

Analysis of the Mechanism Underlying Na⁺/H⁺ Exchanger NHE1-Dependent Calcineurin Activation

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Summary

Cardiac hypertrophy is caused by continuous stress, such as high blood pressure, and by neurohumoral factors, and mostly leads to heart failure, which has poor prognosis. Elucidating the mechanism by which hypertrophy leads to heart failure is very important in preventing the latter. Calcineurin (CaN), a Ca²⁺-dependent phosphatase, is a key molecule that regulates pathological cardiac hypertrophy. CaN dephosphorylates a downstream transcription factor NFAT, which in turn induces hypertrophic gene expression. Recently, we found that an Na⁺-dependent pH-regulating transporter NHE1 activated the CaN-NFAT signaling, leading to cardiomyocyte hypertrophy, which involved the direct binding of CaN to the 6-residue motif (PVITID) in the cytosolic domain of NHE1; we hypothesized that local pH increase produced by NHE1 enhanced the activity of CaN bound to NHE1 by sensitizing Ca²⁺ (grant No. 0836). However, it is unknown how CaN signal is transmitted from NHE1 to NFAT. CaN needs to be released from NHE1 after activation, because CaN-binding to NFAT is essential for its dephosphorylation, with CaN being able to associate with either NHE1 or NFAT at the same binding site. Therefore, we expected that the differences of binding affinity between CaN and NHE1 or NFAT allow CaN to move from NHE1 to NFAT. In this study, we performed detailed mutagenesis study of the CaN-binding site of NHE1. Substitution of the PVITID sequence with a high-affinity sequence (PVIVIT) or a low-affinity sequence (PVIAVN) abolished the CaN-NFAT signaling. In addition, substitution with other high-affinity sequences originating from NFATs (PRIEIT, PSIQIT, or PSIRIT) also abolished the signaling. Alanine-scanning mutagenesis revealed that the original NHE1 sequence showed optimal signal amplification, suggesting that the balanced affinity between NHE1 and CaN is critical for efficient signaling. We consider that such moderate interaction is important for the removal of CaN from NHE1, and for rebinding to downstream target NFAT after NHE1-dependent activation.