ELECTRON PROBE MICROANALYSIS: Na^+/K^+-ATPase, STROPHANTHIN AND CARDIAC ISCHEMIA

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Abstract. Electron probe microanalysis was applied to study the kinetics of changes in potassium and sodium concentration in muscle cells of isolated heart from Wistar rat during experimental ischemia. Hypoxic perfusion without glucose was shown to evoke the potassium deficiency and sodium accumulation in cardiac myocells. Short_term action (10 min) of strophanthin (0.1 mM/l) recovered Na/K balance in ischemic myocells.

Key words: Electron Probe Microanalysis (EPMA), cardiac ischemia, myocell, cytoplasmic K/Na balance, Na^+/K^+-ATPase, strophanthin

In the absence of substrate influx against the background of prolonged hypoxia, one registers growing deenergization of the heart muscle cell [1]. As a result, Na^+/K^+-ATPase inactivates, that causes dissipation of the membrane gradient of electrolytes [2, 3]. As a consequence, the electric potential on the cytoplasmic membrane declines, which comes to be one of the causes of electromechanical uncoupling of contraction [4]. For the described sequence of events, apparently paradoxical is the use strophanthin, Na^+/K^+-ATPase blocker, to stimulate the ischemic heart in clinical practice [5]. Possibly, the explanation of such a phenomenon, when inhibition of active potassium transport stops cardial ischemia, consists in mediated impact of the cardiac glycoside on the cardiomyocyte. Search for a target of such nonspecific action of the drug was the aim of the given work.

Experimental.

Details of specimen preparation and of the method for measuring the cytoplasmic concentration of elements in the rat heart muscle cell have been described by us earlier [1–3]. Briefly, isolated rat (Wistar) heart was perfused according to Langendorff, using a two channel perfusion device to combine different protocols. To stabilize the heart, the first 5 min of perfusion were at 38°C in normoxic conditions. This state was taken as the start for further test impact. Ischemia was modeled by hypoxic perfusion with glucose-free Tyrode`s solution. Deep hypoxia was created by vacuum degassing of the perfusing solution. In tests with strophanthin, the drug was infused by the following scheme. First an isolated heart was subjected to ischemia of varied duration (5, 20, 35 or 50 min); in the subsequent 10 min of perfusion, strophanthin was introduced so that its concentration in the solution was 0.1 mM. The sequence of manipulations in processing a rat heart specimen for electron probe microanalysis of the elemental composition of the cytoplasm is outlined in Fig. 1.

Papillary muscle was isolated from perfused heart and frozen in overcooled liquid propane; 20 μm cryosections were made at –35°C, vacuum freeze-dried, and mounted on the holder of a scanning electron microscope JSM-U3 (JEOL, Japan) equipped with a crystal diffraction spectrometer. Specimen morphology was observed in the secondary electron mode. From the intensity of characteristic X-radiation we calculated the concentration of elements (K, Na) in the cardiomyocyte cytoplasm. For every animal, the result represents the mean concentration over at least 80 cells. Each group comprised five rats. The significance of difference between two groups was assessed by the Mann–Whitney *T*-test.

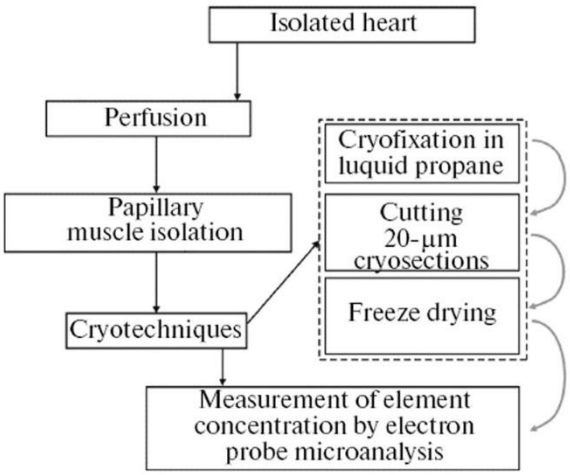

Figure 1 – Scheme of the specimen processing for electron probe microanalysis of potassium and sodium concentrations in the myocyte of isolated rat heart. The dashed line frames the consecutive steps of cryocutting technique

Results and discussion.

The role of strophanthin, a plant cardiac glycoside, in regulation of the cytoplasmic Na/K balance was checked on isolated rat heart in ischemia modeled by hypoxic perfusion without glucose. The cytoplasmic content of K and Na at different intervals of ischemia and the effects of the drug are shown in Fig. 2.

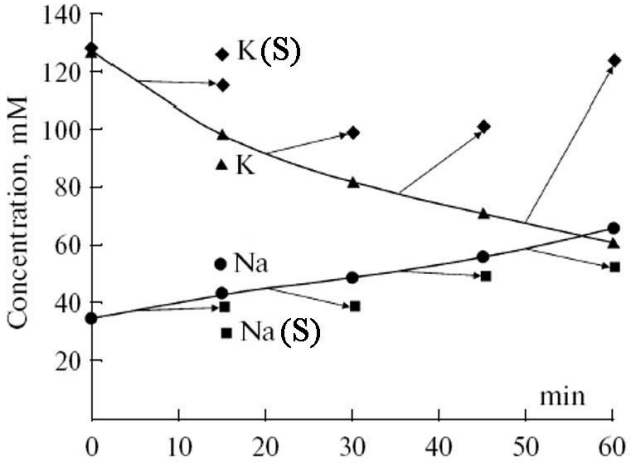

Figure 2 – Measurement of intracellular potassium and sodium concentrations during rat heart hypoxic perfusion without glucose. The arrows show the elemental (K, Na) content at the start of strophanthin (S) exposure and after 10 min of perfusion with 0.1 mM strophanthin . For K^+ the differences with and without strophanthin are significant at all time intervals; for sodium, differences are significant only after 30 min of ischemia. The significance of difference between groups of five animals is $p < 0.05$ in the Mann–Whitney test

