Diversification of Ca^{2+}-mediated signal in a simple model system

Monika Vaneková, Boris Lakatoš, Ľudovít Varečka

Department of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia. E-mail: monika.vanekova@stuba.sk

The most prominent feature of Ca^{2+}-mediated signalling is its pluripotency („Kalzium macht alles...“), which poses important question, how the calcium signal activates the specific pathway determined by the signalling molecule without activating other Ca^{2+}-mediated pathways activated by different signalling molecules. Spatial-temporal properties of Ca^{2+}-signal and Ca^{2+}-binding proteins could participate in the process of diversification of Ca^{2+} signal.

We studied the diversification of the Ca^{2+} signal in artificially induced Ca^{2+} influx in human red blood cells (RBC). Earlier we observed that the activation of the Ca^{2+}-influx by fluoride, or vanadate, led to the activation Na^{+}-permeability. Interestingly, vanadate activated the Na^{+}/H^{+} antiporter sensitive to amiloride, while fluoride activated Na^{+} channel sensitive to tetrodotoxin. This observation represents the example of the Ca^{2+}-signal diversification on the simplest level, where no participation of intracellular structures could be expected. Novel inhibitors of both modes of Ca^{2+}-influx activation were found. Fluoride-activated Ca^{2+} influx was inhibited by a phosphoprotein phosphatase 2B inhibitor – cyclosporin A, and tetrodotoxin, whereas Ca^{2+} influx activated by vanadate was sensitive to Li^{+} ions and dihydropyridine Ca^{2+} blockers.

The fate of fluoride in the RBC suspension was monitored by means of ^{19}F-NMR. Results showed two signals, which correspond to both extracellular and intracellular fluoride, but no signal, which could represent a protein-bound fluoride, was found. The possible role of phosphorylation/dephosphorylation mechanisms in the diversification of Ca^{2+} signal was studied by means of ProQ Diamond staining and by ^{32}P-phosphate incorporation. We could not find any changes in protein phosphorylation induced by both vanadate or fluoride and Ca^{2+} (and other additions), when proteins separated in SDS-PAGE were stained by the ProQ-Diamond dye. However, some changes, which remain to be characterized, were observed by radioactive labelling.

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