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PHYSIOLOGY

Inhibitors of the Cyclooxygenase Oxidation Pathway of Arachidonic Acid Suppress the Stimulating Effect of Glutoxim on Na^+ Transport in Frog Skin

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The amphibian skin and other isolated epithelial systems are classical model objects for investigation of transepithelial ion transport. The amphibian skin and urinary bladder are similar to distal portions of the renal tubules in their transport of electrolyte and reactions to some hormones [1], which allows using data obtained on these objects for clarification of mechanisms of water and ion transport in renal cells.

Recently, new disulfide-containing agents with d-metals as nanoadditives, altering the cell redox state, have been widely used in clinical practice. Thus, the drug Glutoxim® (disodium salt of oxidized gluthathione (GSSG) with a platinum nanoadditive; PHARMA-VAM, Moscow, Russia) has been widely applied in clinical practice as an immunomodulator and a hemostimulant in the integrated therapy of bacterial and viral diseases and psoriasis, as well as radio- and chemotherapies of cancer [2].

We have demonstrated earlier that Na^+ transport in the frog skin is modulated by various oxidizing agents. It has been demonstrated that GSSG and glutoxim applied to the basolateral surface of the frog skin imitate the effect of insulin and stimulate transepithelial Na^+ transport [3]. Henceforth, it has been shown that tyrosine kinases, phosphatidylinositol kinases [4, 5], protein kinase C [6], serine/threonine protein phosphatases PP1/PP2A [7], as well as microtubules and actin filaments [7, 8], are involved in the GSSG and glutoxim regulation of Na^+ transport in the frog skin, as well as in signaling cascades triggered by insulin. However, the molecular mechanisms underlying the regulatory effect of GSSG and glutoxim on the Na^+ transport remain unclear.

It is known that arachidonic acid (AA) and its derivatives are important signaling molecules acting as local hormones and transmitters, playing a key role in the regulation of various physiological and pathophysiological processes [9]. At the same time, AA is one of the major intracellular messengers, mediating various effects of insulin [10]. AA and its derivatives (mainly the products of cyclooxygenase oxidation pathway of AA, prostaglandins) are involved in the regulation of the ion and water transport in kidneys and other reabsorbing epithelia, particularly, the frog skin epithelium [11]. Prostaglandins stimulate the transepithelial Na^+ transport, enhance the secretion of K^+ ions, and increase the water permeability of the apical membrane in the frog skin [11]. Moreover, it has been found that the cyclooxygenase inhibitors diclofenac and ibuprofen suppress the Na^+ transport in the culture of mouse renal collecting tubules [12]. We have earlier shown that treatment of the frog skin with cyclooxygenase inhibitors significantly decreases the basal level of the Na^+ transport [13].

Therefore, there appeared to be sufficient reasons to study the possible role of the cyclooxygenase oxidation pathway of AA in the regulation of the Na^+ transport by glutoxim in the skin of the frog *Rana temporaria*. In the experiments, we used two structurally different inhibitors of cyclooxygenases, meloxicam and indomethacin, used in clinical practice as nonsteroidal anti-inflammatory drugs. It is known that indomethacin suppresses the activity of both forms of cyclooxygenases, cyclooxygenase 1 and cyclooxygenase 2. Meloxicam is a nonsteroidal anti-inflammatory drug of a new generation, significantly more selective to cyclooxygenase 2 than to cyclooxygenase 1. Hence, it has weaker side effects on the kidneys and gastrointestinal tract [14].

The experiments were carried out on male frogs *Rana temporaria* from November 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 experi-

