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The Involvement of Protein Kinase C in the Effect of Oxidized Glutathione and Glutoxim on Na^+ Transport in Frog Skin

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The amphibian skin and other isolated epithelial systems serve as classic model objects to study transepithelial ion transport mechanisms. Na^+ transport in epithelial cells is known to be a complex multicomponent system containing various Na^+ transporting proteins, which may be targets for oxidative stress. Previously, we have demonstrated that Na^+ transport in frog skin can be modulated by various oxidizing agents. It was shown for the first time that oxidized glutathione (GSSG) and its pharmacological analogue glutoxim applied to the basolateral surface of the frog skin imitated the effect of insulin and increased transepithelial Na^+ transport. Furthermore, we elucidated for the first time the involvement of tyrosine kinases and phosphatidylinositol kinases in the stimulatory effect of GSSG and glutoxim on Na^+ transport in frog skin.

It is known that insulin interacts with the receptor with intrinsic tyrosine kinase activity located in the basolateral membrane of epithelial cells. Previously, we have demonstrated the involvement of tyrosine kinases, tyrosine phosphatases, phosphatidylinositol kinases and protein kinase C in the effect of insulin on Na^+ transport in frog skin. Therefore, the purpose of the present research was to study the possible role of protein kinase C in the regulatory effect of GSSG and glutoxim on Na^+ transport in the frog *Rana temporaria* skin.

Using the voltage-clamp technique we studied the influence of the specific protein kinase C inhibitor calphostin C on the effect of GSSG and glutoxim on Na^+ transport in frog skin. To measure I-V relations, transepithelial potential V_T was changed periodically to a series of nonzero values. From skin I-V relations the electrical characteristics of frog 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 ($V_{OC} = V_T$ at the total transepithelial current $I_T = 0$), and transepithelial conductance (g_T). The transepithelial Na^+ transport was measured as amiloride-sensitive I_{SC}.

It was shown that 100 μg/ml GSSG or glutoxim, applied at the basolateral side of the skin, caused a significant increase of Na^+ transport. In a series of ten experiments I_{SC} increased by 40 ± 11% ($P < 0.05$) and 20 ± 1% ($P < 0.01$), and V_{OC} increased by 48 ± 10% ($P < 0.05$) and 20 ± 1% ($P < 0.01$) for GSSG and glutoxim, respectively. The value of g_T did not change. It appeared that the inhibitor of protein kinase C calphostin C (1 μM or 500 nM) significantly reduced the stimulatory effect of GSSG and glutoxim on Na^+ transport in frog skin. Thus, addition of 100 μg/ml glutoxim to the basal side of the skin preincubated with calphostin C produced significantly lower changes of electrical characteristics values: I_{SC} increased by 12 ± 1% ($P < 0.01$) and 17 ± 1% ($P < 0.01$), and V_{OC} increased by 13 ± 2% ($P < 0.01$) and 16 ± 1% ($P < 0.01$) for 1 μM and 500 nM calphostin C, respectively. Similar results were obtained when 100 μg/ml GSSG was added to the basal side of the skin preincubated with calphostin C. The specific inhibitor of epithelial Na^+ channels (ENaC) amiloride (20 μM), applied to the apical solution at the end of each experiment, inhibited I_{SC}, suggesting that the effect of GSSG and glutoxim on Na^+ transport is mostly caused by modulation of the ENaC activity.

Thus, we demonstrated for the first time the involvement of protein kinase C in the stimulatory effect of GSSG and glutoxim on Na^+ transport in the frog *Rana temporaria* skin. The results obtained in this study, as well as our earlier data, suggest that GSSG and glutoxim may interact with cysteine-rich domains of insulin receptor in the basolateral membranes of epithelial cells, transactivate it and trigger a complex signaling cascade, including tyrosine kinases, phosphatidylinositol kinases and protein kinase C. This leads to ENaC activation and Na^+ transport stimulation in frog skin.