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The nM ouabain-induced tissue dehydration as a novel diagnostic marker for neuronal pathology

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Cell hydration is a dynamic parameter determining its functional activity which is realized by hydration-induced changes of intracellular macromolecules' activity by folding-unfolding mechanism [1] and by surface-dependent changes of a number of functionally active membrane proteins, having enzymes [2], receptors [3] and ionic channels forming properties [4]. As membrane is highly permeable for water and intracellular osmotic pressure exceeds the extracellular one, it is assumed that there is a metabolically driven water efflux from the cell in order to balance osmotic water uptake. Previously we have shown that water influx increases membrane permeability for Na^+ (P_{Na}), while water efflux from the cell has opposite effect on it [4] Therefore, water influx as a result of metabolic water efflux impairment increases P_{Na}, which in its turn decreases Na^+ gradient on membrane being a common consequence of any cell pathology. Therefore, it is suggested that the impairment of metabolically driven water efflux, which compensates the osmotically driven water uptake by cell leading to the increase of membrane permeability for P_{Na}, can be considered as a primary gate for generation of cell pathology [5].

It is known that metabolic water efflux is due to membrane ion transporting mechanisms that work in electrogenic regime, such as Na^+/K^+ pump (stoichiometry of $3Na^+:2K^+$) and Na^+/Ca^{2+} exchange in reverse mode (R) (stoichiometry of $3Na^+:1Ca^{2+}$). During the function of these mechanisms cell loses more osmotic particles than accumulates them.

The next mechanism that generates water efflux from the cell is oxidative phosphorylation-induced release of water molecules in cytoplasm [6].

It has been shown that there is a negative close correlation between Na^+/K^+ pump and Na^+/Ca^{2+} exchange and the latter is more sensitive to metabolic factors, such as intracellular messengers [7]. However, the detailed mechanism of the role of intracellular messengers in regulation of Na^+/Ca^{2+} exchange has not been fully elucidated.

It is known that in neuronal and muscle membranes Na^+/K^+-ATPase (working molecules of Na^+/K^+ pump) has three catalytic isoforms (α_1, α_2, α_3) [8] with different affinities to cardiac glycoside ouabain and functional activities: α_1 (with low affinity) and α_2 (with middle affinity) isoforms are involved in transportation of Na^+ and K^+, while α_3 (with high affinity) isn't directly involved in transporting Na^+ and K^+ and has only intracellular signaling function [8,9] through which Na^+/Ca^{2+} exchange is regulated.

The study of dose-dependent effect of ouabain on Na^+ efflux from perfused axon has shown that Na^+ efflux from the cell has ouabain sensitive and ouabain insensitive components. It has been shown that ouabain sensitive component is determined by Na^+/K^+ pump, while ouabain insensitive component is determined by R Na^+/Ca^{2+} exchange [7,8].

Our study performed on intact neurons has shown that extremely low concentrations ($<10^{-9}$M) of ouabain stimulate Na^+ efflux from the cell without inhibiting Na^+/K^+ pump. From this data, it has been concluded that there is an unknown intracellular mechanism responsible for Na^+ efflux from the cell which is not due to Na^+/K^+ pump activity [2]. Later it has been shown that low concentrations of ouabain which are unable to inactivate Na^+/K^+ pump, stimulate Na^+/Ca^{2+} exchange which is accompanied by the increase of intracellular cAMP content [10]. Further studies have revealed that nM ouabain has stimulation effect on cAMP content in different cells [11]. However, after discovering the existence of cAMP-activated Ca^{2+} pump in the membrane of endoplasmic reticulum (ER) which pushes Ca^{2+} from cytoplasm into endoplasm [12] it became clear that cAMP-induced activation of R Na^+/Ca^{2+} exchange is due to the decrease of intracellular Ca^{2+} as a result of stimulation of cAMP-activated Ca^{2+} pump in ER [11].

As Na^+/Ca^{2+} exchanger works in stoichiometry of $3Na^+:1Ca^{2+}$, it was predicted that R Na^+/Ca^{2+} exchange should have dehydration effect on cell, while F Na^+/Ca^{2+} exchange hydration effect on it. However, our recent study has shown that nM ouabain-induced activation of R Na^+/Ca^{2+} exchange leads to cell hydration (instead of cell dehydration) in brain tissues of young animals, while in old animals it has dehydration effect on it. Moreover, this nM ouabain-induced dehydration effect in old animals is also observed in vitro experiments, where metabolic activity of cell is in depressed state [13]. The more detailed investigation has revealed that this age-dependent difference of nM ouabain effect on cell hydration is due to the initial metabolic state of organism. In young animals, the nM ouabain-induced activation of cAMP leads to endogenous water release as a result of activation of oxidative phosphorylation processes in cell, while in old animals the cAMP-dependent activation of intracellular oxidative phosphorylation is depressed as a result of abnormal increase of intracellular Ca^{2+} content inhibiting mitochondrial function. From these data, we have concluded that during cell pathology (including aging) the increase of intracellular Ca^{2+} brings to the dysfunction of mitochondria (depresses oxidative phosphorylation-induced release of H_2O).

Thus, on the basis of the above-mentioned data we suggest that nM

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ouabain-induced dehydration can be considered as a novel diagnostic marker and therapeutic target.

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