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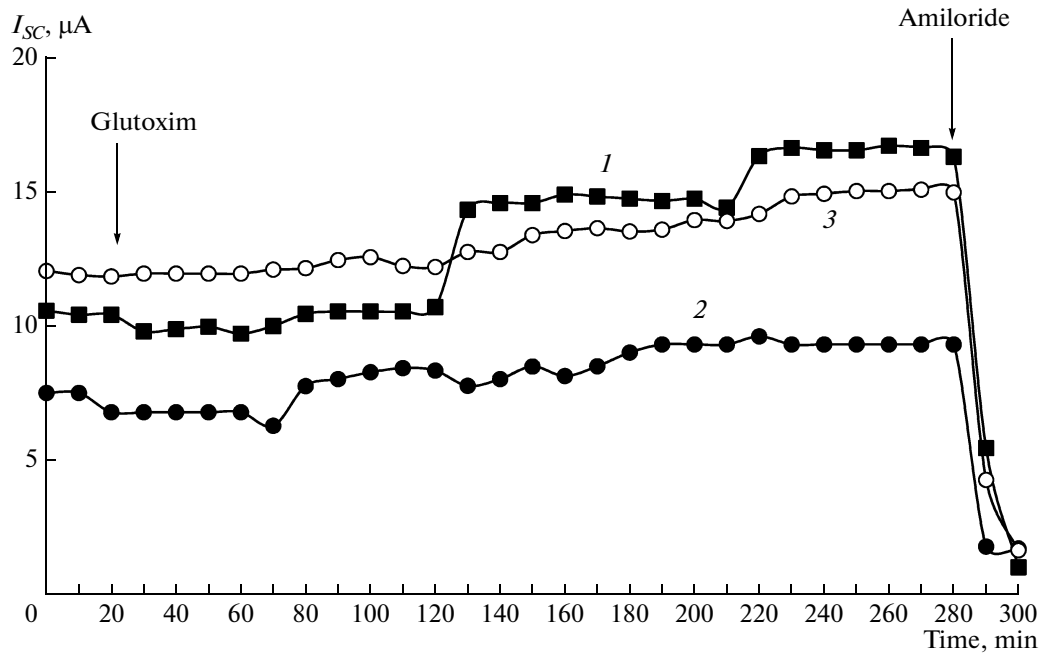

Dependence of the change in short-circuit current I_{SC} through the frog skin in response to the effect of glutoxim on the cyclooxygenase activities: (*1*) I_{SC} after application of 100 μg/mL glutoxim onto the basolateral surface of intact frog skin; (*2*, *3*) I_{SC} after application of glutoxim onto the frog skin pretreated (for 30 min) from the basolateral surface with a cyclooxygenase inhibitor: (*2*) 40 μM indomethacin or (*3*) 40 μM meloxicam; at the end of each experiment, the solution bathing the apical skin surface was supplemented with 20 μM amiloride, an ENaC blocker.

ments were performed at room temperature (22–23°C). The current–voltage characteristics (CVCs) of the frog skin were recorded using an automated device for voltage clamp [3]. The CVCs were used to determine the skin electrical parameters, namely, short-circuit current, I_{SC} ($I_{SC} = I_т$ at $V_т = 0$, where $I_т$ is a transepithelial current); open circuit potential, V_{OC} ($V_{OC} = V_т$ at $I_т = 0$, where $V_т$ is a transepithelial potential); and transepithelial conductance, $g_т$. The Na^+ transport was assessed as an amiloride-sensitive I_{SC}. The inhibitors of cyclooxygenases were added 30–40 min before supplementing the 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 samples (according to the data of 10 experiments) were as follows: $I_{SC} = 13.95 \pm 0.72$ μA, $V_{OC} = -36.17 \pm 2.08$ mV, and $g_т = 0.38 \pm 0.01$ 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 application increased by $34.12 \pm 7.46\%$; V_{OC}, by $36.14 \pm 3.28\%$; and $g_т$ value did not change.

It has been demonstrated that preincubation of basolateral surface of skin with indomethacin (40 μM) or meloxicam (40 μM) for 30 min before the application of 100 μg/mL glutoxim to the same skin surface significantly decreased the stimulating effect of glutoxim on the Na^+ transport (figure). On average (according to the data of 10 experiments), the changes in the electrical characteristics of the frog skin after application of glutoxim onto the skin pretreated with cyclooxygenase inhibitors were as follows: I_{SC} increased by 11.73 ± 2.25 or $16.35 \pm 3.05\%$, and V_{OC}, by 12.04 ± 3.05 or $18.17 \pm 4.12\%$, in experiments with indomethacin and meloxicam, respectively. No changes in the $g_т$ value were observed in any experiment.

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

Thus, the results suggest that the cyclooxygenase oxidation pathway of arachidonic acid is involved in the regulatory effect of glutoxim on the Na^+ transport in the frog skin. The results of this study, as well as of

our earlier studies [3–8, 13], allow us to suggest that glutoxim can interact with cysteine-rich domains of the insulin receptor in the basolateral membrane of epithelial cells, cause its transactivation, and trigger a signaling cascade including tyrosine kinases, phosphatidylinositol kinases, protein kinase C, protein phosphatases, elements of the actin and tubulin cytoskeleton, as well as products and/or enzymes of the cyclooxygenase oxidation pathway of arachidonic acid, which leads to stimulation of the Na^+ transport in the frog skin.

It is known that some clinical cases need combined treatment with glutoxim and nonsteroidal anti-inflammatory drugs. Our results suggest that simultaneous use of these drugs is undesirable, because it may reduce the therapeutic effect of glutoxim.

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