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Biochemistry

Regulation of NaK-ATPase Activity by Neurotransmitters

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(Presented by Academy Member F. Todua)

ABSTRACT. The hitherto unknown NT-regulated mechanism of NaK-ATPase localized in the nerve ending membranes is found. The mechanism certainly has a functional significance and must be involved in the regulation – modulation of chemical synaptic transmission. On the other hand , the availability of the discovered specific protein, regulators (SFa and SFi) of synaptic origin, makes it possible to consider these mechanisms as the possible components of learning and short-term memory processes. *© 2007 Bull. Georg. Natl. Acad. Sci.*

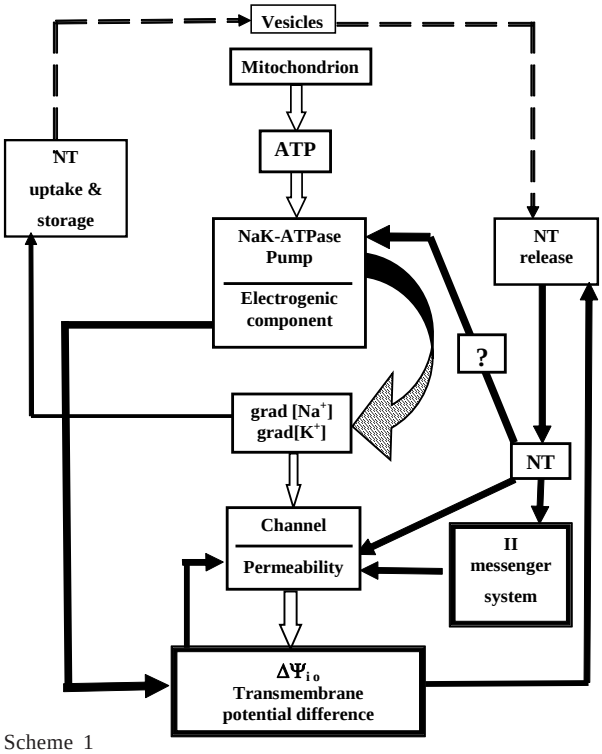
Key words: *NaK-ATPase, neurotransmitters.*

The transmembrane electric potentials difference ($\Delta\psi$) is generated as a result of passive transport of Na^+ and K^+ in the channels possessing specific permeability. In its turn, the enzyme system localized in the membrane, NaK-ATPase (Na/K –pump), creates osmotic energy (grad $[Na^+]$ and grad $[K^+]$) responsible for the Na^+ and K^+ passive transport. Furthermore, NaK-ATPase, because of its electrogenicity, is directly involved in the generation of potentials difference (10-20% of $\Delta\psi$). In terms of the contemporary classic theory, a fast alteration of $\Delta\psi$ having physiological implication (excitation, EPSP, IPSP, etc.) is completely due to changes in permeability of the ionic channels. However, there is also a theoretical possibility of the fast $\Delta\psi$ alteration being due to changes in the electrogenic NaK-ATPase activity (scheme 1). This is possible in the case if an electric or chemical signal elicits a noticeable change in NaK-ATPase activity.

In 1969-1987 the influence of neurotransmitters (NT) on NaK-ATPase activity was explored and major properties specific for this effect [1-10] appeared to be that:

1. Dopamine, noradrenaline, serotonin and acetylcholine inhibit NaK-ATPase activity (≈ 20-60 %) (the effect of saturation being noticeable at NT high concentration). NaK-ATPase appears not to be sensitive to glutamate, GABA, proline, alanine and glycine.

2. NT effect manifests itself only on the NaK-ATPase localized in the synaptic membrane fraction. In the brain



Scheme 1

microsomes the effect is negligible and is not revealed in the protoplasmic membrane fractions of other tissues (the sarcoleme NaK-ATPase inhibition by acetylcholine forms an exception).

3. Dopamine induced NaK-ATPase maximum inhibition is noticeable in the dopaminergic synapses, in the adrenergic by noradrenaline, while in the cholinergic by acetylcholine.

4. NT effect on NaK-ATPase is variable in the synaptic membrane fraction of different brain areas obtained from the rats being at various ontogenetic development and in conventional and socially isolated conditions.

