# The Role of the Inositol 1,4,5-Trisphosphate Receptor in a Signaling Microdomain

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Signaling transduction mediated by protein aggregates within specific signaling microdomains has been receiving increased attention. We previously showed that Na,K-ATPase, partially inhibited by ouabain, induces intracellular calcium ($Ca^{2+}$) oscillations which involves $Ca^{2+}$-release from endoplasmic reticulum (ER) via the inositol 1,4,5-trisphosphate ($InsP_3$) receptor and on $Ca^{2+}$ influx via store operated $Ca^{2+}$ channels.

Recently our group reported that Na,K-ATPase and $InsP_3$ receptor form a cell signaling microdomain that, in the presence of ouabain, generates slow $Ca^{2+}$ oscillations in renal cells. Using fluorescent resonance energy transfer (FRET) measurements, we detected a close spatial proximity between Na,K-ATPase and $InsP_3R$. Ouabain significantly enhanced FRET between Na,K-ATPase and $InsP_3R$. The FRET effect and ouabain-induced $Ca^{2+}$ oscillations were not observed following disruption of the actin cytoskeleton. Partial truncation of the $NH_2$ terminus of Na,K-ATPase catalytic $\alpha$1-subunit abolished $Ca^{2+}$ oscillations and downstream activation of NF-κB. Ouabain-induced $Ca^{2+}$ oscillations occurred in cells expressing an $InsP_3$-sponge and were hence independent of $InsP_3$ generation.

Since there is some experimental evidence that dislocation of Na,K-ATPase may be associated with renal cyst formation, we also examined the possibility that the $Ca^{2+}$-permeable channel protein, polycystin-2 (PC2), the product of the gene mutated in type 2 autosomal dominant polycystic kidney disease, may be involved in the ouabain-induced $Ca^{2+}$ oscillatory response. Application of an antibody raised against the extracellular domain of PC2, and shown to inhibit $Ca^{2+}$ flux via PC2, completely blocked the ouabain-induced $Ca^{2+}$ oscillations. PC2 has been reported to be located in the ER membrane and/or in the plasma membrane. We examined the localization of PC2 with immunostaining in control or ouabain treated fixed cells and in live cells transfected with GFP tagged PC2. Both experimental approaches demonstrated that, under control conditions, PC2 is mainly localized in ER-like structures and that following ouabain incubation there is a translocation of PC2 to the plasma membrane. These results imply a functional association between PC2 and the Na,K-ATPase/$InsP_3R$ signaling microdomain which may have implications for tubule development.

Taken together, our data present novel principles for a cell signaling microdomain where an ion pump serves as a receptor.