THE ROLE OF NON-CONDUCTIVE MEMBRANE MECHANISMS IN NEURONAL SIGNAL TRANSDUCTION

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The electrogenic Na/K pump

One of the main disadvantages of the classic membrane theory is that the signal transduction in neurons is considered as conductive property changes of membrane surface and rejects the direct role of the metabolic controlling non-conductive membrane mechanisms in the generation of membrane potential and its role in signal transduction. One of the first experimental data proving the existence of metabolic component of membrane potential (MP) in normal functioning state of neuron was obtained by the author in Prof. Kostyuk 's laboratory (Airapetian 1969a). On the basis of this fact, it became possible to explain the nature of spontaneous (non-synaptic) inhibition of neurons (Airapetian 1969b) and lower sensitivity of MP to variation in low concentration of potassium ions in medium (Airapetian 1969c). By the further study of the physiological role of electrogenic Na/K pump in neuronal functional activity, a series of potential-independent pathways were discovered, through which the Na/K pump-induced metabolic regulation of membrane function is realized. They are pump-induced cell volume changes, water fluxes through the membrane, intracellular signaling systems, cytoskeleton contractility, electrogenic Na/Ca exchange, membrane lipids composition and fluidity.

The Na/K pump as a cell volume regulator

Another pitfall of the membrane theory is that changes in the active membrane surface during normal cell functioning are not considered and ionic channels in membrane are viewed as homogenous. However, the fact that the membrane surface changes even during a single action potential was demonstrated by Tasaki and co-workers (Tasaki *et al.* 1982), who showed that the membrane depolarization causes a cell swelling while its hyperpolarization leads to the cell shrinkage. Taking into account that the electrogenic character of Na/K pump predicts the membrane surface changing, the purpose of our study was a more detailed investigation of its role in regulation of cell volume.

There are many hypotheses explaining the metabolic regulation of cell volume. An old one is the "Na/K pump hypothesis", according to which pump activation can bring to cell shrinkage while its inactivation — to cell swelling (Ussing, 1949). Nevertheless, this hypothesis was proved experimentally only at the end of the 70s on mammalian brain slices (Cooke, 1978) and on isolated single neuron (Airapetian and Suleymanyan, 1979). The fact that the pump dysfunction is a common consequence of cell pathology including the aging allows us to suggest that it would initiate the activation of cell volume regulatory mechanisms having less-metabolic energy utilizing properties. As the cytoskeleton contractility, electrogenic Na/Ca exchange and membrane lipids rigidity (fluidity) have a crucial role in the neuronal volume regulation, the correlation between pump activity

and the activity of these systems was the subject for our investigation. Since the phosphorylation and de-phosphorylation of actins-like proteins of cytoskeleton has key role in cell volume regulation the correlation between pump activity and intracellular cAMP and cGMP contents and membrane phosphorylation were studied. It was shown that there are close negative and positive correlations between pump activity and intracellular levels of cAMP and cGMP, correspondingly, which are realized through intracellular ATP. The dysfunction of the Na/K pump brings to the accumulation of intracellular Ca ions, which by positive feedback inhibition the Na^+/K^+-ATPase causes accumulation of intracellular ATP. The latter being a substrate for cyclase activity also serves as a positive modulator for it and promotes the cAMP formation and membrane physphorilation (Airapetian *et al*., 1992).

