Na/K-ATPase Ion Transport and Receptor-Mediated Signaling Pathways

Sandrine V. Pierre¹ · Gustavo Blanco²

Accepted: 19 October 2021 / Published online: 1 November 2021

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

While it had been observed that animal cells maintain a steep Na^+ and K^+ gradient across their plasma membrane, the mechanisms involved in this phenomenon remained unexplained for quite some time. It was not until 1957, when Jens Christian Skou from University of Aarhus, Denmark, working with crab nerves, discovered the Na,K-ATPase as an ion transport system (Skou 1957). This fundamental finding made him the recipient of the Nobel prize in Chemistry in 1997, along with two other investigators (J. Walker and P. Boyer), who worked in the mechanisms of action of the mitochondrial proton ATPase and cell bioenergetics (Skou 1998). Skou not only identified the Na,K-ATPase, but he continued by characterizing its biochemical properties and mode of action (Skou, 1957).

The Na,K-ATPase, Na pump, or simply “the pump” belongs to the P-type ATPase family of transport molecules, a series of proteins that use the free energy from the hydrolysis of ATP and become transiently phosphorylated, to actively move ions across cell membranes. Na,K-ATPase exchanges 3 cytoplasmic Na^+ for every 2 extracellular K^+ that move into the cell, through protein conformational changes fueled by ATP. The Na^+ gradient generated by Na,K-ATPase is the force that drives the secondary transport of many other solutes and water in and out of the cells. In addition, the uneven distribution of charges that it maintains across the cell plasma membrane contributes to the cell resting membrane potential and allows excitability in muscle and nerve cells (Horisberger et al. 1991; Jorgensen et al. 2003; Lingrel 1992).

During the second half of the twentieth century, a lot of effort was placed on trying to understand the molecular mechanism of the Na,K-ATPase function. A series of biochemical steps in the catalytic cycle of the Na,K-ATPase were defined. (Apell et al. 1998). The advent of molecular biology allowed a better understanding of the Na,K-ATPase structure and it was recognized that an α subunit, involved in the enzymatic catalysis of ATP, Na^+ and K^+ translocation, and binding of the inhibitor ouabain constituted the catalytic component of the Na,K-ATPase (Apell 2003). A second subunit, the glycosylated β polypeptide, was found to be important for the folding and delivery of the Na,K-ATPase to the plasma membrane (Geering 1991; Lingrel et al. 1990; Martin 2005). However, it was later realized that an additional series of small hydrophobic polypeptides, which have a common FXVD sequence, were also associated to the Na,K-ATPase and are important for the regulation of the function of the transporter (Arystarkhova and Sweadner 2005; Garty and Karlsh 2006; Geering et al. 2003).

Another breakthrough occurred with the finding that not one, but multiple isoforms of Na,K-ATPase existed in mammals, with this molecular diversity including four different α ($\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 4$) and 3 distinct β ($\beta 1$, $\beta 2$ and $\beta 3$) polypeptides expressed in a particular tissue specific pattern and a developmentally regulated manner (Blanco and Mercer 1998; Lingrel 1992; Sweadner 1989). Today, the detailed structure of the $\alpha 1$, $\beta 1$ isoforms is known in detail thanks to crystallographic studies performed under different conditions and conformational states of the proteins, (Nyblom et al. 2013; Toyoshima and Cornelius 2013). In addition, the regulation of function of Na,K-ATPase was described and it was recognized that a dynamic phosphorylation/dephosphorylation of specific residues in the α subunit was able to modify Na,K-ATPase activity or expression levels at the surface of cells.

While a lot of studies have been focused on the function of the Na,K-ATPase as an enzyme and ion transport system, a new astonishing function for the Na,K-ATPase

✉ Gustavo Blanco
gblanco@kumc.edu

¹ Marshall Institute for Interdisciplinary Research (MIIR),
Marshall University, Huntington, WV 25755, USA

