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Editorial

New pathways for an old molecule: The role of the Na^+–K^+ ATPase pump in peripheral neuropathy

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Life began in the ocean. The first organisms, just single cells, needed a way to keep their cytoplasm inside a cell membrane less salty than the surrounding seawater. This was done by some of the first membrane proteins made in the primitive cells; ion channels, transporters, and active ion exchangers like the Na/K ATPase pump. Also called the sodium pump, N/K ATPase transfers three sodium ions out of the cell for every two potassium ions brought into the cell. Using the bond-energy from breaking the terminal phosphate group of ATP to drive these ions against their concentration gradient, N/K ATPase establishes and maintains a resting membrane potential in all cells. The N/K ATPase pump also helps to regulate cell volume. As the osmolarity of the cell is increased by the intracellular build-up of organic substances, causing cell swelling, N/K ATPase compensates by lowering intracellular ion concentrations.

The Na/K ATPase molecule is a heterotrimer composed of alpha, beta, and gamma subunits [1]. The alpha subunit is the largest of the subunit proteins and contains 10 transmembrane, alpha-helical spans which form a ring in the cell membrane. The first functional domain of the alpha subunit is the 'actuator' near the amino terminus and formed by the intracellular segments of the first two loops. The actuator serves as an occlusion or gate for sodium and potassium ions. Farther along there is a 'phosphorylation' domain, which includes a highly-conserved aspartate residue (D376) that is transiently phosphorylated. The 'nucleotide' domain forms a pocket for the ATP molecule which will give up its terminal phosphate group [2].

The beta subunit consists of a single transmembrane spanning peptide, with a short amino terminus inside the cell and a large carboxy end outside the cell. This extracellular segment sports an array of glycosylation sites and is closely associated with the extracellular domains of the alpha subunit. The gamma subunits are the least studied of the three subunits of N/K ATPase. The N/K ATPase pump can function without gamma subunits, but needs both alpha and beta subunits. The gamma subunit was identified as a member of the 'FXYD' protein family. For this reason, sometimes the N/K ATPase protein is said to be a dimer with a variable association of a third (gamma or FXYD family) protein [2].

Phylogenetic analysis of genomes from organisms ranging from one-celled protists to mega-metazoans like humans shows that the Na/K ATPase pump is an ancient molecule and conserved in all animal species examined to date [3]. From analysis of existing organisms, it can be stated that the origin of catalytic alpha subunit of N/K ATPase was prokaryotic and today is expressed in nonmetazoans such as algae, protozoans, and fungi, as well as in all cells of all metazoans. The primitive dinoflagellate found in oceans today represents a pre-metazoan ancestor and it expresses two types of gene coding for the catalytic alpha subunit of N/K ATPase proteins. The first gene type went on to become ubiquitous in all animal cells, and through genome and gene duplication, now exists in four isoforms in most species. The second gene encoding for a different alpha subunit in the dinoflagellate appears not to have survived metazoan lineage. It is found today only in nematodes [3]. While the N/K ATPase pump is found in all unicellular and metazoan animal cells, it is not the same exact protein in all organisms and cell types as there are species differences in the primary sequences of subunits as well as isoforms of each subunit within species. Humans and other mammals express four types of alpha subunits ($alpha_{1-4}$), three beta subunits ($beta_{1-3}$) and seven $gamma_{1-7}$ subunits/$FXYD_{1-7}$ associated proteins [4]. The various isoforms differ slightly in their primary sequence but mainly in their levels of expression in various tissues [5]. The $alpha_1$ isoform has the widest distribution and is found in several tissues, including the kidney, nerves, and lung. The $alpha_2$ isoform is expressed in the skeletal muscle and heart, and the $alpha_3$ isoform mainly in the brain. The $alpha_4$ isoform is restricted to the testes and appears to be specific to the spermatozoa.

The non-stop operation of the N/K ATPase pump is a costly expense for organisms. It is estimated that about 20% of the resting metabolic energy expenditure goes towards the running of the N/K ATPase pumps to maintain membrane potential [5]. For the specialized cells (neurons) of the brain, the energy expenditure for maintaining membrane potential by N/K ATPase is even greater. In one recent study, up to 54% of the energy used by cortical neurons is dedicated to N/K ATPase pumps to maintain resting membrane potential [6]. It is amazing to think that a fifth or more of the food one eats on a daily basis is converted to ATP and routed to N/K ATPase pumps, just to keep the ions inside our cells different than the surrounding seawater (now blood) of their ancestral birthplace.

