Regulation of the Na+/H+ Exchanger by Rat Myocardial Protein Kinases: Phosphorylation by MAP-Kinase and p90rsk

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Abstract

We examined regulation of the Na+/H+ exchanger isoform-1 (NHE1) by phosphorylation in the rat myocardium. We utilized cytosol from adult rat myocardium, adult rat cytosol fractionated using FPLC chromatography and total cytosolic extracts from cultured neonatal cardiac myocytes. The carboxyl-terminal 178 amino acids of the Na+/H+ exchanger was expressed in E. coli as a fusion protein with GST and purified using glutathione Sepharose 4B affinity chromatography. This purified protein was used as a substrate for in vitro phosphorylation and in-gel-kinase assays. Unfractioned cytosolic extracts from neonatal cardiac myocytes or adult hearts phosphorylated the C-terminal domain of the antiporter. Western blot analysis revealed that MAP-kinase (44kDa, 42kDa) and p90rsk (90kDa) were present in specific fractions of cardiac cytosol which phosphorylated the C-terminal protein. In-gel kinase assays confirmed that kinases of approximately 44kDa and 90kDa could phosphorylate this domain. MAP-Kinase and p90rsk dependent phosphorylation of the antiporter was removed by immunoprecipitation of these kinases from extracts of neonatal cardiac myocytes. PD98059, a MEK inhibitor, decreased p90rsk phosphorylation of the antiporter and abolished serum and endothelin 1-stimulated increases in steady-state pHi. These results confirm the presence of MAP-kinase-dependent phosphorylation in the regulation of the Na+/H+ exchanger in the rat myocardium and propose an important role for p90rsk phosphorylation in regulation of the protein in the myocardium.

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