² Department of Molecular and Integrative Physiology,
University of Kansas Medical Center, 3901 Rainbow
Boulevard, Kansas, KS 66160, USA

was unveiled in the late 1990's. Studying the role of Na,K-ATPase in heart hypertrophy, Askari and Xie found that upon binding of glycoside steroids, such as ouabain, Na,K-ATPase responded by activating a series of intracellular messengers in a cascade of events that trigger the phosphorylation of downstream proteins to induce a multiplicity of responses that are cell dependent. This activity was found to be independent from the classical "pumping action" and is today known as the "signaling function" of Na,K-ATPase (Pierre and Xie 2006; Xie and Askari 2002; Xie and Cai 2003). It is through this signaling action that Na,K-ATPase modifies proliferation, apoptosis, necrosis, motility, adhesion and metabolism of cells. The relevance of Na,K-ATPase signaling took another dimension by the finding that ouabain and other glycosides are endogenous substances circulating in the plasma of mammals, including humans (Blaustein and Hamlyn 2020).

Who could have ever imagined that as it was not enough for the Na,K-ATPase system to be in charge of maintaining cell ion balance, it would be performing additional roles, also essential to life? The discovery of a novel function for the pump as the receptor and signal transducer for glycosides opened a new field, which was embraced with enthusiasm, curiosity and sometimes skepticism by many researchers who tried to understand how the integrated pumping and signaling function of Na,K-ATPase supports cell function.

The continuous growth of information in the Na,K-ATPase field gives the impetus to produce a special issue of the *Journal of Membrane Biology* dedicated to this fascinating system. This issue includes a series of manuscripts, covering both the role of Na,K-ATPase as an ion transporter, as well as a target for the effects of cardiac glycosides. Within the work presented, are the results by the group of Vadász, which provides an overview and update on the assembly, maturation and trafficking of the Na,K-ATPase to the plasma membrane, emphasizing the role that the endoplasmic reticulum (ER) and chaperone proteins have in the process (Kryvenko et al. 2021). Importantly, this article points out the importance of the ER microenvironment and how its alteration, due to changes in calcium, ATP levels, or oxidative insults, can affect the co- and post-translational processing of the Na,K-ATPase, preventing its proper maturation. This may help explain the ion changes that are observed under different pathological conditions and opens possibilities for potential interventions with the goal of restoring ionic balance in diseased cells.

Aging is also a physiological situation where Na,K-ATPase function may be modified. This is pointed out by the contribution from Maurya and collaborators (Kumar and Maurya 2021), who confirm a decrease in the Na,K-ATPase from erythrocytes with age and shows that addition of the flavonol quercetin tends to restore the activity of Na,K-ATPase by mechanisms that may involve antioxidant

effects and the capacity that quercetin has as an scavenger of reactive oxygen species.

The detrimental role of oxidative stress on Na,K-ATPase activity is also highlighted by the contribution from the group of Scavone (Kawamoto et al. 2021). In search for the mechanisms involved in neurodegenerative diseases, such as Alzheimer disease (AD), these investigators show that decrease in Na,K-ATPase activity, may contribute to impair the function and augment the vulnerability of neuronal cells to oxidative stress. Working with rat hippocampal slices, they found that amyloid β induced a concentration-dependent increase in nitric oxide (NO) activity and cyclic guanosine monophosphate (cGMP) levels, that reduced Na,K-ATPase activity. This effect could be reverted by treatment with the NO inhibitor N omega-nitro-L-arginine methyl ester (L-NAME), showing the influence of the nitric oxide pathway on the amyloid beta peptide effect on Na,K-ATPase. Moreover, the N-methyl-D-aspartate (NMDA) receptor is an upstream mediator of the response. Interestingly, the reduction in Na,K-ATPase activity was isoform specific, with only the $\alpha 1$, but not $\alpha 2$ or $\alpha 3$ being affected. This study importantly advances our understanding of the molecular mechanisms underlying AD and highlights the different role that isoforms of the Na,K-ATPase have during a pathological condition.

A mechanism that operates modifying Na,K-ATPase activity through oxidative stress, appears to also be implicated in cancer. This topic is addressed by the group of Barbosa, who presents an article showing that a disbalance between the production of free radicals and insufficient levels of antioxidants is correlated with increased Na,K-ATPase activity in head and neck squamous cell carcinoma from patient donors (Toledo et al. 2021). It is possible that this mechanism could extend to other type of malignancies, since augmented Na,K-ATPase had previously been seen in lung and renal cancer, as well as in glioblastoma. The effect is mediated through the Na,K-ATPase $\alpha 1$ isoform, which makes the upregulation of this transporter a potential marker for head and neck cancer and a target to be considered for the development of novel therapies for this disease.