Many readers may already know that the N/K ATPase pump is targeted by cardiac glycosides (e.g. digoxin and ouabain) used to treat congestive heart failure. Inhibition of the activity of N/K ATPase in cardiac muscle cells with digoxin increases the contractions of a failing heart by increasing the intracellular concentration of sodium, which then reverses the sodium–calcium pump to drive more calcium ions into the cell [7]. Cardiac glycosides such as digitalis and ouabain have been used for over 250 years for the treatment of congestive heart failure and were obtained from plants. Perhaps the most surprising is the finding that endogenous ouabain (EO) is present in human blood and other tissues [8]. This endogenous substance is not an ouabain-*like* molecule or mimic; it is identical to ouabain derived from the plant world and is

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a steroid. EO is synthesized in cortical cells of the adrenal gland, like other steroids. There is also EO detected in extracts of the hypothalamus. Physiological blood levels of EO are in the picomolar to nanomolar range which stimulate or activate N/K ATPase signaling transduction pathways. These pathways include interaction with the IP_3 receptor causing release of intracellular calcium, the PI3-kinase/Akt pathway with roles in cell proliferation, and MAPK kinase pathways with various cascading cellular effects. It is only at higher concentrations, such as those resulting from clinical use of ouabain that inhibition of N/K ATPase occurs [8].

In this issue, Dennis Paul and colleagues document the novel role that Na/K ATPase plays in the development of nerve injury (peripheral neuropathy) after an inflammation paw insult in a rat model [9]. From previous research, the authors demonstrated that sodium channels (specifically NaV1.7 channels) are up-regulated in the dorsal root ganglia (DRG) of animals subjected to paw inflammation using an injection of complete Freund's adjuvant (CFA). CFA is commonly used to initiate an inflammatory reaction in animal models and is composed of inactivated and dried mycobacteria, *Mycobacterium tuberculosis*. The authors hypothesized that the influx of sodium cations due to increased NaV1.7 channels with inflammation would need to be countered by an increase in Na/K ATPase molecules in the DRG. It was further hypothesized that the treatment of inflamed animals with ouabain would increase the effects of inflammation and lead to peripheral neuropathy. As hypothesized there was an up-regulation of N/K ATPase in the DRG innervating the inflamed paws compared to the contralateral control DRG using immunohistochemical methods and antibodies to N/K ATPase. Using western blot analysis followed by digital image quantification, the authors showed that both the $alpha_1$ and $alpha_3$ subunits were upregulated in the DRG with paw inflammation. Importantly, the study showed functional relevance of the up-regulated N/K ATPase as inflamed animals treated with ouabain (10 mg/kg, s.c.) after the CFA injection had a significant number of dead DRG neurons compared to saline-treated controls.

This study is important as it demonstrated not only the balancing up-regulation of N/K ATPase in the context of up-regulated Nav1.7 sodium channels as hypothesized but also the fact that these N/K ATPase proteins are essential to preventing neuronal death in the inflammation model. The timing of the Nav1.7 and N/K ATPase up-regulation is such that the authors further hypothesize that there is a common cellular signal inducing simultaneous up-regulation of these membrane proteins. Like so many good scientific papers, this report leads to more questions than it answers. What is the correlation of the observed changes in ouabain-treated animals to ensuing neuropathy? Do these animals show a greater degree of neuropathic pain behaviors such as allodynia or hyperalgesia? What is the dose–response relationship of ouabain to the observed neuronal death and ensuing neuropathy? Can the authors show that low-dose ouabain stimulates N/K ATPase signaling and is this beneficial or detrimental in the peripheral inflammation model? What are the levels of endogenous ouabain and do they change with inflammation?

Other recent reports demonstrate N/K ATPase involvement in psychiatric disorders, suggesting a wider role for N/K ATPase than just pumping cations back and forth across the membrane. There is a role for wild-type or mutant N/K ATPase dysfunction in schizophrenia, autism, depression, mania, and parkinsonism [10–12]. The work from Dennis Paul and others aptly demonstrates that stalwart proteins of the cell membrane, such as sodium channels and N/K ATPase pumps, are complex regulators of cell biology and are themselves highly regulated. As there is a rapidly growing literature on the function and regulation of N/K ATPase pumps, it is certain that research on these ancient molecules will continue to yield new and exciting discoveries.

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