THE HOMEOSTASIS OF SODIUM AND POTASSIUM IN ASTROCYTES, ITS IMPLICATIONS IN INTRACELLULAR SIGNALLING AND UNDER PHTOLOGYCAL CONDITIONS

Viñas Gaza, Helena | Masgrau Juanola, Roser | Department of Biochemistry and Molecular Biology. Universitat Autònoma de Barcelona.

Aims and objectives

- Contribute to the general understanding of neuroglia and its implications in neurological disorders.
- Get to know and understand the structure and functions of astrocytes in relation to neurons.
- Explain the main mechanisms which carry out the Na^+/K^+ homeostasis. Which channels and transporters are implicated, their functioning and regulation.
- Be able to differentiate the homeostasis behaviours under normal circumstances or either under pathological and stressful situations.
- Establish a relation with a gene mutation that codifies for a subunit of the main Na^+/K^+ pump with a disease caused by a dysregulation of the astrocytes; familial hemiplegia migraine type II.

Introduction

The brain has de maximum concentration of Na^+/K^+ATPases which consumes almost the 20% of the energy required by this organ. This pump is situated in the plasma membrane and it generates the electrochemical gradient of Na^+ and K^+. It exports actively 3 molecules of K^+ and imports 2 of Na^+ thanks to the hydrolysis of one molecule of ATP. The correct gradient is important to buffer the K^+ and to provide the energy for the cotransporters and channels of other ions and neurotransmitters.

The enzyme has two kinds of sunubits:

- **α-subunit** (4 isoforms) containing the binding sites for Na^+, K^+, ATP and the specific inhibitor ouabain.
- **β-subunit** (3 isoforms)

There is an additional H^+ -binding site located on the *C-terminus* that further supports the ion transport.

Recent studies established the correlation between mutations in the Na^+/K^+ATPase α isoforms and neurological disorders.

Neuroglia

Neuroglial cells do not participate direclty in synaptic interactions and electrical signalling. They are more numerous than nerve cells but smaller and they lack axons and dendrites. There are three types of glial cells (Figure 1) in the central nervous system:

1. Astrocytes
2. Oligodendrocytes
3. Microglia

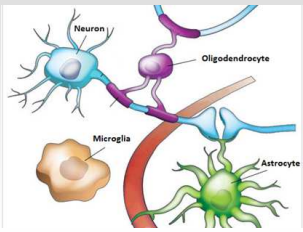

Figure 1: Schematic representation of nerve and gilial cells. Modified from **(Toustrup-Jensen et al. 2013)**

Astrocytes contribute to the formation and preservation of a secure blood-brain barrier. Because of their organization around microvasculature they have a major role in the supply of glucose. They are a reservoir of glycogen and ther are also involved in the control of ionic and osmotic homeostasis as well as in the removing of several neurotransmitters from the synaptic cleft. They can modulate the activity of adjacent cells because they are connected to each other by gap junctions.

Structure of the Na^+/K^+ATPase

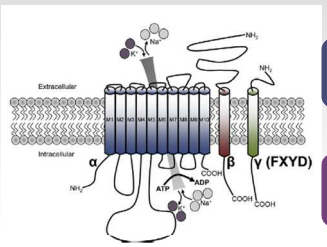

Figure 2: Schematic illustration of the Na^+/K^+ ATPase. **(Bøttger et al. 2012)**

The structure consists of a large catayltic α-subunit which traverses the membrane 10 times (αM1-αM10) and a smaller glycosylated β-subunit.

α-subunit
- There are four isoforms (α_1, α_2, α_3, α_4)
- Has three cytoplasmatic doamins; actuator (A),nucleotide-binding (N) and phosphorylation (P) (Figure 3).

β-subunit
- It makes direct contact with αM7 and αM10 of the α-subunit
- There are three isoforms (β_1, β_2, β_3).

γ-subunit
- It belongs to the FXYD family.
- It is not essential for the well funcion of the pump.

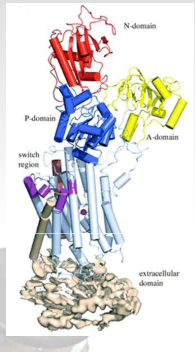

Figure 3. A domain overview of the of Na^+/K^+ ATPase. **(Morth et al. 2007)**

Physiology of the Na^+/K^+ATPase

When stimuli are applied to astrocytes they induce the Ca^{2+} release from the intracellular stores of the cell. This elevation of Ca^{2+} is followed by the released of glutamate, transiently increasing the effective glutamate concentration in the synaptic cleft, and ATP.

