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AUTOCOMMENTARY

Heat shock proteins and the Na^+/H^+ exchanger

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Na^+/H^+ exchanger isoform one (NHE1) is a pH regulatory protein that is virtually ubiquitous in mammalian tissues and serves to remove protons from within cells and protect against intracellular acidification. Human NHE1 has a large 315 amino acid cytosolic regulatory domain that regulates the catalytic membrane domain of 500 amino acids (Fig. 1). NHE1 is important in cell growth, is a trigger in heart disease, and is an important trigger of breast cancer.[1,2] In heart disease it contributes to ischemia/reperfusion damage in the myocardium through elevated activity that leads to increased intracellular sodium, which causes reversal of the Na^+/Ca^{2+} exchanger. This leads to intracellular calcium overload and cell damage. Additionally, elevated NHE1 activity contributes to cardiac hypertrophy.[1] In triple negative breast cancer, abnormal elevated NHE1 activity is a trigger for metastasis.[2] Regulation of NHE1 is thus of critical interest in human disease.

NHE1 is regulated by both protein kinase mediated phosphorylation and by interaction with regulatory proteins that bind to the cytosolic regulatory domain. To elucidate which regulatory proteins are critical in NHE1 regulation, we recently examined protein-mediated regulation of NHE1 in the kidney.[3] NHE1 is present in different renal cell lines and in whole kidney itself. We used expressed and purified C-terminus of NHE1 to do affinity chromatography with whole kidney extracts. This yielded several NHE1 binding proteins and Hsp70 and Hsp90 were prominent among these. The interaction of these heat shock proteins with NHE1 was confirmed by immunoprecipitation. AKT is a regulatory kinase that complexes with Hsp90.[4] We therefore examined the physiologic role of both Hsp90 and AKT in regulation of NHE1 activity. Treatment of cells with either Hsp90 inhibitor (17-AAG), or AKT inhibitor (MK2206) resulted in decreased NHE1 activity and decreased levels of phosphorylation of NHE1. In addition, treatment of cells with both 17-AAG and MK2206 showed no additive inhibitory effect on NHE1 activity indicating that both Hsp90 and AKT acted through a similar regulatory mechanism. These results demonstrated that Hsp90 and its associated regulatory kinase AKT, can regulate the activity of NHE1.[3]

Hsp70 has been shown to interact with NHE1 in fibroblasts,[5] in macrophages[6] and in brain and heart[7] while the association of NHE1 with Hsp90 has not been characterized earlier. Our results are the first demonstration of the mechanism by which heat shock proteins are involved in protein-mediated regulation of NHE1 by Hsp90. However, there still remains the question of how this regulation affects the activity of the protein and what the downstream effects are on cellular function.

Our results also showed that inhibition of AKT decreased NHE1 activity and phosphorylation levels. Thus phosphorylation by AKT was stimulatory in our assay. Previous reports have shown that AKT mediated phosphorylation occurs at serine 648 and that it can be either inhibitory or stimulatory, depending on the cell type[8] (and reviewed in[1]). NHE1 contains calmodulin binding domains at amino acids 626–653 and 658–686. The first of these, is a higher affinity calmodulin binding site that acts as an autoinhibitor of NHE1 when calcium-calmodulin complex is not bound.[1] The phosphorylaton site of AKT is within the