Cardiac glycosides have been shown to have cytotoxic effects and they can trigger different mechanisms of cell death, depending on the cell type considered. This has focused the attention on these compounds as potential anti-cancer drugs; however, their cardiotoxic effects severely limit their use. The steroidal nucleus and lactone ring in their structure have the most prominent role for the activity of these compounds. Therefore, these chemical moieties have been the target of modifications, with the idea of generating compounds that will be more efficient and selective toward cancer cells (Ayogu and Odoh 2020). The paper by the group of Cortes presents data on the effect of synthetic modifications of the cardiac glycoside digitoxin on tumoral cell lines

(de Oliveira et al. 2021). The results show that γ -benzylidene derivatives have reduced toxicity toward cells compared to the parent compound and required much higher concentrations to inhibit Na,K-ATPase activity in Hela, human lung carcinoma and human fibroblast cells. In addition, the compounds had an isoform specific effect. The group of Barbosa also explored the role of a digoxin derivative in cancer cells and find that these drugs can induce changes in the lipid profile of the membrane of those cells (Silva et al. 2021). While these γ -benzylidene digoxin derivatives may not be currently effective against tumoral cells, they represent compounds that could be better tolerated and could be used in association with other approaches to inhibit cancer growth. Moreover, the modifications performed provide important information on the structure function relationship of glycosides that will allow to continue exploring further chemical changes to enhance desired effects.

Cardiotonic steroids have been shown to have pleiotropic effects, an event that could be attributed to their mechanisms of action, both on the “pumping” or the “signaling” action of the Na,K-ATPase (Riganti et al. 2011). Most of the times, it is hard to differentiate between those effects and in addition, glycoside induced and Na,K-ATPase mediated pathways frequently involve a multiplicity of intermediates and effectors. The group led by Shapiro present a provoking review article that may help explain the unexpected cardiorenal protective effects of the sodium glucose transport system 2 (SGLT-2) inhibitors in the context of Na,K-ATPase signaling (Liu et al. 2021). While the function of Na,K-ATPase in the maintenance of the transmembrane Na^+ gradient driving SGLT-2 activity has been well demonstrated, this effect falls short in explaining the cardiac and renal enhancing effects of SGLT-2 inhibitors. Na,K-ATPase signaling is presented as an alternate mechanism, which by favoring natriuresis and blood pressure decrease in the kidney, or reducing fibrosis in the heart, acts in a synergistic manner with the SGLT-2 inhibitors.

The crucial role of Na,K-ATPase requires that the transporter is tightly regulated (Ewart and Klip 1995; McDonough and Farley 1993; Pirkmajer and Chibalin 2016). The work by the group of Chibalin describes the modulation of phosphorylation of the Na,K-ATPase $\alpha 1$ subunit on tyrosine 10 by AMPK and ouabain. The authors show that the effect is via the activation of the EGFR receptor. This highlights the link between cellular metabolism and Na,K-ATPase regulation (Petric et al. 2021).

Finally, we would like to remark the importance of the molecular diversity of Na,K-ATPase, which appears to have developed as an evolutionary advantage to serve the needs of specific cells. Our group has been working for quite some time on Na,K-ATPase $\alpha 4$, a polypeptide which expression is limited to testis male germ cells and is abundant in fully differentiated spermatozoa (Jimenez et al. 2011). In this special

issue, we used a double genetic and pharmacologic approach to first switch the ouabain affinity of both of the Na,K-ATPase α subunits expressed in spermatozoa ($\alpha 1$ and $\alpha 4$), to then allow selective blockage of their function with ouabain (McDermott et al. 2021). This approach confirmed that Na,K-ATPase $\alpha 4$ is the main Na^+ and K^+ active transporter involved in sperm motility, while Na,K-ATPase $\alpha 1$ exerts a housekeeping role, maintaining the basic ion balance in the cell. In addition, since the mutation of the ouabain affinity site in Na,K-ATPase $\alpha 1$ and Na,K-ATPase $\alpha 4$ did not interfered with fertility of the mouse, our results show that the ouabain affinity site in the Na,K-ATPase is not an essential requirement for sperm production or its fertilizing capacity. These data once again point to the exquisite refinement that nature undergoes to achieve proper cell specific tasks.