Another mechanism through which the pump dysfunction-induced elevation of intracellular cAMP brings to neuronal dehydration is the electrogenic Na/Ca exchange (Airapetian 2001). It is characterized by cell swelling and shrinkage in regimes: 3Na influx and 1Ca efflux and 1Ca uptake to the 3Na efflux, correspondingly. It was also demonstrated that besides the electrochemical gradients of these ions on membrane, the correlation between Na/K pump and Na/Ca a exchange are realized also by intracellular cAMP and cGMP. The elevation of cAMP activates the Na/Ca exchange in regime of Na efflux and Ca influx (Sagian *et al*., 1996) while the increase of intracellular cGMP leads to its activation of in reversal mode (Azatian *et al*., 1998). The above mentioned data on the pump inhibition-induced cAMP-dependent contraction of cytoskeleton and activation of Na/Ca exchange in regime of Na efflux and Ca influx, allow us to assume about the biphasic effect of dysfunction on cell hydration: first, cell swelling and then cell shrinkage which could initiate the cascades bringing to neuronal apoptosis. However, it is known that increase of intracellular Ca can stimulate the formation of cGMP in cells via Ca-calmoduline activation of NO production and phospholipase A_2 activation. Since according to our data, the cGMP has a stimulating effect on Ca efflux through 3Na/Ca exchange, having hydration effect on neurons this pathway is supposed to be a protective reaction of neuronal metabolism which weakens in aging.

Thus, from the obtained data a conclusion can be made that the active membrane surface is a dynamic neuronal parameter, which could change in response to the disturbance of membrane selective permeability and activity of metabolic mechanisms controlling osmotic gradients on membrane. Consequently, the purpose of our *next* study was the elucidation of the physiological role of cell hydration in regulation of neuronal function.

Neuronal hydration and membrane function

Although the important physiological role of water in cell functional activity is widely accepted, its messenger role in signal transduction and the generation of

various diseases, including aging-induced memory loss and the nerve disorder risk increase, has not been paid an adequate attention to by investigators. The study of the membrane surface dependence of the membrane function activity brings us to a very important discovery that a number of functional active protein molecules in membrane having enzymatic, receptive, and channel forming properties are functionally in active and inactive (reserve) states, depending on the membrane surface (membrane packing) (Airapetian 1980). By these mechanisms, the pump-induced volume changes in the auto-regulation of pump activity (Airapetian *et al.*1984) as well as regulation of the number of functioning ionic channels (Airapetian *et al.* 1988) and chemoreceptors (Airapetian & Arvanov 1979) in membrane have been realized. As the cell volume changes are accompanied by water efflux and influx through the membrane, the objective of our following work was to study the water flux effect on membrane ionic currents. It has been shown that water fluxes have activation or inactivation effects on ionic currents in the case of the same or opposite directions, respectively (Airapetian *et al*. 1988). By these experiments, performed on snail neurones and on squid axon, potential dependant ionic channels in membrane were established to be heterogeneous depending on their sensitivity to water fluxes. These data allow us to explain the Na/K pump-induced spontaneous neuronal inhibition not only by membrane hyperpolarization but also by pump-induced water efflux and cell shrinkage-induced decrease in the number of functionally active ionic channels in membrane (Kogima *et al.*, 1984).

Cell volume and under-threshold signal transduction

In order to activate the potential dependent or agonist activated ionic channels, a couple of mv. and more than 10^{-10} M agonist, correspondingly, are necessary to apply to membrane. But there is an accumulation of great number of experimental data indicating that the physical and chemical signals having intensity much lower than the thresholds of ionic channels activation can modulate membrane conductive properties. Our study has shown that the extremely low (below 10^{-10} M) concentration of synaptic transmitters (LDST), unable to activate receptor-binding ionic channels exerts strong modulatory effects on membrane currents in neurons produced by higher concentration of agonists, potential-activated ionic channels and electrogenic Na/K pump (Airapetian & Carpenter 1991a,b). It was established that the LDST–induced modulation of membrane current is realized via increase in the frequency of single channel activity, without changing its individual characteristics (Arvanov et al. 1988). This effect of LDST has a metabolic character and is due to modulation of intracellular signaling systems (Airapetian & Carpenter 1991b). It was shown that the ouabain–specific inhibitor of Na/K pump at low concentration (below 10^{-8} M), which does not affect the pump activity, modulates the agonist induced

