From the department of Women's and Children's Health
Karolinska Institutet, Stockholm, Sweden

A special role of Na,K-ATPase and its molecular partners for astrocyte function

THESIS FOR DOCTORAL DEGREE (Ph.D.)

by

Nina Illarionova

Monday 2nd of June 2014, 14:00

Scandiasalen 1tr, Astrid Lindgren Children Hospital, Solna

Principal Supervisor:
MD PhD Eli Gunnarson
Karolinska Institutet
Department of Women's and Children's Health

Co-supervisor(s):
Professor Anita Aperia
Karolinska Institutet
Department of Women's and Children's Health

PhD Sergey Zelenin
KTH Royal Institute of Technology
School of Biotechnology

Opponent:
Professor Mahmood Amiry-Moghaddam
Institute of Basic Medical Sciences
Department of Anatomy

Examination Board:
Professor Agneta Nordberg
Karolinska Institutet
Department of Neurobiology
Division of Care Sciences and Society

Professor Milos Pekny
University of Gothenburg
Department of Clinical Neuroscience and Rehabilitation
Division of Neuroscience

Associate Professor Alexander V. Chibalin
Karolinska Institutet
Department of Molecular Medicine and Surgery
Division of Integrative Physiology

ABSTRACT

Astrocytes are glial cells that express several specific transporters and channels with specialized functions to maintain water, ion and neurotransmitter concentrations in order to preserve normal neuronal function. Astrocytes express two catalytic isoforms of plasma membrane enzyme Sodium Potassium-ATPase (Na,K-ATPase), that are essential for several of astrocyte's functions. In each cycle Na,K-ATPase actively transports 3 Na^+ ions out of the cell and 2 K^+ ions into the cell, using the energy of one ATP molecule. Na,K-ATPase establishes transmembrane Na^+ gradient that allows for efficient Na^+-coupled transport, including glutamate uptake. Na,K-ATPase is also responsible for active K^+ uptake from extracellular space and therefore maintenance of extracellular K^+ homeostasis. The overall goal of this thesis was to study the molecular and functional interactions between the Na,K-ATPase and other proteins responsible for astrocyte function.

The water channel aquaporin 4 (AQP4) is abundantly expressed in astrocytes. Emerging evidence suggests that AQP4 facilitates extracellular K^+ clearance by astrocytes and contributes to recovery of neuronal excitability. We found that AQP4 can assemble with its regulator metabotropic glutamate receptor 5 (mGluR5) and with Na,K-ATPase. The AQP4 NH_2-terminus was shown to interact with Na,K-ATPase catalytic α subunit and with mGluR5. Föster resonance energy transfer (FRET) studies in primary astrocytes derived from rat striatum showed that interaction occurs in intact cells. Thus AQP4/Na,K-ATPase/mGluR5 can form a macromolecular complex in astrocytes, that may be of functional importance for the regulation of water and K^+ homeostasis in the brain.

Astrocytes express two isoforms of the Na,K-ATPase catalytic α subunit: the ubiquitous α1 and the α2, which in the brain is predominantly expressed in astrocytes. The α2 isoform has lower Na^+ affinity than α1. We explored the relative roles of the α1 and α2 isoforms for the support of Na^+-coupled glutamate uptake in primary astrocytes. We found that the α2 isoform contributes to a more efficient restoration of increases in intracellular sodium concentration ($[Na^+]_i$) evoked by the Na^+-coupled glutamate uptake. Both α1 and α2 interacted molecularly with glutamate transporters via the 1st intracellular loop, but the interaction with α2 appeared stronger. The study points to a specific role for α2 in the handling of $[Na^+]_i$ transients in astrocytes and suggests that α1 and α2 may differ with regard to capacity to interact with the glutamate transporters.

Mutations in the Na,K-ATPase α2 subunit are associated with the neurological disease familial hemiplegic migraine type 2 (FHM2). In this study we determined α1 and α2 abundance and glutamate uptake in primary cultures from heterozygous and homozygous α2 mutant mice carrying the human knock-in FHM2-mutation G301R. Both α2 abundance and glutamate uptake were significantly reduced in astrocytes expressing the mutant α2. The plasma membrane expression of mutant Venus-tagged α2 was also reduced in comparison to wild type α2. The results suggest that reduced capacity of astrocytes expressing α2 G301R mutant to take up glutamate, may lead to temporary increases in ambient glutamate concentration that, at least to some extent, may contribute to the symptoms in FHM2.