5. Ca^{++}, cAMP and phosphokinase do not influence NT inhibitory effect of NaK-ATPase.

6. As a result of treatment with detergents and NaI, NaK-ATPase specific activity increases, while NT induced inhibition gets sharply diminished.

Thus, on the strength of this data a conclusion can be drawn that some NT (dopamine, noradrenaline, acetylcholine and serotonin) inhibit NaK-ATPase activity localized only in the synaptic membranes and the effect has a functional significance. It is remarkable that NaK-ATPase is not sensitive to glutamate, GABA, proline, alanine and glycine.

In 1987-1996 [11-18] regulation of NT effect on NaK-ATPase activity by protein factors of synaptic origin (SF_a and SF_i) was studied.

1. The high-molecular (100 kD) SFa and low-molecular (10-15 kD) SFi soluble proteins are isolated from the nerve endings cytosole. SFi inhibits NaK-ATPase activity (the effect being not NT-dependent). SFa has no direct effect on NaK-ATPase, but in the presence of dopamine and noradrenaline, it results in a robust increase of NaK-ATPase activity. The isolated SFa and SFi fractions are heterogeneous.

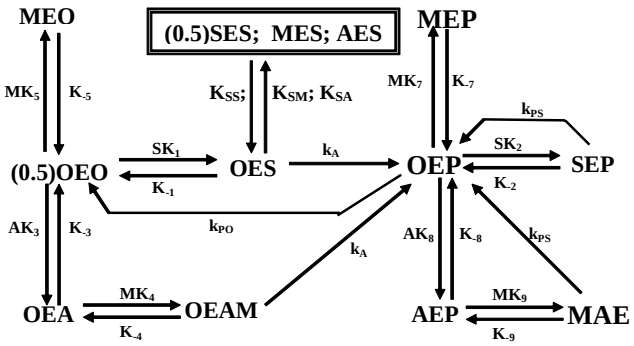
2. No proteins having SFa–like action are found in the cell extract (microsomal fraction supernatant) from the brain and kidney.

3. The serotonin and acetylcholine–dependent, SFa –stimulated NaK-ATPase activation is weakly expressed and is not statistically significant.

4. The noradrenaline and dopamine-dependent, SFa –stimulated NaK-ATPase activation is relatively strong, the effect , however, being revealed only on the NaK-ATPase localized in the synaptic membrane fraction.

5. NT and SFa have no effect on the synaptic membrane fraction localized enzymes: acetylcholinesterase, Ca,Mg-ATPase and Mg-ATPase.

6. The pNPPase activity of NaK-ATPase is inhibited by NT and activated by (NT+ SFa)

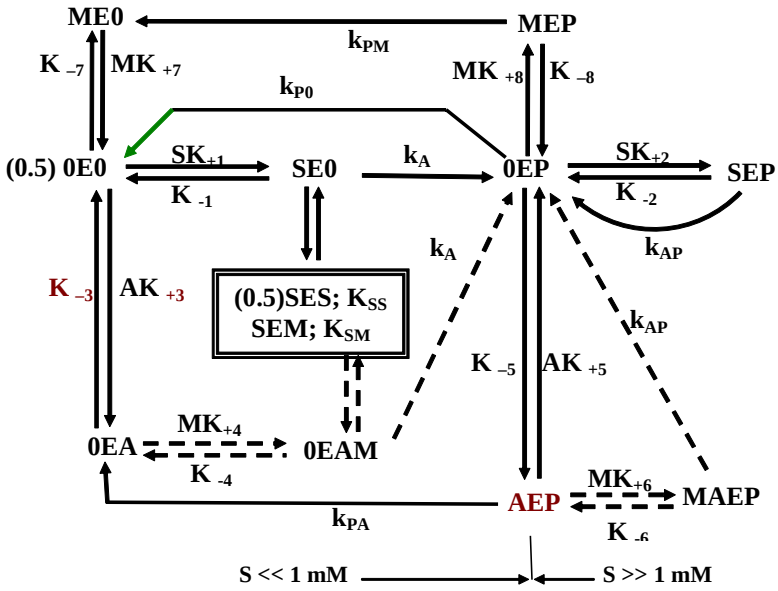


Scheme 2. (M ≡ Mg^{++}, A ≡ ATP_f, S ≡ MgATP)

7. Vanadate was shown not to be involved in the NT and SFa effect on NaK-ATPase. It is a NaK-ATPase independent inhibitor.

