Minireview

Relevance of Na,K-ATPase to local extracellular potassium homeostasis and modulation of synaptic transmission

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Abstract The ion gradients generated by the Na,K-ATPase are essential for Na^+-coupled transport systems, osmoregulation and restoration of ion concentrations in excitable tissues. Indirectly, the sodium pump controls intracellular Ca^{2+} concentration through the Na/Ca exchanger. In the nervous system various neurotransmitters can modulate Na,K-ATPase activity. The great diversity of Na,K-ATPase subunit isoforms, their complex spatial and temporal regulation of expression and their cellular localisation imply a functional role of the sodium pump in different regulatory pathways. Among these, potassium homeostasis and modulation of synaptic transmission are discussed here.

Key words: Na,K-ATPase; Regulation; Ionic homeostasis; Nervous system

1. Introduction

The sodium-potassium-activated adenosine triphosphatase (sodium pump or Na,K-ATPase; EC 3.6.1.37) is responsible for establishing the electrochemical gradient of Na^+ and K^+ ions across the plasma membrane. The Na,K-ATPase is found in the plasma membrane of all higher eukaryotes, but it is not present in lower eukaryotes such as yeast. The enzyme is a member of the P-type family of ATPases to which also the Ca-ATPase, the H-ATPase and the gastric H,K-ATPase belong. The ion gradients formed by the enzyme are necessary for Na^+-coupled transport of nutrients into cells, for osmotic balance and cell volume regulation, and for maintenance and restoration of the resting membrane potential in excitable cells [1]. The Na,K-ATPase controls the intracellular Ca^{2+} concentration through the Na^+/Ca^{2+} exchanger in heart, skeletal muscles and in the nervous system and therefore indirectly influences muscle contraction and neurotransmitter release.

The Na,K-ATPase is composed of two subunits. The catalytic α subunit, a 110 kDa transmembrane protein traversing the plasma membrane 8–10 times, carries the binding sites for sodium, potassium, ATP and the specific inhibitor ouabain [2]. The β subunit is a glycoprotein with a single transmembrane spanning segment, a large carboxy-terminal extracellular domain of about 220–250 amino acids and a core protein weight of 32–35 kDa [3,4]. The β subunit induces conformational stability of the α subunit in the endoplasmic reticulum and is needed for transport of the functionally active enzyme to the plasma membrane. Without β, α cannot acquire activity [5].

Three genes encoding α subunits with more than 90% similarity have been identified in mammals [6]. In contrast, the three β subunit isoforms share only 39–48% sequence identity [3,4]. However, despite this large sequence divergence, β isoforms appear to be functionally equivalent for transport of the holoenzyme to the plasma membrane. In fact, all isoenzymes with β1 or β2 (the recently found β3 isoform has not yet been examined) can be reconstituted in vitro [7], underscoring the conservation of major structural domains and an inherent common fold of each β isoform. However, subtle differences have been found regarding assembly [7,8] and catalytic activity [9,10], pointing to preferential formation of particular isoenzymes and specific functional implications in vivo. An interesting role, not directly related to pump activity, has been assigned to the mouse β2 isoform: β2-mediated binding of neurons to astrocytes and increased neurite outgrowth suggest a role for this isoform in transcellular interactions [11,12].

2. Subunit expression

α and β subunit isoforms exhibit a complex tissue-specific expression pattern [4,13–19]. All α and β isoforms are expressed in the nervous system. α1 is almost ubiquitously distributed with highest expression levels found in kidney, α2 predominates in skeletal muscle, brain and heart, and α3 appears to be the most abundant form in brain, but it is also present in heart. Highest β1 expression is found in kidney, followed by that of brain. The β2 subunit is very strongly expressed in brain and moderate expression is found e.g. in spleen. Strong expression of β3 has been discovered in testis [4]. The temporal and spatial expression in brain demonstrates that multiple α and β isoforms are expressed in the same cell type [16,17,20], indicating the simultaneous presence of several sodium pump isoenzymes. For example, brain microvessels and epithelial cells of the choroid plexus express (at least) five isoforms [21], giving rise, theoretically, to six different isoenzymes. It remains to be demonstrated, however, which isoenzymes are actually present and active in vivo.

3. Regulation of Na,K-ATPase activity

Given the many isoforms and their complex expression patterns, sodium pump regulation seems pertinent and could be triggered by exogenous stimuli such as hormones, growth factors and neurotransmitters. There is evidence that the hyperpolarising action of noradrenaline is exerted by Na,K-ATPase stimulation [22]. Dopamine has been implicated in Na,K-

