OUABAIN PRECONDITIONING PROTECTS CARDIAC NA,K-ATPASE AGAINST ISCHEMIA/REPERFUSION INJURY

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Background: Ouabain and other digitalis drugs are specific inhibitors of the purified Na,K-ATPase, the ubiquitous enzyme that transports Na and K across the plasma membrane by hydrolysis of ATP. Independent of changes in ion pumping activity, ouabain binding to the cardiac Na,K-ATPase also triggers the assembly and activation of a cardioprotective signaling cascade initiated by the formation of the Na,K-ATPase/c-Src binary receptor. Activation of this complex by low doses of ouabain before ischemia/reperfusion (IR) protects the heart against infarction, a phenomenon called ouabain preconditioning (Pierre et al., Cardiovasc Res., 2007).

The aim of this study was to investigate whether ouabain preconditioning affects the fate of the cardiac membrane Na,K-ATPase enzyme complex following IR injury.

Methods: Crude homogenates from 3 groups of Langendorff-perfused rat hearts were prepared: control (continuous perfusion), IR (30 min Ischemia and 5 or 30 min reperfusion), and OPC (ouabain 10 µM before 30 min ischemia, followed by 5 or 30 min reperfusion). Na,K-ATPase enzyme activity was measured by the ouabain-sensitive ATP hydrolysis, in the presence or absence of increasing concentrations of the ionophore alamethicin. Expression levels of the 2 cardiac isoforms of the Na,K-ATPase catalytic subunit were compared using SDS-PAGE and immunodetection. Differential centrifugation was also used to access the presence of Na,K-ATPase in endosome enriched fractions.

Results: Na,K-ATPase activity was significantly decreased in all IR groups (p<0.01 vs. control). Total Na,K-ATPase isoforms expression was not altered after 5 min of reperfusion, but was significantly decreased after 30 min. However, treatment with alamethicin suggested that cellular distribution of Na,K-ATPase was altered as early as 5 min after the beginning of reperfusion. This was confirmed by immunodetection following differential cell fractionation, which showed that Na,K-ATPase α1 content increased in fractions enriched with the Early Endosome Antigen 1 (EEA1) marker in the IR groups. IR-induced alterations of Na,K-ATPase expression and activity were prevented by ouabain preconditioning.

Conclusion: Taken together, this suggests that, during the first 5 min of reperfusion that follow 30 min of ischemia, the Na,K-ATPase complex is redistributed into intracellular compartments. At 30 min, part of the Na,K-ATPase pool has been degraded. Such redistribution may explain previous reports of decreased sarcolemmal activity without changes in total protein contents and contribute to the increase in intracellular Na observed during ischemia/reperfusion injury.