+ transport was assessed as an amiloride-sensitive I_{SC}. The agents causing microtubule rearrangement were added 30–40 min before supplementing solution with glutoxim. Student's *t*-test was used for statistical processing; the data are shown as $x \pm s_x$. The figure shows the results of typical experiments.

The average values of electrical characteristics of the frog skin in the control (according to the data of 10 experiments) were as follows: $I_{SC} = 14.58 \pm 0.91$ μA, $V_{OC} = -38.01 \pm 2.74$ mV, and $g_T = 0.38 \pm 0.01$ mS. It has been shown that glutoxim (100 μg/mL) applied to the basolateral surface of intact frog skin stimulates the Na^+ transport. On average (according to the data of 10 experiments), the I_{SC} value after glutoxim appli-

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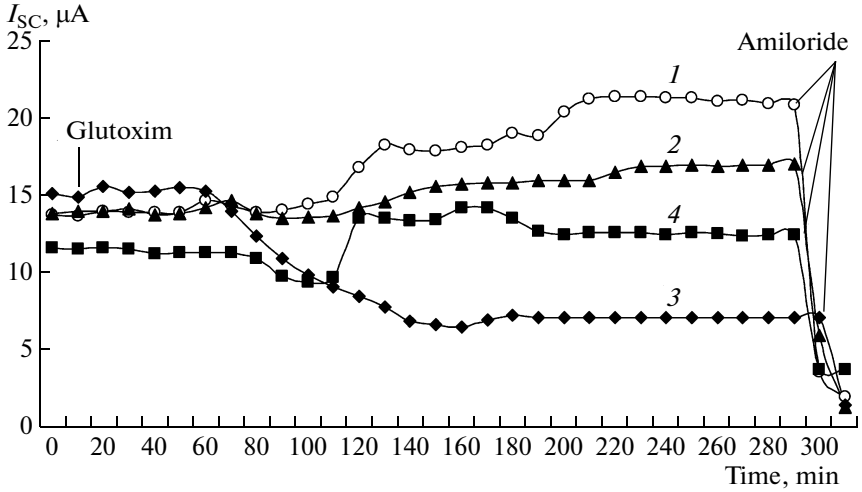

Dependence of the changes in short-circuit current, I_{SC}, through the frog skin in response to glutoxim on integrity of the tubulin cytoskeleton: (*1*) I_{SC} after applying 100 μg/mL glutoxim onto the basolateral surface of intact frog skin; (*2–4*) I_{SC} after applying glutoxim onto the frog skin pretreated (for 30 min) from the apical side with (*2*) 25 μM nocodazole, (*3*) 25 μM colcemid, or (*4*) 50 μM taxol; at the end of each experiment, the solution bathing the apical skin surface was supplemented with 20 μM amiloride, an ENaC blocker.

cation increased by 31.24 ± 8.32%; V_{OC}, by 38.04 ± 5.15%; and g_T did not change.

Nocodazole, colcemid, or taxol significantly decreased the glutoxim stimulatory effect on Na^+ transport (figure). On average (according to the data of 10 experiments), the electrical characteristics of frog skin after applying glutoxim when the apical skin surface was pretreated with nocodazole at various concentrations for 30 min changed in the following manner: I_{SC} increased by 12.85 ± 3.13 and 8.75 ± 1.10% and V_{OC}, by 11.31 ± 2.04 and 9.45 ± 1.08% for nocodazole at concentrations of 10 and 25 μM, respectively. The pretreatment of the apical skin surface with colcemid at various concentrations not only completely inhibited the stimulatory effect of glutoxim on Na^+ transport, but also considerably decreased the skin electrical characteristics. In particular, I_{SC} decreased by 20.12 ± 3.18 and 29.12 ± 5.03% and V_{OC}, by 23.14 ± 4.05 and 31.34 ± 6.12% at colcemid concentrations of 25 and 50 μM, respectively. If the apical surface of frog skin was preincubated with taxol, I_{SC} increased after glutoxim application by 9.13 ± 2.08 and 5.06 ± 1.32% and V_{OC}, by 10.14 ± 3.12 and 8.41 ± 2.81% for taxol concentrations of 20 and 50 μM, respectively. Any changes in the g_T value were not observed in any experiment.

It has been also found that nocodazole, colcemid, and taxol modulate the effect of glutoxim on Na^+ transport. We have earlier demonstrated that GSSG or glutoxim applied to the basolateral frog skin surface imitated the effect of insulin and stimulate Na^+ transepithelial transport, inducing a two-phase increase in I_{SC} [4]. The figure shows the changes in the I_{SC} through the frog skin after applying 100 μg/mL glutoxim onto the basolateral surface of intact skin (figure, curve *1*), as well as the skin pretreated (for 30 min) from the apical side with 25 μM nocodazole (figure, curve *2*), 25 μM colcemid (figure, curve *3*), or 50 μM taxol (figure, curve *4*). It is evident that nocodazole and colcemid completely inhibit both phases in the glutoxim stimulatory effect, characteristic of this oxidant, on the Na^+ transport in frog skin. The skin pretreatment with taxol also completely inhibits the second phase in glutoxim action but almost does not change the initial phase in the glutoxim-induced stimulation of Na^+ transport in frog skin (figure, curve *4*).

We have earlier shown that the Na^+ transport in frog skin depends on the structural and functional organization of actin and tubulin cytoskeleton [11, 12]. It has been also demonstrated that antimitotic agents (colchicine, colcemid, and vinblastine) inhibit I_{SC} in the *R. temporaria* skin. According to the literature data, disturbance of the microtubule structure by antimitotic agents decreases a stimulatory effect of various hormones (aldosterone and vasopressin) on the Na^+ transepithelial transport [10]. In addition, destruction of microtubules completely prevents many effects of insulin on cells [13]. Presumably, microtubules play an important role in exocytosis and endocytosis, being involved in the regulation of the ENaC density in the apical membrane. On the other hand, some data suggest that the sulfhydryl residues of tubulin are targets for oxidants. It has been shown that GSSG at concentrations exceeding physiological ones inhibits in vitro assembly of microtubules [14]. Our data also suggest that the tubulin cytoskeleton is involved in the glutoxim regulation of Na^+ transport in the frog skin.

ENaC play the key role in the Na^+ transport in reabsorbing epithelia. Numerous cysteine residues located in different ENaC segments determine the ENaC sensitivity to the redox state and represent the target for intracellular and extracellular oxidants and reducing agents [2, 15]. Addition of an ENaC blocker, amiloride (20 μM), at the end of each experiment to the medium bathing the apical surface of frog skin caused a complete inhibition of I_{SC} (figure), suggesting that the glutoxim effect on Na^+ transport is mainly associated with ENaC activity modulation.

Thus, our results suggest that microtubules are involved in the action of glutoxim on the Na^+ transport in frog skin and that any changes in the structure of tubulin cytoskeleton, be it stabilization of depolymerization, lead to a considerable decrease in the stimulatory effect of glutoxim on the Na^+ transport. The results of this work, as well as of our earlier studies [4–8, 11], suggest that glutoxim interacts with the cysteine-rich domains of the insulin receptor in the basolateral membrane of epithelial cells, causes its transactivation, and triggers a signaling cascade involving tyrosine kinases, phosphatidylinositol kinases, protein kinase C, protein phosphatases, and elements of the actin and tubulin cytoskeleton, thereby leading to stimulation of the Na^+ transport in frog skin.

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