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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Sigma-1 Receptor Antagonists Haloperidol and Chlorpromazine Modulate the Effect of Glutoxim on Na^+ Transport in Frog Skin

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Abstract— Using voltage-clamp technique, the involvement of sigma-1 receptors in the regulation of Na^+ transport in frog skin by the immunomodulatory drug glutoxim was investigated. We have shown for the first time that preincubation of the frog skin with the sigma-1 receptor antagonists haloperidol and chlorpromazine attenuates the stimulatory effect of glutoxim on the Na^+ transport. The results suggest the possible involvement of the sigma-1 receptors in the regulation of Na^+ transport in frog skin epithelium by glutoxim.

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The skin of amphibians and other isolated epithelial systems are classic model objects for the study of ion transport mechanisms 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 the distal renal tubules [1], which allows the data obtained on these objects to be used to determine the transport mechanisms of water and ions in kidney cells.

Amiloride-sensitive epithelial Na^+ channels (ENaC) play the key role in Na^+ transport in reabsorbing epithelia. These channels are located in the apical membranes of epithelial cells and are members of the large superfamily degenerins/epithelial Na^+ channels (Deg/ENaC), which unites the ligand-gated Na^+ channels that are blocked by the diuretic amiloride [2, 3].

Previously [4], we found that Na^+ transport in frog skin is modulated by various oxidizing and reducing agents. In the cited paper, we for the first time showed that oxidized glutathione (GSSG) and drug Glutoxim® (G, disodium salt of GSSG with a nano additive of a *d*-metal, PHARMA-VAM, Russia), when applied to the basolateral surface of the frog skin, mimic the effect of insulin and stimulate the transepithelial transport of Na^+.

Sigma-1 receptors are unique ligand-regulated molecular chaperones located in the plasma membrane and endoplasmic reticulum membrane at the boundary with the mitochondria. These receptors are widely expressed in the central nervous system and in peripheral tissues, including the kidney and liver cells [5, 6]. Their ligands are endogenous steroids, antidepressants, antipsychotics, anticonvulsants, and analgesics [7]. Sigma-1 receptors interact with numerous target proteins, including ion channels and receptors, as well as participate in modulation of many cellular processes [8].

We have previously shown that sigma-1 receptor antagonists—antipsychotics haloperidol (HP) and chlorpromazine (CP)—inhibit Na^+ transport in the frog skin [9]. It is known that some of the clinical cases require concomitant use of immunomodulators and neuroleptics. In this regard, it was appropriate to study a possible involvement of sigma-1 receptor in the effect of G on the Na^+ transport in the frog skin epithelium, which was the subject of this communication. In the experiments, we used the sigma-1 receptor antagonists—the phenothiazine derivative CP [10] and the butyrophenone derivative HP [11].

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 an inner opening diameter of 12 mm. The experiments were performed at room temperature (22–23°C). The current–voltage characteristics (I–V relations) of the frog skin were recorded using an automated voltage-clamp device [4]. On the basis of I–V relations, the electrical parameters of the skin were determined: 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. The transport of Na^+ ions was assessed by the magnitude of the amiloride-sensitive I_{SC}. The reagents used in the experiments were from

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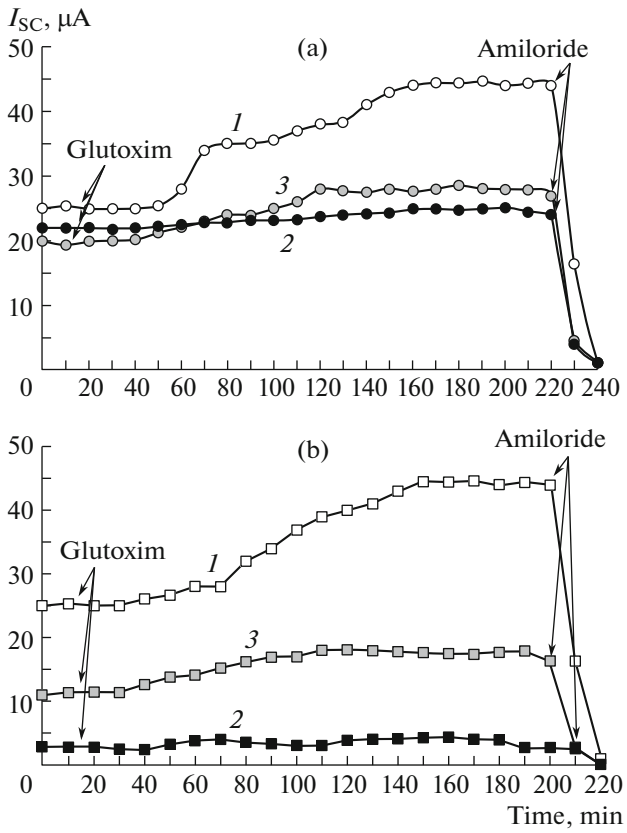

Fig. 1. Kinetics of changes in the short-circuit current I_{SC} through the frog skin in response to glutoxim (G) and sigma-1 receptor antagonists chlorpromazine (CP) and haloperidol (HP) applied from (a) apical or (b) basolateral skin surface. Designations: (*1*) I_{SC} after the addition of 100 μg/mL G to the basolateral surface of intact skin; (*2*) I_{SC} after the addition of G to the frog skin pretreated with 100 μg/mL CP for 30 min; (*3*) I_{SC} after the addition of G to the frog skin pretreated with 100 μg/mL HP for 30 min. At the end of each experiment, the ENaC blocker amiloride (20 μM) was added the solution bathing the apical skin surface. The figure shows the results of typical experiments.

