Some novel pathways for IP_3 receptor activation and their medical implications

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Na,K-ATPase is an integral membrane protein, expressed in all eukaryotic cell, where it serves to create the electrochemical gradient required for virtually all sodium coupled transports systems. The plant-derived steroid, digoxin, is a specific inhibitor of Na,K-ATPase and has been used for centuries in treatment of heart disease. Soon after this collaborative project was initiated, our group made the discovery that ouabain can trigger slow intracellular calcium oscillations. We showed that in renal epithelial cells ouabain, in doses causing no or only partial Na,K-ATPase inhibition, can trigger regular, low-frequency intracellular calcium oscillations that activate the transcription factor, NF-κB [Aizman O et al. PNAS 98:13420, 2001]. Partial inhibition of Na,K-ATPase using low extracellular K+ and depolarization of cells, did not have these effects. Incubation of cells with an IP_3 receptor antagonist and depletion of intracellular calcium stores abolished the oscillations indicating that the IP_3 receptor plays a key role for this effect. Ouabain-triggered calcium oscillations were not observed in cells expressing an IP_3 sponge, and were hence independent of IP_3 generation. In contrast, bradykinin-induced calcium spikes were abolished in IP_3 sponge expressing cells.

In our continued studies we could demonstrate that the ouabain-triggered calcium oscillations were initiated from a cell signaling microdomain formed by Na,K-ATPase and the IP_3 receptor [Miyakawa-Naito A & Uhlén P et al. J Biol Chem, in press]. Using fluorescent resonance energy transfer (FRET) measurements, we detected a close spatial proximity between Na,K-ATPase and InsP(3)R. Ouabain significantly enhanced FRET between Na,K-ATPase and InsP(3)R. The FRET effect and ouabain-induced calcium oscillations were not observed following disruption of the actin cytoskeleton. Partial truncation of the NH(2)-terminus of Na,K-ATPase catalytic α1-subunit abolished oscillations and downstream activation of NF-κB. Thus, a novel principle for a cell signaling microdomain where an ion pump serves as a receptor was identified.

In ongoing studies we are exploring two possible medical implications of our findings. In both projects our preliminary results support our hypothesis. *Project 1:* Since calcium can be a death signal, and since there are empirical observations that ouabain may act as an anti-cancer agent, we are studying the effect of ouabain in a human cancer cell line and in human hyper-nephroma cells in primary culture. The molecular mechanism for this signal and its subsequent effect on mitochondrial calcium are compared to findings in human kidney cells in primary culture. So far we have found that in cancer cells but not in rat renal cells primary culture, low doses of ouabain causes massive apoptosis. The apoptotic effect is calcium dependent. The formation of calcium oscillations are, in addition to IP_3 also dependent on store operated calcium channels (SOC), that are sensitive to changes in calcium concentrations within the endoplasmatic reticulum.

Project 2: We have hypothesized that polycystin 2 (PC2) may act as a SOC in kidney cells. PC2 is one of the two proteins that are mutated in polycystic kidney disease, and it has been shown to have the properties of a calcium channel. So far we have found that ouabain causes translocation of PC2 to the plasma membrane and that a PC2 blocking antibody can abolish the ouabain-induced calcium oscillations.