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**Na/K-ATPase amplification of oxidant stress; a universal but unrecognized clinical target?**
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Abstract

The Na/K-ATPase has a signaling function which appears to be separate from its ion pumping function. This signaling function refers to the transduction of conformational changes in the Na/K-ATPase α1 subunit. These changes activate Src’s tyrosine kinase activity, triggering a cascade which generates reactive oxygen species (ROS), modulates other signaling pathways, and causes many physiological and pathophysiological effects. We have recently observed that ROS themselves as well as cardiotonic steroids can actually initiate the signal by directly inducing conformational changes in α1. It therefore appears that the Na/K-ATPase signal cascade can serve as a feed forward amplification for ROS with circulating cardiotonic steroids setting the gain. Work in both cellular and animal models of disease suggest that this amplification process is activated in conditions characterized by oxidant stress ranging from cancer to obesity/metabolic syndrome and may serve as a potential clinical target for interventions.

Keywords:

Na/K-ATPase, oxidant stress, hypertension, fibrosis, renal failure, obesity
The sodium potassium adenosine triphosphatase (Na/K-ATPase) was discovered by Jens Skou in the 1950s and found to be the predominant ion pump in animal tissues.\(^1\) The Na/K-ATPase consists of at least 2 peptides named \(\alpha\) and \(\beta\) which each possess different isoforms. The \(\alpha_1\) isoform appears to be displayed ubiquitously in different animal tissues, and for many years was considered to be less important in terms of cell signaling than other \(\alpha\) isoforms (e.g., \(\alpha_2\) and \(\alpha_3\)) which are expressed more selectively.\(^2\) Although considerable attention has been devoted to the study of the Na/K-ATPase and its isoforms, the discovery that conformational changes in \(\alpha_1\) can induce a signal cascade initiated within caveolae and/or lipid rafts has only occurred within the last 20 years.\(^1\) Our group has demonstrated that the Na/K-ATPase \(\alpha_1\) subunit regulates the activity of membrane associated Src, and that conformational changes in \(\alpha_1\) may disinhibit Src’s tyrosine kinase, leading to activation of a signal cascade which generates reactive oxygen species (ROS), activates mitogen activated protein kinase (MAPK), mammalian target of rapamycin (mTOR) as well as many other cellular processes.\(^3\)\(^-\)\(^6\) The contribution of other \(\alpha\) isoforms to signaling is less clear at present.

More recently, we have observed that in addition to generating ROS, the signal cascade can be initiated by H2O2, creating a feed forward amplification of oxidant stress.\(^7\) Specifically, it appears that either cardiotonic steroids or oxidant stress directly can cause the reversible carbonylation of the \(\alpha_1\) subunit at specific sites.\(^7\) As this process can also be initiated by the binding of cardiotonic steroids to the Na/K-ATPase \(\alpha_1\) subunit, we would argue that these cardiotonic steroids set the gain for the amplification of ROS. Specifically, increases in the concentration of cardiotonic steroids might allow for greater amplification of ROS generated by other cellular process related to signaling and/or energy metabolism. A schematic of this process is shown in Figure 1.
Figure 1: Schematic showing Na/K-ATPase as reactive oxygen species (ROS) amplifier system. CTS-cardiotonic steroids, Orange dimer is Na/K-ATPase. pNaKtide refers to peptide formed from epitope of Na/K-ATPase α1 subunit known to bind Src tyrosine kinase fused with TAT leader sequence.

Using this framework, we have identified that activation of this oxidant amplification occurs in settings ranging from cancer growth to organ fibrosis.8-13 We find that we can interfere with this process by downregulation of the expression of the Na/K-ATPase, elimination of caveolar structure, antibodies to cardiotonic steroids, pharmacological antagonists of cardiotonic steroid binding to the Na/K-ATPase, Src inhibitors and perhaps most directly, by a peptide developed from the α1 Na/K-ATPase subunit merged with a TAT leader sequence to allow for cellular uptake and membrane distribution called pNaKtide.3, 13-17 We propose that virtually any of these strategies might have potential clinical application in conditions associated with oxidant stress.

Testing our hypothesis is non-trivial. First, it is likely if not certain that normal physiological functions are regulated in part by this oxidant amplification. Specifically, we know that urinary sodium excretion is impacted by this pathway in rodents, and we have reason to believe that fetal growth and organ development may also require the participation of this pathway.18 Any clinical testing would need to anticipate these potential toxicities. Second, existing pharmacological agents which antagonize the cardiotonic steroid regulation of this amplifier system may be promiscuous. Spironolactone and its major metabolite, canrenone, while satisfying pharmacological evidence for being competitive antagonists of cardiotonic steroid binding to the Na/K-ATPase, are also well known to have effects on the mineralocorticoid receptor.12 Lastly, most agents which target this system including humanized antibodies to cardiotonic steroids or peptides like pNaKtide have yet to be
subjected to rigorous toxicological analysis. Clearly, testing of these agents in actual clinical scenarios will need additional preclinical work to ensure safe study.

That said, we believe there is widespread potential application. Accumulation of oxidant injury appears to be central to the pathogenesis of many clinical problems, some of which have been effectively recalcitrant to current therapies. It is our contention that addressing the cellular amplification of oxidant stress may allow for novel therapies that might effectively treat some of these clinical problems.
References


