

The Effect of Neurotransmitter and Synaptosomal Factor on the Synaptic Membrane Anion-Activated ATPases

Sopio Dzneladze* and Eka Nozadze*

**Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia*

(Presented by Academy Member David Mikeladze)

The anion activated ATPases represent the transporting enzyme systems which are activated by anions and stimulated by magnesium ion. They play a principal role in the regulation of intracellular pH, cell volume, and determination of rest potential. The neurotransmitters 5-hydroxytryptamine (5-HT), dopamine (DA) and synaptosomal factor (SF) represent modifiers of Na, K-ATPase system. The effects of dopamine (DA) and endogenous synaptosomal factors on anion Mg-dependent ATPases (Cl-activated ATPase E.C.3.6.3.11, HCO₃⁻-activated ATPase E.C. 3.6.1.3.) were studied in synaptosomal fraction of rat brain. NT and SF separately inhibit Cl-ATPase, whereas their combined action (SF/NT) results in the withdrawal of the inhibitory effect and passes into activation. The effect of NT and SF on Mg²⁺-dependent HCO₃⁻-ATPases is not manifested. © 2020 Bull. Georg. Natl. Acad. Sci.

Cl-activated ATPase, HCO₃⁻-ATPase, neurotransmitter, synaptosomal factor

Cl-activated ATPase and HCO₃⁻-ATPase are known as Mg-dependent ATPases. They are found in the plasma membrane of animal and plant cells [1-3]. They represent multi-sited enzyme systems, regulating cellular level of these anions [4]. So, as Na, K-ATPase, which is well studied, these ATPases also belong to the transport ATPases. Investigation of an impact of various neurotransmitters (NT) on Na, K-ATPase has shown that one group of NT, such as acetylcholine, noradrenaline, dopamine and serotonin change its activity in a concentration dependent manner [4, 5]. Later it was found that the NT effect, in its turn, is regulated by the

synaptosomal factor (SF) from a supernatant obtained after osmotic shock of the crude mitochondrial fraction [6, 7]. Its edition into the reaction medium inhibits the Na, K-ATPase activity. In a case of its introduction into the reaction medium together with NT, inhibition of Na, K-ATPase either by SF or NT is abolished and enzyme is sharply activated [8]. This effect is not manifested in other transport ATPases. The subject of our interest was identification of NT and SF effects on anion activated ATPases (Cl-ATPase, HCO₃⁻-ATPase).

Materials and Methods

The synaptic fraction obtained from the brain of the adult albino rats of either sex served as an investigation material. The fraction was obtained by differential centrifugation between the 1.2-0.9 M sucrose layers [9]. The protein concentration was evaluated by the Lowry method [10], inorganic phosphorus by the modified Fiske-Subbarow [11] and Kazennov-Maslova methods [12]. ATPase activity (V) was assessed by the amount of isolated inorganic phosphorus per mg protein per hour. HCO₃-ATPase and Cl-ATPase activity was determined by the activity difference in medium with and without HCO₃ and Cl ions. The synaptosomal factor was isolated from the regions rich in nerve-endings, by means of osmotic shock (9 ml of water per gr of tissue) of crude mitochondrial fraction. The supernatant was subjected to heat treatment (82°C, 5 min) and sedimentation of 30 min 25000 g. With an aim of isolating the constituent components of the SF the following methods: dialysis (dialysis bags of LKB) and gel-filtration (on the LKB-type device) were used.

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Results and Discussion

On the one hand the influence of Dopamine (DA) and SF and on the other hand their combined effect on the Cl-ATPase and HCO₃-ATPase was studied. The incubation medium for HCO₃-ATPase contained: 30mM tris-HCl buffer; [ATP_f] = [Mg²⁺] = 0.31 mM; [MgATP] = 1.69mM; 0.2mM ouabain. For Cl-ATPase – 30mM tris-malat buffer; [ATP_f] = [Mg²⁺] = 0.31 mM; [MgATP] = 1.69 mM; 0.3mM etacrinic acid.

Table. Dependence of Cl-ATPase activity (V), on Dopamine's (DA) different concentrations

[DA]	V	%
0	7.70 ± 0.20	100
0.01 mM	7.09 ± 0.42	93.38
0.05 mM	3.47 ± 1.07	45.06
0.1 mM	3.25 ± 1.28	42.21

The Table represents influence of DA various concentrations on Cl-ATPase activity. From the experimental data it is evident that DA inhibits enzyme system in concentration dependant manner. Increase of DA concentration deepens inhibitory effect of neurotransmitter. Fig. 1 and Fig. 2 show influence of Dopamine (DA) and SF only and their combined effect on Cl-ATPase and HCO₃-ATPase activity. DA and SF separately inhibit enzyme systems, while combined administration in reaction medium abolishes inhibition and Cl-ATPase is activated. HCO₃-ATPase is not sensitive to NT and SF effect. Therefore, from the experiment results, it is evident that DA effect on transport ATPases is not characterized with high specificity and besides Na, K-ATPase Cl activated ATPases also show NT sensitivity.