The cardiac glycoside was shown to affect Na^+/K^+-ATPase in isolated cells [6, 7]. Bearing in mind the specific effect of the inhibitor, one should expect, upon suppression of active transport in the cardiomyocyte, a still more pronounced K^+ deficit and Na^+ accumulation as compared with ischemia. Contrary to such expectation, strophanthin exerts an opposite effect, raising the K^+ and reducing the Na^+ levels (Fig. 2). In other words, a relatively short action of strophanthin (10 min) stops the disturbance in Na/K balance emerging in ischemia. Therewith the intracellular concentration of elements (K, Na) tends to the initial level, which is especially obvious at the late terms of ischemia. Such a tendency may signify restoration of the state of the heart in the presence of strophanthin. Possibly, the difference in the effect of the drug at different steps of hypoxic perfusion without glucose is caused by activation of different transmembrane ion transport complexes over time [2, 4, 8]. Thus, low doses of Na^+/K^+-ATPase inhibitor, contrary to expectations, have a stimulating effect on cardiac function, which is consistent with clinical practice. So what does stipulate the nonspecific action ofstrophanthin on the cardiomyocyte in ischemia? Possibly, the reason hides in active involvement of the capillary endothelium in regulating the myocyte homeostasis (Fig. 3).

Figure 3a demonstrates the cascade of events evoked by ischemia, which causes a deficit of K^+ in the heart muscle cell. First of all, the K^+ level rises in the intercellular space because of te exit of K^+ from cardiomyocyte through a system of channels [2, 8]. As a result, there takes place activation of Na^+/K^+-ATPase of the endothelial cell at the side of the basal membranes, with subsequent passive transcellular transport of potassium into the capillary lumen. Figure 3b illustrates the situation developing in the presence of strophanthin. Upon short-term perfusion of the heart with a solution containing the drug at low concentration, the target of its action in the first places comes to be the more spatially accessible Na^+/K^+-ATPase of the capillary wall endothelium. As the result, the endothelial cell loses the function of active regulator of the ionic homeostasis of the interstitium. In the given case, K^+ from the intercellular space into the capillary lumen is interrupted, and endothelium becomes a morphological barrier to potassium leakage from the muscle tissue into blood [9]. In the described scenario, not only conservation of the state is possible but also reverse diffusion of K^+ into the intercellular space through the fenestrae between endothelial cells. Passive transport of substances from the capillary into the intercellular space together with active Na^+/K^+-ATPase on the membrane of the myocyte promote restitution promote restitution in the latter of the Na/K balance in ischemia with short-term exposure to strophanthin (Fig. 2). Such an effect of low doses of cardiac glycoside has been denoted by us as a “mechanism of mediated impact”. For its realization, necessary is the fulfillment of the following conditions: (1) inhibition of Na^+/K^+-ATPase on the membrane of the capillary wall endothelial cell; (2) diffusion of substances from the capillary lumen into the extracellular space of the muscle cells; (3) active Na^+/K^+-ATPase on the cardiomyocyte membrane. To attain direct action of strophanthin on the myocyte in an isolated heart perfusion experiment, one perhaps should raise the drug concentration above that used in cell culture (0.1 mM) and/or prolong the exposure.

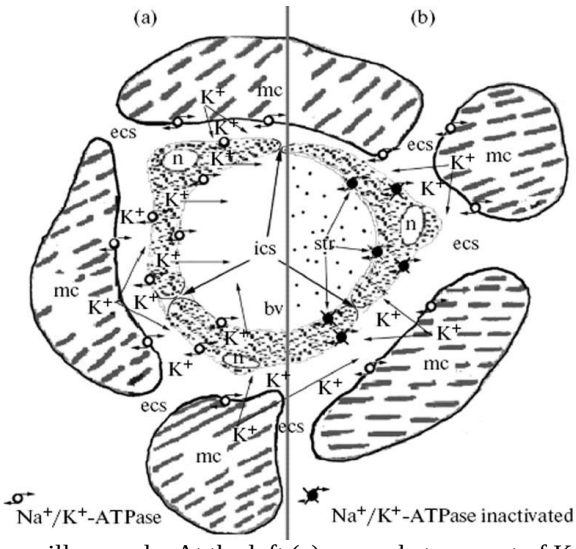

Figure 3 – Scheme of cardiac capillary node. At the left (a), cascade transport of K+ from the muscle cell via the intercellular space and endothelium into the capillary lumen under ischemia. At the right (b), nonspecific effect of short-term exposure to strophanthin, caused by inhibition of endotheliocyte membrane Na^+/K^+-ATPase. Designations: bv – blood vessel; *n* – nucleus of vascular endotheliocyte; ecs – extracellular space; mc – muscle cell; str – strophanthin in the capillary lumen; ics – pores in the capillary endothelium

In conclusion, the task of microanalysis of elements in the cytoplasm of the heart muscle cell can be regarded as solved; this model allows testing the influence of various substances on the state of cardiomyocyte by changes in the intracellular elemental composition.

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