Na^+ pump gene regulation in cultured renal tubule cells by changes in intracellular ionic concentrations

Shigeaki Muto, Yukio Miyata
Department of Nephrology, Jichi Medical School

Summary

In a wide variety of cell systems, increases in cell Na^+ ($[Na^+]i$) lead to an induction of Na^+, K^+-ATPase mRNA expression. On the other hand, the increase in $[Na^+]i$ can also induce a rise in cell Ca^{2+} ($[Ca^{2+}]i$) through a secondary inhibition of Na^+/Ca^{2+} exchange and a decrease in cell pH (pHi) through a secondary inhibition of Na^+/H^+ exchange. It is not known whether $[Na^+]i$, $[Ca^{2+}]i$, and/or pHi directly modulates N^+, K^+-ATPase mRNA expression. Therefore, we used normal rat kidney epithelial cells (NRK) to examine the effects of ouabain on Na^+, K^+-ATPase α1- and β1-mRNA accumulation by Northern blot analysis and the relationship between the mRNAs accumulation and $[Na^+]i$, $[Ca^{2+}]i$, or pHi. $[Na^+]i$, $[Ca^{2+}]i$, and pHi were measured using a Na^+-sensitive fluorescent dye (SBFI), a Ca^{2+}-sensitive fluorescent dye (Fura-2), and a pH-sensitive fluorescent dye (BCECF), respectively. Ouabain (1 mM) significantly increased $[Na^+]i$. Upon addition of ouabain, α1-mRNA levels increased to 2.3 times the control level at 3 hr, with a maximum 3.3-fold elevations at 12 hr. β1-mRNA levels also increased to 2.4 times the control level at 3 hr, with a maximum 3.3-fold increase at 12 hr. The ouabain-mediated α1- and β1-mRNA induction was inhibited by both the RNA transcription inhibitor (actinomycin D) and the protein synthesis inhibitor (cycloheximide). Ouabain at 3 hr caused an increase in $[Ca^{2+}]i$. Similar increases in $[Ca^{2+}]i$, which were elicited by the Ca^{2+} ionophore (ionomycin) in the presence of extracellular Ca^{2+}, had no effect on α1- or β1-mRNA levels. In Ca^{2+}-free medium treated with EGTA, ouabain at 3 hr caused a significant increase in $[Na^+]i$ without any changes in $[Ca^{2+}]i$, and also increased α1- and β1-mRNA levels. Ouabain at 3 hr caused a significant decrease in pHi. Similar decreases in pHi, which were elicited by the specific inhibitor of Na^+/H^+ exchange (ethylisopropylamiloride), caused no effect on α1- or β1-mRNA levels. Exposure of NRK to the Na^+ ionophore (monensin) in the absence of extracellular Ca^{2+} increased $[Na^+]i$ and α1- and β1-mRNA levels. The increases in α1- and β1-mRNA levels upon addition of ouabain were associated with significant increases in α1- and β1-subunit proteins. We conclude that in NRK, ouabain causes an increase in $[Na^+]i$, which directly modulates Na^+, K^+-ATPase α1- and β1-mRNA accumulation.