With these collection of manuscripts, we now invite the reader to dive into the world of the Na,K-ATPase, a critical system, with multiple functions that supports life and the proper function of cells.

Acknowledgements We would like to thank Dr. Alexey S. Ladokhin from the department of Biochemistry and Molecular Biology of University of Kansas Medical Center for giving us the opportunity to serve as Guest Editors for this special issue and for all his help during the process.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

- Apell HJ (2003) Structure-function relationship in P-type ATPases—a biophysical approach. *Rev Physiol Biochem Pharmacol* 150:1–35
- Apell HJ, Schneeberger A, Sokolov VS (1998) Partial reactions of the Na, K-ATPase: kinetic analysis and transport properties. *Acta Physiol Scand Suppl* 643:235–245
- Arystarkhova E, Sweadner KJ (2005) Splice variants of the gamma subunit (FXD2) and their significance in regulation of the Na, K-ATPase in kidney. *J Bioenerg Biomembr* 37:381–386
- Ayogu JI, Odoh AS (2020) Prospects and therapeutic applications of cardiac glycosides in cancer remediation. *ACS Comb Sci* 22:543–553
- Blanco G, Mercer RW (1998) Isozymes of the Na-K-ATPase: heterogeneity in structure, diversity in function. *Am J Physiol* 275:F633–F650
- Blaustein MP, Hamlyn JM (2020) Ouabain, endogenous ouabain and ouabain-like factors: the Na(+) pump/ouabain receptor, its linkage to NCX, and its myriad functions. *Cell Calcium* 86:102159
- de Oliveira GC, Rocha SC, da Silva Lopes MA, Paixão N, Alves SLG, Pessoa MTC, Noël F, Quintas LEM, Barbosa LA, Villar JAFP, Cortes VF (2021) Implications of synthetic modifications of the cardiotonic steroid lactone ring on cytotoxicity. *J Membr Biol* 2021:1–11