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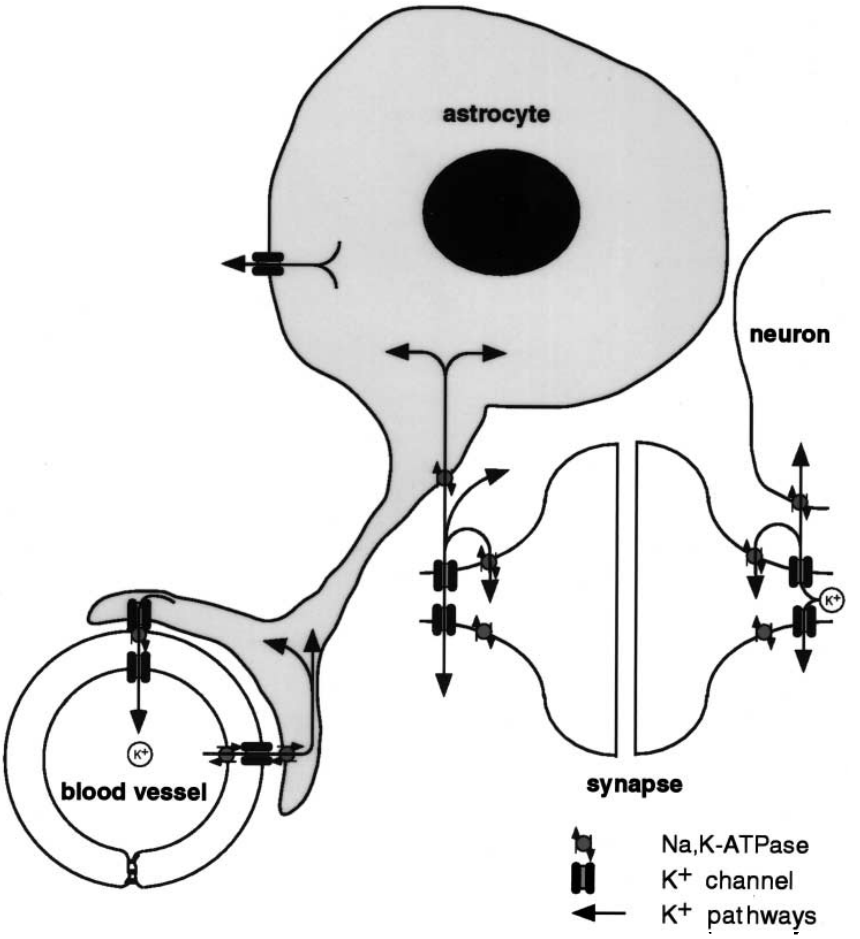

Fig. 1. Scheme for removal of K^+ from sites of high concentration. K^+ can be taken up directly at sites of release, e.g. at synapses, or redistributed among neighbouring cells. Particular pathways can include pumps, channels, gap junctions or orthogonal arrays of particles at the boundary between astrocytic endfeet and blood vessels. For details see text.

ATPase inhibition in neostriatal neurons [23]. Norepinephrine depletion leads to 40% reduction of ouabain binding sites in cerebral microvessels in vivo [24]. Serotonin reduces Na,K-ATPase activity of the choroid plexus [25]. Glutamate, through the NMDA (*N*-methyl-D-aspartate) type receptor, leads to alteration of cGMP levels and this effect is potentiated by ouabain in cerebellar extracts [26]. A direct relation between glutamate action and Na,K-ATPase activity can be inferred from the observation that glutamate, through metabotropic receptors, is able to increase Na,K-ATPase activity in Purkinje neurons [27]. Such changes of Na,K-ATPase activity could possibly de- or hyperpolarise neural cells to an extent sufficient to influence electric excitability and synaptic transmission.

Still little is known about the intracellular signalling cascades leading to neurotransmitter-mediated modulation of sodium pump activity. One mechanism could be through phosphorylation of the α subunit. In recent years various reports have demonstrated Ser/Thr phosphorylation of α subunit isoforms at various sites. α subunits can be phosphorylated in homogenates of *Xenopus* oocytes after activation of protein kinases A and C [28]. Protein kinase C also phosphorylates Na,K-ATPase α subunits in vivo in sciatic nerves [29]. In shark rectal glands and rat renal cortex, phosphorylation by protein kinase A or C leads to inhibition of enzyme activity [30,31]. A linkage between neurotransmitter modulation and α subunit phosphorylation has been found in the choroid plexus: serotonin reduces pump activity and increases the amount of the phosphorylated α [25]. Along with phosphorylation of α subunits by protein kinases, the actual level of phosphorylation is thought to be modulated by protein phosphatases too [32]. Although the exact intracellular signalling cascades have yet to be elucidated, the level of phosphorylation appears to be an important determinant of Na,K-ATPase activity.

4. Functional aspects

The data presented so far suggest that specific Na,K-ATPase isozymes could be necessary to fulfil different physiological roles. For example, an important function assigned to glial cells, which might also depend on Na,K-ATPase isoenzyme activity, is the regulation of potassium concentration in the nervous system. A sufficiently high increase in extracellular potassium during neuronal activity can cause depolarisation of the neuronal membrane, prevent repetitive action potentials and lead to shrinkage of the extracellular space [33]. Spatial buffering, the influx of potassium ions into glial cells at sites of high extracellular concentration and efflux of an equal amount of potassium at distant sites of lower concen-