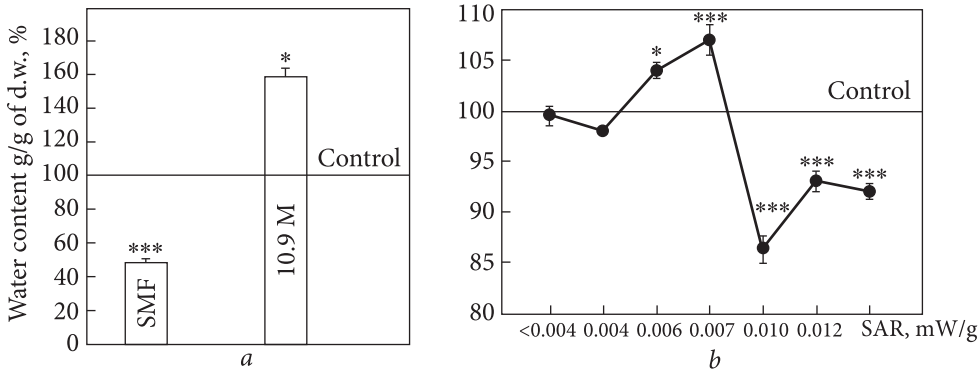

Fig. 1. The effect of 15 min 2,5 mT SMF and 10^{-9} M ouabain (*a*) and dose-dependent MMW; expose on hydration of rat brain cortex cells (*b*)

membrane current also realized by activation of cAMP/cGMP dependent Na/Ca exchange activity (Airapetian & Carpenter 1991 b).

Earlier study of the doze dependant curve of ouabain binding with membrane, distinguished among saturated and linear components, from which only the latter is responsible for pump inhibition (Airapetian *et al.* 1984). By isotope measurements of membrane ionic fluxes, it has been shown that the function of ouabaine receptors having the highest affinity is connected with activation of cyclic dependent electrogenic Na/Ca exchange (Sagian *et al.*, 1997). The study of the membrane sensitivity to LDTS and ouabaine binding with membrane of *xenopus oocytes,* preliminary injected different specific mRNA, has revealed the absence of both LDTS membrane sensitivity and saturated component of ouabain binding with membrane (Airapetian 1998). The difference in the degrees of sensitivity to LDST between neuronal and oocytes membranes and the absence of high affinity of ouabain receptors in oocyte membrane seems extremely interesting from the point of understanding the functional differences between excitable and non-excitable membranes, which can be a subject for future more detail investigation.

Another dynamic parameter membrane capable to regulate the membrane function is the membrane lipid fluidity, which is increased by cell swelling and decreased by cell shrinkage. Our study showed that both currier- and channel-driving, ion transporting systems in membrane are highly lipid fluidity UA-dependent (Sulyemainian et al.,1986).

During the last decade our study has demonstrated that the intracellular cyclic nucleotides dependent cell hydration regulating mechanisms are sensitive even to non-ionizing radiation-induced structural changes of cell bathing medium (Airapetian 2006). By our recent study, all above mentioned experimental results on the sensitivity euronal of hydration to weak chemical and physical signals, performed on snail neurons are fully reproducible in mammalian cells (Danielyan et al.2000, Musegyan et al., 2009).

As seen in Fig. 1, the extreme sensitivity of rat brine cortex cell hydration to low concentration ouabaine (10^{-9} M), 2.5 T static magnetic field (SMF) (A) and millimeter waves (MMW) (B) exposure. The close correlation between brain cortex cell hydration and pain thresholds to "hot plate" in rats has been shown (Museghyan *et al*. 2009). The brain cortex cells dehydration has analgesic while hydration algesic effect on rats (Museghyan et al 2009).

Thus, on the basis of above presented brief review of our data, it can be concluded that the non-conductive membrane mechanisms regulating the number and activity of membrane protein molecules serve as pathways for under channel threshold signals transduction in neurons. It is supposed that the pump dysfunction-induced neuronal dehydration and its functional depression are the main pathways through which the cascade apoptosis in nerve disorder and aging are realized.

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