- **Glutamate** plays an important role in the propagation of the Na^+ through gap junctions (Figure 4).
- **ATP** binds to purinoreceptors on neighbouring astrocytes, which induce the propagation of Ca^{2+}. The breakdown of ATP will increase glycolysis and uptake of glucose via GLUT-1.

The GABA released in the extracellular space is removed by Na^+-dependent GAT's.

The Na^{2+} dependant activation of Na^+/K^+ ATPase initiates the astrocyte-neurone lactate shuttle (**Kirischuk et al. 2012**).

Intracellular Na^+ can affect the functional activity of proteins by binding directly to them.

Changes in intracellular Na^+ concentration modulate $H^+/OH^-/HCO_3^-$ transport systems, which are fundamental for both intra and extracellular pH homeostasis.

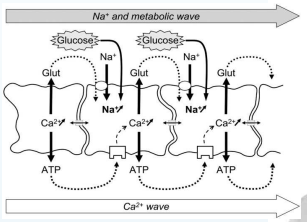

Figure 4: Model for Na^+ and metabolic wave transmission mechanism **(Bernardinelli et al. 2004)**

Regulation of extracellular K^+ homeostasis fundamental .The K^+ uptake by the astrocyte is carried out through passive uptake via cotransporters, active uptake using the Na^+/K^+ATPase and/or spatial buffering.
Astrocytes may also buffer K^+ by releasing K^+ directly into the blood stream

Familial hemiplegia migraine type II (FHM2)

Familial hemiplegic migraine (FHM) is a rare type of migraine with aura. There are three types of FHM; type 1 associated with mutations in the neuronal calcium channels gene (CACNA1A) and is the most prevalent form, type 2 is caused by mutations present in ATP1A2 gene, and type 3 is a rare form of FHM related to mutations in the sodium channel gene (SCN1A).

FMH2 is caused by mutations in the ATPS1A2 gene located on the long (q) arm of chromosome 1 at position 23.2. The migraine aura is known to be caused by cortical spreading depression (CSD). CSD is a wave of continual strong neuronal depolarization that slowly progresses across the cortex.

There are two hypothesized mechanisms for the effects of ATP1A2 (**Gritz & Radcliffe 2013**). First, the mutations cause an increase in extracellular potassium, which can result in the impaired clearance of potassium ions and therefore induce CSD. Second, since the distribution of ATP1A2 is co-localized with the Na^+/Ca^{2+} exchanger, the mutations to ATP1A2 would cause intracellular sodium to increase, which increases intracellular calcium levels through the Na^+/Ca^{2+} exchanger, resulting in glutamate release and a decrease in glutamate clearance and thus lowering the threshold for cortical spreading depression. Both hypotheses result in making the brain more susceptible to CSD and therefore migraines with aura.

CONCLUSIONS:

- Astrocytes are complex cells that respond to a variety of external stimuli. They have to be capable to both perceived and correctly respond to those stimuli such as changes in the energy supplies, neuronal activity, changes in the concentration of ions or NT, among others.
- The Na^+/K^+ ATPase pump has a complex expression pattern. Each one of its units has different isoforms related to its location and function. Its normal activity is capital to control the homeostasis, mainly of Na^+ and K^+, but not without affecting the transients of other ions and NT.
- Astrocytes have been found to be implicated in several disorders of the CNS, including FHM2, a rare type of FHM caused by a mutation in the α_2 Na^+/K^+ ATPase.
- It would be highly recommendable to go further in research and investigation to contribute to our understanding of astrocyte physiology and its role in neuron-glia interaction under physiological and pathophysiological conditions in the brain.

REFERENCES:

Bøttger, P., Doğanlı, C. & Lykke-Hartmann, K. Migraine- and dystonia-related disease-mutations of Na+/K+-ATPases: relevance of behavioral studies in mice to disease symptoms and neurological manifestations in humans. *Neurosci. Biobehav. Rev.* **36,** 855–71 (2012).

Bernardinelli, Y., Magistretti, P. J. & Chatton, J.-Y. Astrocytes generate Na+-mediated metabolic waves. *Proc. Natl. Acad. Sci. U. S. A.* **101,** 14937–42 (2004).

Morth, J. P. *et al.* The structure of the Na+,K+-ATPase and mapping of isoform differences and disease-related mutations. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **364,** 217–27 (2009).

Kirischuk, S., Parpura, V. & Verkhratsky, A. Sodium dynamics: another key to astroglial excitability? *Trends Neurosci.* **35,** 497–506 (2012).

Toustrup-Jensen, M. S. *et al.* Relationship between intracellular Na+ concentration and reduced Na+ affinity in Na+,K+-ATPase mutants causing neurological disease. *J. Biol. Chem.* (2013). doi:10.1074/jbc.M113.543272