tration [34], and potassium accumulation, involving local simultaneous uptake of potassium, chloride and water [35] are mechanisms by which the extracellular potassium concentration can be restored. The respective contributions of these mechanisms to total potassium uptake are not exactly known and at least a partial involvement of glial cell Na,K-ATPase isozymes seems likely, especially at sites of high cell packing density and high neuronal activity. Astrocytes are not only in close vicinity to neurons but they also contact blood microvessels. The concentration of potassium in the blood is nearly twice as high as in the extracellular space of the CNS and potassium is actively extruded at the blood–brain barrier by mechanisms including the Na,K-ATPase [36] (see Fig. 1). Evidence supporting the role of the sodium pump as a controller of ionic homeostasis comes from mice deficient for the β2 gene, which show massively enlarged ventricles and astrocytic endfeet around blood vessels, indicative for vasogenic and cytotoxic edema, respectively [37]. These animals die around day 18. The formation of large ventricles is noteworthy, because in the choroid plexus, which is the primary source of cerebrospinal fluid, apical Na,K-ATPase accounts for sodium-driven cerebrospinal fluid flow into the ventricles [25]. Therefore, the opposite effect, i.e. shrinkage of the ventricle, would be expected to occur in the absence of a Na,K-ATPase subunit isoform usually present in this tissue. However, it has yet to be demonstrated whether absence of β2 in choroid plexus in fact reduces total pump activity or whether an absence of β2 leads to overcompensation by other β isoforms. Alternatively, effects secondary to altered pump activity may ultimately determine the phenotype.

Both glial and neuronal Na,K-ATPase isoenzymes could be implicated in synaptic transmission. Glial cells tightly surround synaptic regions thereby generating a locally restricted microcompartment. This means that changes in glial pump activity are likely to influence transmission, e.g. by altering transmitter uptake or the level of the membrane potential. The relevance of neuronal Na,K-ATPase molecules to the regulation of synaptic transmission could even be more specific. For example, the outward current that is measured between bursts of action potentials in dopaminergic neurons is inhibited by ouabain [38], demonstrating a direct influence on synaptic transmission patterns. The identification of a functional linkage of Na,K-ATPase isoenzymes, carbon monoxide, cGMP, protein kinase G and glutamate in Purkinje neurons [27] provides further evidence for this notion. The authors suggest an inhibitory effect on axonal firing caused by inhibition of the Na,K-ATPase. Excitatory synapses can undergo a persistent enhancement of synaptic efficacy after repetitive stimulation and the facilitated state can last for several hours (long-term potentiation (LTP)) [39]. The induction needs an incoming signal of sufficient strength and delivery at high frequency. LTP occurs most prominently in the hippocampus and serves as in vitro paradigm to study information storage [40]. Depolarisation induced by elevated extracellular potassium concentrations produces electrophysiological changes resembling LTP [41], but depolarisation of the postsynaptic membrane alone is not sufficient for induction. At least in the CA1 region of the hippocampus stimulation of the NMDA receptor is also needed [39] and this ion channel is blocked by Mg^{2+} ions at highly negative membrane potentials. Thus, pump activity-dependent changes of the membrane potential could influence the state of the channel. Ca^{2+}/calmodulin-dependent protein kinase II and protein kinase C are activated by glutamate and participate in LTP [41]. Since, as mentioned above, both glutamate and protein kinase C can also regulate Na,K-ATPase activity, phosphorylation of sodium pump α subunit isoforms could be a mechanism contributing to changes of synaptic activity, in particular when large amounts of potassium are released from the postsynaptic cell following strong activation [40]. Protein kinase A, which also can regulate Na,K-ATPase activity, appears to be important for the late period of LTP [42], an observation compatible with the view that modulation of Na,K-ATPase activity could be relevant during different periods of synaptic activity.

In this frame, yet another pathway has to be considered: NMDA receptor stimulation releases nitric oxide and this messenger has been shown to affect Na,K-ATPase activity in a subunit-specific manner [27,43]. The rapid diffusion of this gas could make all forms of sodium pumps in close vicinity of the release site likely targets of isoenzyme-specific responses.

5. Conclusions and outlook

High neuronal activity requires a simultaneous regulation of neuronal sodium pumps of active and neighbouring inactive neurons as well as regulation of glial enzymes to control ionic and neurotransmitter homeostasis. Rapid control of activity-caused imbalances certainly gains high priority during excitotoxic stress [44]. Supporting this notion is the observation that partial inhibition of pump activity can potentiate glutamate-induced excitotoxicity [45]. To get on well with these conditions, the existence of various isoforms of each subunit, the complex expression pattern, which in addition may be combined with the recently shown subunit-specific assembly, the diverse intracellular pathways (*cis* interaction) leading to bidirectional alteration of Na,K-ATPase activity and, finally, subcellular compartmentalisation of isoforms [27,46] are essential prerequisites. In addition, the ability of the β2 subunit isoform to act as a signal transducer suggests that Na,K-ATPase isoenzymes could be subjected to direct regulation involving the large extracellular loop of β isoforms (*trans* interaction).

Given the overall high similarity, in particular between the α subunit isoforms, functional analysis could prove to be a complex task. However, the use of refined approaches of protein function analysis such as inducible knock-outs and replacement strategies should nevertheless allow such issues to be addressed in a physiological context.

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