Reverse mode Na\(^{+}/\)Ca\(^{2+}\) exchange-induced cell dehydration as a primary mechanism for cell pathology

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Na\(^{+}/\)K\(^{-}\) pump is a fundamental metabolic mechanism in cell membrane which controls cell functional activity. It generates Na\(^{+}\) gradient on cell membrane and serves as an energy source for a number of secondary ion transporters in membrane, such as Na\(^{+}/\)Ca\(^{2+}\) and Na\(^{+}/\)H\(^{+}\) exchangers, Na\(^{+}\)sugars, amino acids and different osmolytes [1]. It is known that Na\(^{+}/\)K\(^{-}\) pump, with the function of controlling intracellular ionic homeostasis, works in electrogenic regime, generates the metabolic component of membrane potential [2-4] and has a crucial role in cell volume regulation [5,6]. The activation of Na\(^{+}/\)K\(^{-}\) pump leads to generation of water efflux from the cells by a) push out of 3Na\(^{+}\) and uptake of 2K\(^{+}\) and b) release of H\(_2\)O in cytoplasm (42 H\(_2\)O for one molecule glucose oxidation) as a result of activation of intracellular oxidative phosphorylation [7]. Such a Na\(^{+}/\)K\(^{-}\) pump-induced water efflux has a great physiological meaning as it balances the osmotic water uptake [5] by cell and inactivates Na\(^{+}\) and Ca\(^{2+}\) inward currents through the membrane [8,9].

We have previously shown that Na\(^{+}/\)K\(^{-}\) pump-dependent regulation of cell volume is a powerful metabolic mechanism through which both the auto-regulation of Na\(^{+}/\)K\(^{-}\) pump [10] and the regulation of membrane chemosensitivity [11] and excitability [8] are realized by changing surface-dependent number of functionally active proteins in membrane.

It is known that the dysfunction of Na\(^{+}/\)K\(^{-}\) pump, which is accompanied by the increase of intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{i}\)), is a common consequence of any cell pathology (including aging). Traditionally, the increase of [Ca\(^{2+}\)]\(_{i}\), which is accompanied by Na\(^{+}/\)K\(^{-}\) pump inactivation, is considered as a result of intracellular Na\(^{+}\) concentration ([Na\(^{+}\)]\(_{i}\)) increase which stimulates Ca\(^{2+}\) uptake through the membrane [8,9]. However, our previous study has shown that cGMP and cAMP modulate Na\(^{+}/\)Ca\(^{2+}\) exchange activity without significantly changing Na\(^{+}/\)K\(^{-}\) pump activity (where K\(^{-}\) was replaced by 86Rb\(^{+}\)) (Figure 2B). Na\(^{+}/\)K\(^{-}\) pump-independent Na\(^{+}\) efflux is activated by the increase of extracellular Ca\(^{2+}\) concentration, which is due to Na\(^{+}\) exchange with 45Ca\(^{2+}\) uptake (Figure 3) [26]. It is interesting to note that in pioneer work of Prof. Baker and his co-authors on fundamental study of Na\(^{+}/\)Ca\(^{2+}\) exchange, which was performed on internally perfused squid axon, ouabain-sensitive (Na\(^{+}/\)K\(^{-}\) pump) and ouabain-insensitive (Na\(^{+}/\)Ca\(^{2+}\) pump) components of Na\(^{+}\) efflux were identified [12]. Thus, the absence of low ouabain-induced activation of Na\(^{+}/\)Ca\(^{2+}\) exchange in internally perfused axon (which is present in intact neurons) indicates the involvement of a cytoplasmatic mechanism(s) in ouabain-induced activation of R Na\(^{+}/\)K\(^{-}\) pump exchange.

This activation effect on Na\(^{+}\) efflux takes places without significantly changing Na\(^{+}/\)Rb\(^{+}\) pump activity (where K\(^{-}\) was replaced by 86Rb\(^{+}\)) (Figure 2B). Na\(^{+}/\)K\(^{-}\) pump-independent Na\(^{+}\) efflux is activated by the increase of extracellular Ca\(^{2+}\) concentration, which is due to Na\(^{+}\) exchange with 45Ca\(^{2+}\) uptake (Figure 3) [26]. It is interesting to note that in pioneer work of Prof. Baker and his co-authors on fundamental study of Na\(^{+}/\)Ca\(^{2+}\) exchange, which was performed on internally perfused squid axon, ouabain-sensitive (Na\(^{+}/\)K\(^{-}\) pump) and ouabain-insensitive (Na\(^{+}/\)Ca\(^{2+}\) pump) components of Na\(^{+}\) efflux were identified [12]. Thus, the absence of low ouabain-induced activation of Na\(^{+}/\)Ca\(^{2+}\) exchange in internally perfused axon (which is present in intact neurons) indicates the involvement of a cytoplasmatic mechanism(s) in ouabain-induced activation of R Na\(^{+}/\)K\(^{-}\) pump exchange.