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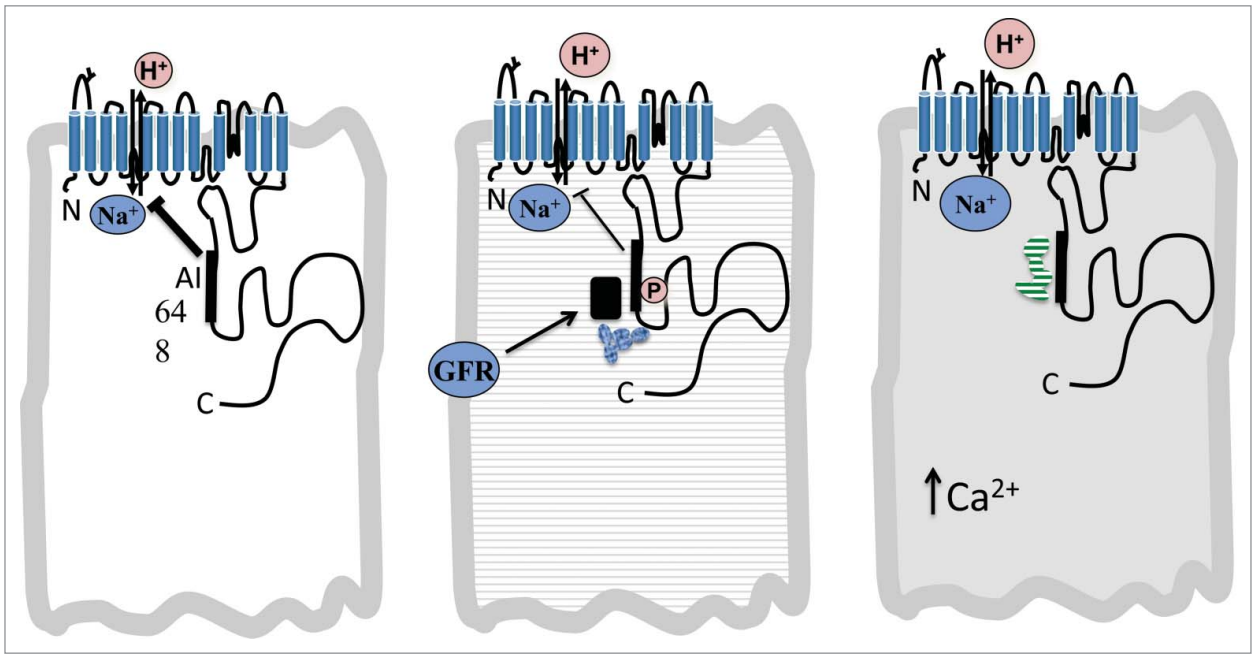

Figure 1. Schematic diagram of proposed mechanism of regulation of NHE1. AI, auto-inhibitory domain on the regulatory tail (indicated by thick line). AI contains amino acid 648. Left panel, absence of phosphorylation or calmodulin binding inhibits NHE1. Center panel, in a cell with a stimulated growth factor receptor (GFR), AKT kinase (rectangle) binds to AI domain and phosphorylates amino acid 648 reducing auto-inhibition. Hsp90 (stippled) guides AKT to the NHE1 tail. Right panel, in high intracellular calcium, and in the absence of phosphorylation, calmodulin (striped) binds to AI and prevents auto-inhibition.

high affinity calmodulin binding/autoinhibitory domain. Snabaitus et al.[8] demonstrated that phosphorylation of serine inhibits calcium/calmodulin binding. We suggest that AKT-Hsp90 mediated phosphorylation reduces auto-inhibition by this domain, but not as effectively as calcium/calmodulin binding. Therefore, in cells with higher endogenous levels of calcium in the presence of calmodulin, the auto inhibitory site is permanently blocked by calcium/calmodulin. This elevates NHE1 activity. Phosphorylation by AKT may be inhibitory in these cases preventing calcium/calmodulin binding and reducing the blockage of the auto-inhibitory domain. On the other hand, in tissues with lower calcium levels, phosphorylation may reduce auto inhibition significantly, (though not as effectively as complete blockage mediated by the presence of a calcium/calmodulin complex). Figure 1 illustrates this hypothesis. The role of Hsp90 in this mechanism may be to more efficiently target AKT to the tail of NHE1 and amino acid 648.

A number of experiments could be done to test this theory. Firstly, the site of action of the auto inhibitory domain on the membrane domain needs to be clearly identified. Then the relative affinity of phosphorylated vs. unphosphorylated inhibitory domain could be determined, including in the presence of calcium/calmodulin. Alteration of the intracellular level of calcium combined with measurement of calmodulin association with the NHE1 tail *in vivo*, would also be instructive. Additionally, the association of NHE1 with Hsp90, AKT and calmodulin in the disease state are of interest. Finally, identification of the Hsp90 binding site on NHE1 would be desirable. Future experiments may pursue these studies.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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