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Dissertations-Kurzfassung

Nucleoside Diphosphate Kinase B and C are Essential Regulators of G protein Content and cAMP Production in Cardiomyocytes

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Nucleoside diphosphate kinase B (NDPK B) regulates cAMP signaling by complex formation with heterotrimeric Gs proteins, key transducer of β-adrenoceptor (βAR) signals in cardiomyocytes. Thus, the function of NDPK B was explored in more detail to unravel the underlying mechanisms. The loss of NDPK B in embryonic fibroblasts (MEFs) from NDPK B-depleted mice is associated with a severe reduction in membranous Gs protein and carveolin-1 content. As this reduction is associated with a marked decrease in basal and βAR agonist (ISO) -induced cAMP formation, the re-expression of wild-type NDPK B (WT) or its catalytically inactive H118N mutant were compared in their rescue ability. Both, re-expression of WT- and H118N-NDPK B induced the re-appearance of Gs and caveolin-1 at the plasma membrane. Accordingly, WT- and H118N-NDPK B similarly enhanced ISO-induced cAMP formation. Interestingly, the catalytically inactive H118N-NDPK B was however less potent and less effective in rescuing basal cAMP production in MEFs. As identical results were obtained in neonatal rat cardiac myocytes (NRCM) after siRNA-induced knockdown and adenoviral re-expression of NDPK B, the data demonstrate a dual role of NDPK B in Gs protein regulation: The stabilization at the plasma membrane is a scaffold function of NDPK B. In addition, the catalytic activity of the enzyme is required for βAR-independent Gs activation.

Interestingly, the expression of a close relative of NDPK B, NDPK C is increased in failing human hearts and chronic treatment of rats with ISO induced an up-regulation of cardiac NDPK C expression. Upon ISO stimulation of isolated cardiomyocytes, NDPK C translocated from the cytosol to the plasma membrane within 3 h and this was accompanied by an enhanced complex formation of NDPK C with Gs proteins and NDPK B. In accordance, adenoviral overexpression of NDPK C in NRCM increased basal and ISO-induced cAMP synthesis, whereas siRNA mediated knockdown of endogenous NDPK C decreased cAMP levels by 50%. Importantly, the depletion of the NDPK C ortholog in the zebrafish resulted in severely reduced fractional shortening in the ventricle of the heart. As the contractile dysfunction was accompanied by a strong reduction in cAMP formation, the NDPK C expression level apparently regulates cAMP-dependent cardiac contractility in a similar manner as NDPK B. In contrast to the much higher expressed NDPK B, NDPK C has a hydrophobic N-terminal motif which is suspected to form a membrane insertion domain. Our data are therefore consistent with a model in which the amount of NDPK C in cardiomyocytes determines the amount of NDPK – G protein complexes at the plasma membrane thereby regulates Gs protein function, cAMP formation and thus basal as well as βAR-stimulated contractility in the heart.