Thus we can conclude that Na,K-ATPase was most sensitive to avermectins on the later stages on the development loach embryos. Owing to these essential functions of ATPases (nerve transmission, coordination, metabolism, motility, respiratory system and organ functions in general), it is possible that some specific effect of avermectin may partially result from the inhibition of these enzymes. The embryotoxic of avermectin can be realized to inhibition of enzyme activity ATPases, such as Na,K-ATPase. Membranes are first barrier on drug penetrate in cell. Thus investigate effect drug on structure and function membranes are important for understanding interacting between drug and cell.

THE ROLE OF TYROSINE KINASES IN THE EFFECT OF OXIDIZED GLUTATHIONE AND GLUTOXIM ON \( \text{Na}^+ \) TRANSPORT IN FROG SKIN


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The skin and urinary bladder of amphibians are useful model objects to study the transepithelial ion transport mechanisms. In epithelial systems sodium ions enter into epithelial cells through amiloride-sensitive \( \text{Na}^+ \)-channels (ENA) in the apical membrane and are then extruded at the basolateral membrane by the Na,K-ATPase. The rate-limiting step of vectorial sodium transport is mainly attributed to the activity of ENA, which is the target for hormonal control and is involved in \( \text{Na}^+ \) reabsorption in epithelia. The extracellular loop of ENA contains domains rich in highly conserved cystein residues. These extracellular cystein residues of ENA are likely to be implicated in the formation of disulfide bonds important for the proper folding of the protein. Cystein residues are also found in the transmembrane segments and in the amino and carboxyl termini of \( \alpha \), \( \beta \) and \( \gamma \) ENA subunits. Na/K-ATPase and the insulin receptor in the basolateral membrane of epithelial cells also have multiple cystein residues, which may be targets for various thiol-modifying reagents.

Oxidized glutathione (GSSG) is a low-molecular-weight thiol found in all cells and extracellular space. Recent studies have shown that GSSG could have a receptor-mediated effect on cellular processes. In addition, a pharmacological analogue of GSSG, the drug Glutoxim, is used in clinics as an immunomodulator and hemostimulator for integrated treatment of bacterial and viral diseases, psoriasis and radio- and chemotherapy in oncology.

Previously, we showed that GSSG and Glutoxim applied to the basolateral surface of the frog skin increased \( \text{Na}^+ \) transport [1]. However, the mechanisms that mediate the effect of GSSG and Glutoxim on \( \text{Na}^+ \) transport are still unclear. It was shown in experiments on human carcinoma A431 cells that GSSG and Glutoxim induced transactivation of epidermal growth factor receptor and activation of its intrinsic tyrosine kinase activity. To test the hypothesis that GSSG and Glutoxim may transactivate insulin receptor in the basolateral membrane and activate its intrinsic tyrosine kinase activity we studied the possible role of tyrosine kinases in the regulatory effect of these oxidizing agents on \( \text{Na}^+ \) transport in frog skin.

Fig. 1. (a) – Kinetics of changes in the short-circuit current (\( I_{sc} \)) after the treatment of the basolateral surface of the frog Rana temporaria skin with 100 \( \mu \)g/ml oxidized glutathione (GSSG). (b) – the skin was preincubated for 20 min with 100 \( \mu \)g/ml genistein (Gen, applied apically) and then 100 \( \mu \)g/ml GSSG was added to the basolateral solution. The ENA blocker 20 \( \mu \)M amiloride (A) was added to the apical solution at the end of all experiments. The results of typical experiments are presented.

Fig. 2. (a) – Kinetics of changes in the short-circuit current (\( I_{sc} \)) after the treatment of the basolateral surface of the frog Rana temporaria skin with 100 \( \mu \)g/ml Glutoxim; (b) – the skin was preincubated for 20 min with 100 \( \mu \)g/ml genistein (Gen, applied apically) and then 100 \( \mu \)g/ml Glutoxim was added to the basolateral solution. The ENA blocker 20 \( \mu \)M amiloride (A) was added to the apical solution at the end of all experiments. The results of typical experiments are presented.

With the use of the voltage clamp technique we studied the influence of tyrosine kinase inhibitor genistein on the regulatory effect of GSSG and Glutoxim on \( \text{Na}^+ \) transport in the frog Rana temporaria skin. To measure \( I-V \) relations, transepithelial potential \( V_T \) was changed periodically to a series of non-zero values. From skin \( I-V \) relations the electrical characteristics of frog skin were determined: the short – circuit current (\( I_{sc} \)), the open – circuit potential (\( V_{oc} \), at the total transepithelial current \( I_T = 0 \)), and transepithelial conductance (\( g_T \)). The transepithelial \( \text{Na}^+ \) transport was measured as amiloride-sensitive short – circuit current (\( I_{sc} \)). To ensure that \( \text{Na}^+ \) transport was the source of \( I_{sc} \), the ENA blocker, amiloride (20 \( \mu \)M) was added to the apical bath at the end of all experiments.

In a series of ten experiments the control values of electrical characteristics of frog skin were: \( I_{sc} = 10.45 \pm 2.98 \mu \text{A}, V_{oc} = -30.04 \pm 2.85 \text{mV}, g_T = 0.36 \pm 0.01 \text{mS.m.} \) Basal application (280-20 min) of 100 \( \mu \)g/ml GSSG caused a signifi-
cant increase of both $I_{sc}$ and $V_{oc}$ by as follows: $49.45 \pm 10.17\%$ ($P<0.05$) and $50.28 \pm 12.01\%$ ($P<0.05$). Addition of 100 μg/ml GSSG to the basal side of the skin preincubated with 100 μM genistein (30-50 min, applied apically) produced significantly lower changes of electrical characteristics values: $I_{sc}$ decreased by 10.05 $\pm$ 2.11 $\%$ ($P<0.05$), $V_{oc}$ by 30.25 $\pm$ 4.32 $\%$ ($P<0.05$), $g_{f}$ did not change.