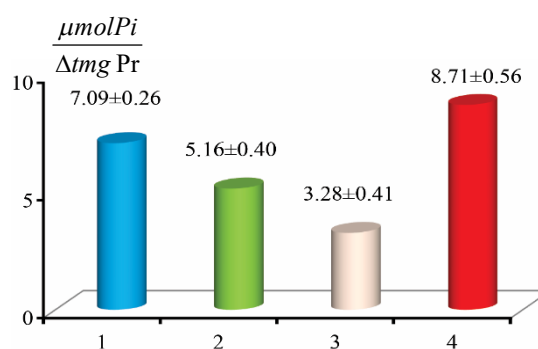


Fig. 1. Dependence of albino rat brain synaptic membrane fraction Cl-ATPase activity (V), on Dopamine (DA) and synaptosomal factor (SR) concentration. 1 – [DA] = 0; [SF]=0; 2 – [DA]=0.01mM; [SF]=0; 3 – [SF]=0.03 mg/ml; [DA]=0; 4 – [DA]=0.01mM; [SF]=0.03mg/ml.

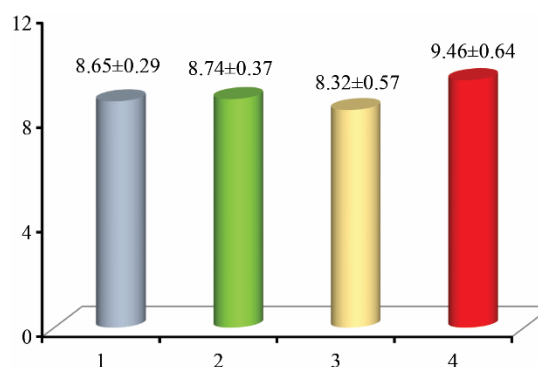


Fig. 2. Dependence of albino rat brain synaptic membrane fraction HCO₃-ATPase activity (V), on Dopamine (DA) and synaptosomal factor (SF) concentration. 1 – [DA]=0; [SF]=0; 2 – [DA]=0.01mM; [SF]=0; 3 – [SF]=0.03 mg/ml; [DA]=0; 4 – [DA]=0.01mM; [SF]=0.03mg/ml.

Conclusion

Experimental results showed that DA and SF are modifiers of Cl-ATPase and do not effect HCO_3^- -ATPase. Regulation of Cl-ATPase by the neurotransmitter and SF is represented by several mechanisms, the unity of which makes one whole regulatory system. These mechanisms are: a) DA dependent ATPase inhibitory mechanism; b) DA

dependent ATPase activator mechanism, one of the constituents of which is SF; c) DA independent inhibitory mechanism, determined by action of SF.

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ბიოქიმია

ნეიროტრანსმიტერისა და სინაფსური ფაქტორის გავლენა სინაფსური მემბრანის ანიონურ ATPაზებზე

ს. ძნელაძე* და ე. ნოზაძე*

* ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო

(წარმოდგენილია აკადემიის წევრის დ. მიქელაძის მიერ)

ანიონური ATPაზები წარმოადგენს ფერმენტულ სატრანსპორტო სისტემებს, რომელიც აქტივირდება ანიონებით და სტიმულირდება მაგნიუმის იონებით. ისინი გადამწყვეტ როლს თამაშობენ უჯრედშიდა pH-ის, უჯრედის მოცულობის რეგულაციასა და სინაფსური გადაცემის მექანიზმში. ნეიროტრანსმიტერები 5-ჰიდროქსი-ტრიპტამინი (5-HT), დოფამინი (DA) და სინაფსური ფაქტორი (SF) წარმოადგენს მოდიფიკატორებს Na,K-ATPაზური სისტემისათვის. წარმოდგენილ ნაშრომში შესწავლილია დოფამინისა და ენდოგენური სინაფსური ფაქტორის გავლენა Mg-დამოკიდებულ ანიონურ ATPაზებზე (Cl-აქტივირებული ATPაზა E.C.3.6.3.11, HCO_3^- -აქტივირებული ATPაზა E.C. 3.6.1.3.) თეთრი ვირთაგვას თავის ტვინის სინაფსურ ფრაქციაში. ნეიროტრანსმიტერი და სინაფსური ფაქტორი ცალ-ცალკე ინჰიბირებს Cl-ATPაზას, მაშინ, როცა მათ ერთობლივ მოქმედებას ინჰიბიციური ეფექტი გადაჰყავს აქტივაციურში. ნეიროტრანსმიტერისა და სინაფსური ფაქტორის მოქმედება Mg^{2+} -დამოკიდებულ HCO_3^- -ATPაზაზე არ ვლინდება.

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