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System Analysis of Na-Dependent Regulation of Intracellular Mg Ion Concentration

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Summary

We investigated regulation of intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$) by Mg^{2+} permeable channels /transporters and the Na^+-Mg^{2+} exchange transport in cardiac muscle. Single ventricular myocytes enzymatically isolated from rats were loaded with the fluorescent Mg^{2+} indicator furaptra, and $[Mg^{2+}]_i$ was measured. In some experiments, intracellular Na^+ concentration ($[Na^+]_i$) was similarly measured with the fluorescent indicator SBFI. Contribution of intracellular ATP to the Na^+-Mg^{2+} exchange was studied. After treatment of the cells with either FCCP, carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone, or KCN, intracellular depletion of ATP induced a rise of $[Mg^{2+}]_i$ up to 2.5 - 3 mM and shortened cell length (due to rigor contraction). The relative initial rates of decrease in $[Mg^{2+}]_i$ upon introduction of extracellular Na^+ (Mg^{2+} efflux by the Na^+-Mg^{2+} exchange) were markedly (by ~90%) reduced in the cells depleted of ATP, compared with that in the Mg^{2+}-loaded cells. The slowed Mg efflux was not attributed to an increase in $[Na^+]_i$, because $[Na^+]_i$ measured with a Na^+ indicator SBFI was, on average, 5.0 - 10.5 mM (n = 4) within the time range for initial $\Delta[Mg^{2+}]_i/\Delta t$ measurements, while $[Na^+]_i$ at the half inhibition of the Mg^{2+} efflux is about 40 mM. To cancel intracellular acidosis caused by metabolic inhibition, application of nigericin, a proton ionophore, did not reverse the FCCP- or KNC-induced inhibition of the Mg^{2+} efflux. These results suggest requirement of cellular ATP for the Na^+-dependent Mg^{2+} transport in cardiac myocytes. Mechanism of ATP action was further studied by measuring Mg^{2+} efflux rate at different temperatures between 15°C and 35°C. Temperature dependence (Q_{10}) of the Na^+-Mg^{2+} exchange transport was estimated to be 1.56, which is lower than that expected for processes directly coupled to ATP hydrolysis.

Preliminary experiments were carried out on the Mg^{2+} efflux pathway. When the cells were superfused with a low-Na^+, high-Mg^{2+} solution, $[Mg^{2+}]_i$ quickly and linearly increased to very high levels at 35°C, but no significant rise of $[Mg^{2+}]_i$ was observed at 25°C. This high temperature dependence could be a good signature of the Mg^{2+} channels/transporters which are responsible for Mg^{2+} influx. Experiments were now ongoing to identify the Mg^{2+} influx pathways in cardiac myocytes.