For Glutoxim we obtained the following results. In a series of ten experiments the control values of electrical characteristics of frog skin were: $I_{sc} = 11.25 \pm 1.17\mu A$, $V_{oc} = -31.65 \pm 2.19\ mV$, $g_{f} = 0.36 \pm 0.01\ mSm$. Basal application (280-20 min) of 100 μg/ml Glutoxim caused an increase of both $I_{sc}$ and $V_{oc}$ by as follows: $16.04 \pm 1.37\%$ ($P<0.05$) and $17.12 \pm 2.02\%$ ($P<0.05$). Addition of 100 μg/ml Glutoxim to the basal side of the skin preincubated with 100 μM genistein (30-50 min, applied apically) produced significantly lower changes of electrical characteristics values: $I_{sc}$ increased by 10.05 $\pm$ 1.48 $\%$ ($P<0.05$), $V_{oc}$ by 11.05 $\pm$ 1.25 $\%$ ($P<0.05$). No changes in $g_{f}$ were observed in any experiment.

Thus, according to our results the inhibitor of tyrosine kinases genistein significantly reduced the stimulatory effect of GSSG and Glutoxim on Na$^+$ transport in frog skin (Figs. 1 and 2). The data suggest the involvement of tyrosine kinases in the regulatory effect of GSSG and Glutoxim on Na$^+$ transport in frog skin.

The data are compatible with the proposal that basally applied GSSG and Glutoxim may interact with cystein-rich domains of insulin receptor, transactivate it and activate its intrinsic tyrosine kinase activity. This leads to ENaC activation and Na$^+$ transport stimulation in frog skin.

References


ADAPTATION TO OXYGEN INSUFFICIENCY, THE ROLE OF MITOCHONDRIAL ATP DEPENDENT POTASSIUM CHANNEL

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Cell mechanisms of adaptation to hypoxia are intensively studied in many laboratories and clinics. Ischemic preconditioning is one of the classical ways of adaptation; it consists of one or more brief episodes of coronary artery occlusion separated by brief periods of reperfusion. Such procedures paradoxically protect the heart against necrosis, arrhythmia and the functional damage caused by the subsequent prolonged ischemia. In heart, the opening of the mitochondrial ATP-dependent potassium channel (mitoK$_{ATP}$) is the key step of IP, since the inhibitors of the channel abolish the positive effect of such adaptation, whereas the channel activators imitate it.

The mechanisms of this adaptation are intensively studied in many laboratories and clinics all over the world. The protective effect is supposed to be three-staged and includes different extracellular trigger mechanisms and intracellular signal mechanisms, mainly with protein kinases. The end effectors of ad-

aptation, however, would be always the mitoK$_{ATP}$ channel – no matter what intra- or extra cellular mechanisms are involved.

There are two types of K$_{ATP}$ channels in the cell: cell-membrane and mitochondrial. Both channels is inhibited by physiological concentration of ATP and consist of two subunits: the channel-forming subunit which forms the channel pore, and the regulatory one.

The presence of common and different modulators for the cell and mitochondrial potassium channels indicates the community but not identity of the structure of these channels.

It is generally accepted now that the main function of both channels is to maintain, together with the H/K exchanger, the mitochondrial volume. For the last 10 years, widely discussed in literature is the role of mitoK$_{ATP}$ in cardioprotection. The high effectiveness of pharmacological activators of the channel in preventing heart ischemic injury brings up a question on their immediate application in medicine. However, as often revealed in clinical trial, the effect of a drug is accompanied by nonspecific side effects. On the other hands, the activation of mitoK$_{ATP}$ during IP suggests that at this moment, a metabolic channel activator appears in the cell.

With this suggestion in mind, we have been seeking for natural activators of mitoK$_{ATP}$ for the last few years. Finally we have discovered an effective natural activator of mitoK$_{ATP}$, uridine-5'-diphosphate (UDP). Micromolar concentrations of this nucleotide reactivate the BLM-reconstituted mitoK$_{ATP}$ pre-inhibited with ATP.

Studies were also carried out on intact mitochondria, where both subunits of the channel are presented. In intact mitochondria, 30 μM UDP reactivated the ATP-sensitive K$^+$ transport inhibited by ATP. The effect of UDP is specific, since the addition of the mitoK$_{ATP}$ blocker 5-HD abolishes this reactivation. According to this data, UDP concentration in the cell can regulate the extent of the channel opening.

Taking into account all the aforesaid, we examined the cardioprotective effect of UDP precursors. We found that uridine and UMP possess clear anti-ischemic properties on the model of acute myocardial ischemia in rats, namely the 60-min occlusion of left coronary artery without reperfusion. Both preparations greatly decrease the index of ischemic alteration, which estimates the sizes of the infarction zone. UMP decreases the infarction zone almost 4 times. The K$_{ATP}$ channel inhibitors (glibenclamide and 5-HD) reverse the positive effect of UMP.

In addition to the anti-ischemic action, uridine and UMP demonstrated a significant anti-arrhythmic effect and glibenclamide completely abolished the positive effect of uridine and UMP. The effect of the specific mitoK$_{ATP}$ inhibitor 5-HD was less pronounced. This suggests that the anti-arrhythmic effect of uridine and UMP is mediated by both channels, but mostly by the cellK$_{ATP}$.

According to our data, the rate, with which UDP precursor uridine disappear from blood, is increased during myocardial ischemia. This stimulates the UDP accumulation in heart during ischemia, which promotes the mitoK$_{ATP}$ activation.