The study of dose-dependent [H]-ouabain binding with neuronal membrane in isotonic, hypertonic and hypotonic solutions has
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Figure 1. The effects of MV on 45Ca2+ uptake by neuronal ganglia in normal (A) and K+-free PS (B) [23].

Figure 2. A. The rate constant as a function of the concentration of ouabain in the medium. The dashed line indicates the rate constant in normal Ringer’s solution. The values of the rate constant are equivalent to the values at 5 min. The limits of ±SE do not exceed the diameter of the symbols [10]. B. 86Rubidium uptake as a function of ouabain concentration. Results shown are the means of ±SEM of ten experiments, each period from study of ten pooled ganglia. The open circle at the left represents 86Rb uptake in the absence of ouabain, and this level is indicated in the dashed line [14].

Figure 3. A. The rate constant of 22Na+ efflux as a function of ouabain concentration in K+-free physiological solution containing 2 (1), 7 (2) and 20 (3) mM Ca2+. B. 45Ca2+ uptake in K+-free medium containing 2, 7 and 20 mM Ca2+ [26].
shown, that compared with the number of ouabain receptors in cell membrane incubated in isotonic solution, both the numbers of pump units (α, isoforms) and ouabain receptors responsible for activation of R Na+/Ca2+ exchange (α2/α3 isoforms) are higher in hypotonic and lower in hypertonic solutions (Figure 4) [10]. It is notable that the osmosensitivity of high affinity ouabain binding sides is more pronounced that of high affinity ones (>10^{-3} M).

It is known that the differences of electrochemical gradients of Na+ and Ca2+ serve as energy sources for Na+/Ca2+ exchange [12]. Traditionally, both low and high ouabain concentrations-induced stimulations of Na+/Ca2+ exchange are explained by Na+/K+ pump inhibition leading to the increase of [Na+]i, [24]. However, as the above presented data indicate, low concentrations of ouabain stimulate Na+/Ca2+ exchange without notable changes in Na+/K+ pump activity [14]. As the activities of Na+/Ca2+ exchange and Na+/K+ pump are realized by different proteins, it is suggested that the close-talking correlation between them can be realized not only by the changes of [Na+]i, but also by the changes of [Ca2+]i. It is obvious that [Ca2+]i can be decreased through [Ca2+]i sorption by intracellular structure and [Ca2+]i dilution by increasing intracellular water contents.

As it is noted above, low ouabain-induced activation of R Na+/Ca2+ exchange is accompanied by the increase of intracellular cAMP content in neurons [14]. It has also been shown that the activation of R Na+/Ca2+ exchange, which is accompanied by the increase of intracellular cAMP, takes place upon the effect of extremely low concentrations of biologically active substances (such as synaptic transmitters, H2O2) [27,28] and physical factors (such as background radiation, microwave with non-thermal intensity) [16,23] which are unable to activate ionic channels in membrane [16]. There is a great number of literature data showing that nM ouabain elevates intracellular cAMP content in different tissues, including dog renal cortex, goldfish intestinal mucosa, mouse pancreatic islets, murine epithelioid and fibroblastic cell lines, rat brain, rat renal collecting tubule cells in culture and astrocytes [29]. It has been shown that intracellular cAMP has activation effect on Ca2+ pump localized in endoplasmatic reticulum (ER) membrane transporting Ca2+ from cytoplasm into ER [30]. It is known that Na+/Ca2+ exchange functions in stoichiometry of 3Na+:1Ca2+ [12]. Therefore, it is predicted that the activation of R Na+/Ca2+ exchange should bring to cell dehydration. However, by our previous study performed on brain and heart muscle tissues of young (healthy) rats it has been shown that nM ouabain-induced elevation of intracellular cAMP leads to activation of R Na+/Ca2+ exchange, which is accompanied by cell hydration. It has also been shown that both the rate of R Na+/Ca2+ exchange and cell hydration have a metabolic nature and age-dependent weakening, reverse character [19,31].

Thus, based on the aforementioned data, it can be suggested that in healthy animals there are minimum two mechanisms through which cAMP activates R Na+/Ca2+ exchange as a result [Ca2+]i decrease. They are a) the activation of Ca2+ pump in ER membrane and b) increase of intracellular water content leading to dilution of [Ca2+]i. However, in old and unhealthy animals Na+/K+ pump dysfunction increases [Na+]i, which in its turn stimulates R Na+/Ca2+ exchange bringing to the increase of [Ca2+]i. As the latter has multi-poisoning effect on cell metabolism, the activation of R Na+/Ca2+ exchange is accompanied by cell dehydration. Therefore, it is suggested that R Na+/Ca2+ exchange-induced cell dehydration can be considered as a primary mechanism for cell pathology.

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Figure 4. Binding of [3H] ouabain to Helix pomatia cell membranes as a function of the concentration of glycine solutions in different tonicities. Binding was measured after 2 hr of exposure to [3H] ouabain at 22°C (O) Cells exposed to glycine in hypotonic solution (0mM sucrose; T=0.5); (●) cells exposed in isotonic solutions (63mM sucrose; T=1); (■) cells exposed in hypertonic solutions (189mM sucrose; T=2). Both axes are logarithmic [10].


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