Sigma-Aldrich (United States). HP (100 μg/mL) and CP (100 μg/mL) were added 30–40 min before the addition of G to the solution. Statistical analysis was performed using Student's *t* test.

The values of the electrical characteristics of the frog skin in the control were as follows (hereinafter, data are represented as $M \pm m$, n (number of tests) = 10): $I_{SC} = 24.46 \pm 4.08$ μA, $V_{OC} = -80.67 \pm 12.35$ mV, and $g_T = 0.27 \pm 0.12$ mS. We established that G (100 μg/mL) applied to the basolateral surface of the frog skin, similarly to insulin, stimulates Na^+ transport (Figs. 1a, 1b, curve *1*). After the application of G, I_{SC} increased by $41.13 \pm 8.01\%$, V_{OC} increased by $49.31 \pm 8.34\%$, and the g_T value did not change.

We found that the pretreatment of frog skin with HP (100 μg/mL) or CP (100 μg/mL) for 30 min before the addition of 100 μg/mL G to the basolateral surface of the skin reduced the stimulatory effect of G on Na^+ transport (Table 1, Fig. 1). The comparison of the effects of the studied sigma-1 receptor antagonists showed that HP and CP differed in the degree of inhibition of the effect of G, which also depended on the application of the agents from the apical or basolateral surface of the skin. The results presented in Table 1 and Fig. 1 showed that CP decreased the stimulatory effect of G on Na^+ transport much more significantly. In addition, the inhibitory effect of HP and CP was more pronounced when the agents were applied from the apical surface of the frog skin. For example, the application of CP to the apical skin surface completely suppressed the stimulatory effect of G on Na^+ transport (Table 1; Fig. 1, curve *2*). The inhibitory effect of HP was also more pronounced when the agent was applied from the apical surface of the skin. However, skin pretreatment with HP caused a reduction, but not suppression, of the stimulatory effect of G (Table 1; Fig. 1, curve *3*).

Thus, in this study, we for the first time showed using the frog skin epithelium that two structurally different antagonists of sigma-1 receptors modulate the effect of G on Na^+ transport, which indicates the involvement of sigma-1 receptors in the signaling cascades triggered by G in the frog skin epithelium and leading to Na^+ transport stimulation.

Our results are consistent with the published data. For example, recent data indicate that sigma-1 receptors modulate the activity of ion channels of different types, including the proton-gated (acid-sensing) ion channels (ASICs), a member of the Deg/ENaC superfamily, to which ENaC also belong. It was shown [12] that sigma-1 receptors and ASICs can interact both directly (to form a sigma-1 receptor/ASIC subunit complex with a 1 : 1 stoichiometry) and indirectly (the effect of sigma-1 receptor agonists/antagonists on ASICs may be mediated by additional signal molecules such as heterotrimeric G proteins and the complex of calcineurin with the AKAP150 adapter protein) [13]. Our data that the inhibitory effect of HP and CP is much more pronounced when they are added from the apical surface of the skin suggest that the main targets of the action of sigma-1 receptor antagonists are localized in the apical, rather than basolateral, membranes of frog skin epithelial cells.

It is known that many Na^+-transport proteins contain numerous cysteine residues, which are targets for intracellular and extracellular oxidizing and reducing agents [14, 15]. The addition of the ENaC blocker amiloride (20 μM) to the solution bathing the apical skin surface caused a complete suppression of Na^+ transport (Fig. 1). This fact indicates that the effect of G on Na^+ transport is determined primarily by the modulation of the ENaC activity.

Thus, in this study we for the first time demonstrated the modulatory influence of sigma-1 receptor

Table 1. The effect of glutoxim (G) on the electrical characteristics of frog skin

Blocker, concentration	Electrical characteristics	Changes in electrical characteristics after application of G to the frog skin pretreated with sigma-1 receptor antagonists from the apical surface, %	Changes in electrical characteristics after application of G to the frog skin pretreated with sigma-1 receptor antagonists from the basolateral surface, %
Haloperidol, 100 μg/mL	I_{SC}	↑ 25.34 ± 7.12	↑ 30.02 ± 9.34
	V_{OC}	↑ 32.19 ± 8.41	↑ 29.76 ± 7.48
	g_T	↑ 6.37 ± 2.07	↑ 9.13 ± 2.09
Chlorpromazine, 100 μg/mL	I_{SC}	↑ 2.35 ± 0.15	↑ 19.45± 4.12
	V_{OC}	↑ 5.52 ± 1.09	↑ 17.37 ± 3.21
	g_T	↓ 7.27 ± 2.13	↑ 4.55 ± 1.74

The arrows indicate the increase (↑) or decrease (↓) in the electrical characteristics of the skin after the application of G as compared to the control. Data are represented as $M \pm m$, $n = 10$.

antagonists HP and CP on the effect of G on Na^+ transport in the frog skin epithelium. The results also suggest that a combined use of the drug G and neuroleptics HP and CP in clinical practice is undesirable.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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