Thus, these data lend support to the conclusion that SFa has no direct action on NaK-ATPase, but in the presence of dopamine and noradrenaline it produces a robust increase (≅300%) of NaK-ATPase. Likewise NT inhibition of NaK-ATPase, NT-SFa effect on NaK-ATPase is specific and is in close relation with the functioning of a synapse, with chemical synaptic transmission in particular.

It is known that in different tissues and subcellular fraction membranes different isomers of NaK-ATPase are found. We have demonstrated that the NaK-ATPase molecular mechanism localized in the brain and kidney microsomal fraction (scheme 2) is identical, whereas the mechanism of NaK-ATPase activity in the synaptic fraction is different (scheme 3). The essential difference consists in that the phosphorylated intermediates (ME_2P and AE_2P) bound with Mg^{++} (M) or free ATP (A) in the synaptic membranes possess a catalytic capacity and



Scheme 3. (M ≡ Mg^{++}, A ≡ ATP_f, S ≡ MgATP)

these intermediates, as distinct from the microsomal NaK ATPase, form no dead-end branches. At the same time, in the case of ME_2P catalysis the standard transport stoichiometry $Na^+ \div K^+ = 3 \div 2$ is altered and occurs $Na^+ \div K^+ = 3 \div 1$ or $Na^+ \div K^+ = 3 \div 0$. (scheme 2 & 3).

Thus, in the molecular mechanism of NaK-ATPase activity the following operation modes may be distinguished:

(OPO)OEO↔OES→OEP→OEO; ($Na^+ \div K^+ = 3 \div 2$).
(OPS) OEP↔SEP→PEO ($Na^+ \div K^+ = 3 \div 2$).
(OPA)OEO↔OES→OEP↔AEP→AEO↔ OEO ($Na^+ \div K^+ = 3 \div 2$).
(OPM) OEO↔OES→OEP↔MEP→ MEO↔ OEO ($Na^+ \div K^+ = 3 \div 1$) or **($Na^+ \div K^+ = 3 \div 0$).**

From this, OPA and OPM occur only in the synaptic NaK-ATPase fraction.

Study of the molecular mechanisms of NT and SFa effect on NaK-ATPase [19-26] provides a basis for concluding that:

1. The NT+SFa regulation of the Na,K-ATPase activity is not provoked by the secondary messenger system. cAMP ($d \leq 1$mM), cGTP ($d \leq 0.5$mM), GTP ($d \leq 0.1$mM), G-proteins general activator 5-GIP ($d \leq 0.1$mM), triptoperasine ($d \leq 0.1$mM), theophyline ($d \leq 0.1$mM), and farbol ether ($d \leq 10^{-8}$ mM),have no influence on the NaK-ATPase NT- SFa effect. Ca^{++} is not implicated in the realization of NT- SFa effect, either.

2. As a result of kinetic analysis of activity curves V=f(NT, SFa), it has been established that only a certain part of NaK-ATPase total amount is sensitive to NT+SFa; that there is one or more linkage transmission chain SFa interaction with which results in the conversion of NT-dependent inhibition mechanism of NaK-ATPase into NT-dependent activation mechanism of NaK-ATPase.

3. A thorough kinetic analysis has demonstrated that in the synaptic membrane fraction obtained from the brain of rats being at different ontogenetic development and in conventional and socially isolated conditions an abrupt change is noted in the quantitative ratio of activating and inhibiting NT-dependent mechanism.