- Ewart HS, Klip A (1995) Hormonal regulation of the Na(+)-K(+)-ATPase: mechanisms underlying rapid and sustained changes in pump activity. *Am J Physiol* 269:C295–C311
- Garty H, Karlish SJ (2006) Role of FXYD proteins in ion transport. *Annu Rev Physiol* 68:431–459
- Geering K (1991) The functional role of the beta-subunit in the maturation and intracellular transport of Na, K-ATPase. *FEBS Lett* 285:189–193
- Geering K, Beguin P, Garty H, Karlish S, Fuzesi M, Horisberger JD, Crambert G (2003) FXYD proteins: new tissue- and isoform-specific regulators of Na, K-ATPase. *Ann N Y Acad Sci* 986:388–394
- Horisberger JD, Lemas V, Kraehenbuhl JP, Rossier BC (1991) Structure-function relationship of Na, K-ATPase. *Annu Rev Physiol* 53:565–584
- Jimenez T, McDermott JP, Sanchez G, Blanco G (2011) Na, K-ATPase alpha4 isoform is essential for sperm fertility. *Proc Natl Acad Sci U S A* 108:644–649
- Jorgensen PL, Hakansson KO, Karlish SJ (2003) Structure and mechanism of Na, K-ATPase: functional sites and their interactions. *Annu Rev Physiol* 65:817–849
- Kawamoto EM, Cararo-Lopes MM, Kinoshita PF, Quintas LEM, Lima LS, Andreotti DZ, Scavone C (2021) Influence of nitric oxide-cyclic GMP and oxidative STRESS on amyloid- β peptide induced decrease of Na,K-ATPase activity in rat hippocampal slices. *J Membr Biol*. <https://doi.org/10.1007/s00232-021-00196-9>
- Kryvenko V, Vagin O, Dada LA, Sznajder JI, Vadász I (2021) Maturation of the Na,K-ATPase in the endoplasmic reticulum in health and disease. *J Membr Biol*. <https://doi.org/10.1007/s00232-021-00184-z>
- Kumar A, Maurya PK (2021) Quercetin mitigates red blood cell membrane bound Na⁺, K⁺-ATPase transporter during human aging. *J Membr Biol*. <https://doi.org/10.1007/s00232-021-00200-2>
- Lingrel JB (1992) Na, K-ATPase: isoform structure, function, and expression. *J Bioenerg Biomembr* 24:263–270
- Lingrel JB, Orlowski J, Shull MM, Price EM (1990) Molecular genetics of Na, K-ATPase. *Prog Nucleic Acid Res Mol Biol* 38:37–89
- Liu J, Tian J, Sodhi K, Shapiro JI (2021) The Na/K-ATPase signaling and SGLT2 inhibitor-mediated cardiorenal protection: a crossed road? *J Membr Biol*. <https://doi.org/10.1007/s00232-021-00192-z>
- Martin DW (2005) Structure-function relationships in the Na⁺, K⁺-pump. *Semin Nephrol* 25:282–291
- McDermott JP, Sánchez G, Mitra A, Numata S, Liu LC, Blanco G (2021) Na,K-ATPase $\alpha 4$, and not Na,K-ATPase $\alpha 1$, is the main contributor to sperm motility, but its high ouabain binding affinity site is not required for male fertility in mice. *J Membr Biol*. <https://doi.org/10.1007/s00232-021-00181-2>
- McDonough AA, Farley RA (1993) Regulation of Na, K-ATPase activity. *Curr Opin Nephrol Hypertens* 2:725–734
- Nyblom M, Poulsen H, Gourdon P, Reinhard L, Andersson M, Lindahl E, Fedosova N, Nissen P (2013) Crystal structure of Na⁺, K⁺-ATPase in the Na(+)-bound state. *Science* 342:123–127
- Petric M, Vidovic A, Dolinar K, Mis K, Chibalin A, Pirkmajer S. (2021). Phosphorylation of Na⁺,K⁺-ATPase at Tyr10 of the $\alpha 1$ -subunit is suppressed by AMPK and enhanced by ouabain in cultured kidney cells. *J Memb Biol*
- Pierre SV, Xie Z (2006) The Na, K-ATPase receptor complex: its organization and membership. *Cell Biochem Biophys* 46:303–316
- Pirkmajer S, Chibalin AV (2016) Na, K-ATPase regulation in skeletal muscle. *Am J Physiol Endocrinol Metab* 311:E1–E31
- Riganti C, Campia I, Kopecka J, Gazzano E, Doublier S, Aldieri E, Bosia A, Ghigo D (2011) Pleiotropic effects of cardioactive glycosides. *Curr Med Chem* 18:872–885
- Silva L, Garcia I, Valadares J, Toledo MM, Pessoa MT, Machado M, Busch M, Rocha I, Villar J, Atella G, Santos H, Cortes V, Barbosa (2021). Evaluation of Cardiotonic Steroid Modulation of Cellular Cholesterol and Phospholipid. *J Memb Biol*
- Skou JC (1957) The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim Biophys Acta* 23:394–401
- Skou JC (1998) The identification of the sodium-potassium pump (nobel lecture). *Angew Chem Int Ed* 37:2320–2328
- Sweadner KJ (1989) Isozymes of the Na⁺/K⁺-ATPase. *Biochim Biophys Acta* 988:185–220
- Toledo MM, De Souza Gonçalves B, Colodette NM, Chaves ALF, Muniz LV, De Ribeiro RIMA, Dos Santos HB, Cortes VF, Soares JMA, De Lima Santos H, Barbosa LA (2021) Tumor tissue oxidative stress changes and Na, K-ATPase evaluation in head and neck squamous cell carcinoma. *J Membr Biol* 2021:1–12
- Toyoshima C, Cornelius F (2013) New crystal structures of PII-type ATPases: excitement continues. *Curr Opin Struct Biol* 23:507–514
- Xie Z, Askari A (2002) Na(+)/K(+)-ATPase as a signal transducer. *Eur J Biochem* 269:2434–2439
- Xie Z, Cai T (2003) Na⁺-K⁺-ATPase-mediated signal transduction: from protein interaction to cellular function. *Mol Interv* 3:157–168

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.