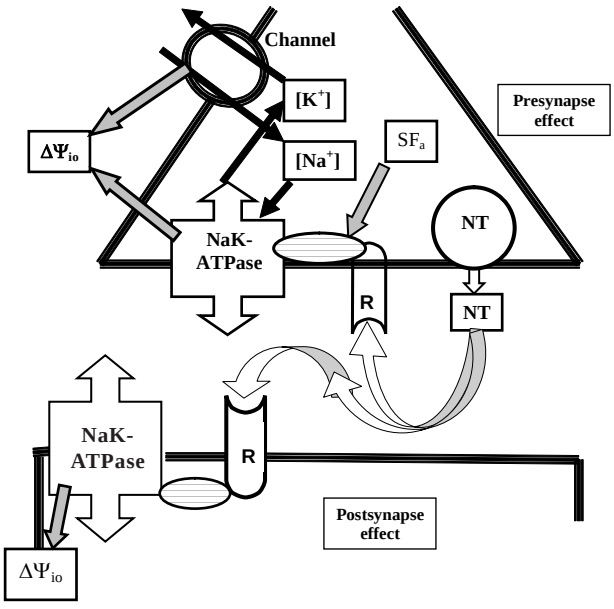
4. Analysis of kinetic curves of complex geometric shape enabled to establish that the NaK-ATPase localized in the synaptic membranes being in OPS operation mode under NT influence passes into OPM mode. The affinity of the phosphorylated subunit dramatically decreases to a substrate and increases to Mg^{++}.

5. As a result of NT influence the transport stoichiometry alters, instead of $Na^+ \div K^+ = 3 \div 2$ becoming $Na^+ \div K^+ = 3 \div 1$ or $Na^+ \div K^+ = 3 \div 0$, i.e. increases the electrogenicity coefficient. NT in respect to K^+, as an activator, is an inhibitor with constant affinity, while in respect to Na^+, as an activator, is an inhibitor with double effect.

6. With a simultaneous action of NT+SFa the operation mode of the NaK_ATPase localized in the synaptic membranes does not alter. The transport stoichiometry remains unchanged, $Na^+ \div K^+ = 32$. SFa represents an activator with constant affinity.

Scheme 4 shows the possible arrangement of NaK-ATPase NT-dependent inhibitory and NaK-ATPase NT-dependent SFa –stimulated activating mechanisms in the axo-somatic, axo-dendritic and axo- axonal chemical synaptic transmission. Certainly, NT acts outside the membrane, while SF_a, being found only on the synaptic terminals, inside it. Postsynaptic activating effect can occur only in the case of axo-axonal chemical synaptic transmission.

Thus, on the strength of the experimental material described above, it is beyond doubt that two mechanisms of regulation are found in the chemical synapses: NT-dependent NaK-ATPase inhibition and (NT+SFa)-dependent NaK-ATPase activation. Statistically significant NaK-ATPase inhibition was elicited only by dopamine, noradrenaline, serotonin and acetylcholine, whereas activation was elicited by dopamine and noradrenaline. These mechanisms no doubt have a certain functional significance. It may be supposed that this is expressed in the regulation-modulation of chemical synaptic transmission. If we share the opinion that ouabain (NaK-ATPase specific inhibitor) has an inhibitory effect on short-term memory [25, 26] and post-tetanic potentiation[27], then these NT-dependent mechanisms may be associated with the learning and short-term memory processes.



Scheme 4

ბიოქიმია

NaK-ATPაზას აქტივობის ნეიროტრანსმიტერებით რეგულაცია

ზურაბ ქომეთიანი

ი. ბერიტაშვილის ფიზიოლოგიის ინსტიტუტი, თბილისი

(წარმოდგენილია აკადემიკოს ფ. თოდუას მიერ)

აღმოჩენილია ნერვული დაბოლოების მემბრანებში ლოკალიზებული NaK-ATPაზას ნეიროტრანსმიტერებით რეგულაციის დღემდე უცნობი მექანიზმები, რომლებსაც უდავოდ გააჩნიათ ფუნქციური დატვირთვა. ეს მექანიზმები მონაწილეობას უნდა ღებულობდნენ ქიმიური სინაფსური გადაცემის რეგულაცია-მოდულაციაში, ხოლო აღმოჩენილი სინაფსური წარმოშობის სპეციფიკური ცილა-რეგულატორების (SFa და SFi) არსებობა შესაძლებელს ხდის განვიხილოთ ეს მექანიზმები როგორც დასწავლისა და ხანმოკლე მეხსიერების პროცესების შესაძლო კომპონენტები.

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