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S10.P6

Do native and mutated forms of huntingtin distinguish human VDAC isoforms?

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The metabolite exchange between mitochondria and cytoplasm is supported by VDAC (voltage-dependent anion selective channel). Studies concerning the role of VDAC have proved that the channel participates in ATP rationing, Ca^{2+} homeostasis, intracellular redox state regulation, communication between mitochondria and nucleus and apoptosis execution. Thus, the channel is regarded as crucial for mitochondria functioning and consequently for cell life or death. In mitochondria of different organisms VDAC may be present as isoforms encoded by separated genes, displaying different channel-forming activities and probably playing different roles. *Saccharomyces cerevisiae* mitochondria express two VDAC isoforms (yVDAC1 and yVDAC2), of which only yVDAC1, encoded by POR1 gene, has been proved to form a channel with properties highly conserved in other species. In human mitochondria, as in the case of other vertebrates, three isoforms of VDAC (hVDAC1-hVDAC3) able to form functional channels have been identified. They are expressed in different tissues and organs at different levels. Huntington's disease (HD) is an autosomal-dominant neurodegenerative hereditary disorder that gradually robs affected individuals of memory, cognitive skills and normal movements. It is originated by the mutation of the gene encoding the huntingtin-protein (Htt). Htt with an abnormal stretch of above 35 glutamines in the N terminus (mHtt) results in HD. The observed symptoms correlate with the selective loss of neurons within the central nervous system, not only in the striatum but also in the cerebral cortex. At present increasing amount of data indicates that mitochondrial functioning is affected by mHtt and the resulting mitochondrial impairments may occur early enough to contribute to mHtt-induced toxicity and the HD pathogenic mechanism. In our studies, we focused on the interaction of hVDAC1-hVDAC3 with Htt and mHtt. Therefore, we examined the effect of GST-Htt exon 1 fusion proteins containing 28 (Htt) and 74 (mHtt) glutamines on channel properties of the VDAC proteins isolated from Δpor1hVDAC1, Δpor1hVDAC2 and Δpor1hVDAC3 *S. cerevisiae* cells as well as from neuroblastoma cells. Obtained results indicate that Htt and mHtt directly and differently modulate human VDAC. This in turn could be important for development of new therapeutic strategies concerning HD. Acknowledgements: the studies were supported by the grant: NCN 2011/01/B/NZ3/00359.

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Stochastic model of glycine transporter and its application to amino acid transport in mammalian neurons

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Neurotransmitter uptake is quite essential in various metabolic and functional processes in neural tissue. Inhibitory glycinergic neurotransmission is terminated by specific glycine transporters GlyTs (GlyT1 and GlyT2), which actively reuptake glycine from the synaptic cleft. In physiological conditions the pool of this amino acid is regulated by a cascade of metabolic reactions and by membrane transport via specific transporters as well. The re-uptake of glycine into presynaptic terminals and surrounding glia is obligatory for the maintenance of low synaptic levels of the transmitter in the synaptic cleft. Glycine transporter type 2 (GlyT2) presented in the presynaptic membrane is a member of Na^+/Cl^--dependent transport proteins family, which share a common structure with 12 transmembrane domains. Transport of one glycine molecule involves the transport of one Cl^- and 3 Na^+ per transport cycle. The main goal of the present study was to develop a computer simulator of GlyT2 activity based on known experimental data for quantitative estimation of membrane glycine transport. A sequence of elemental events happening during the cycle of GlyT2 activity was summarized as a single scheme, which became a basis of an original software. The algorithm of transporter simulator was developed using the probability approach describing the behavior of a single protein. As a result of such computations the number of translocated glycine molecules per time period has been evaluated. The computer experiments were carried out under different environmental conditions such as ion and glycine concentrations. As the major equilibrium constants of the transport steps are still unmeasured the reversibility degree of the glycine transport is also considered as a variable parameter. Using described software the time dependences of glycine, sodium and chloride ions amounts were obtained. Ligand cooperativity was observed for sodium ions (Hill coefficient is 3.6). The developed software based on proposed probability algorithm can be used for a virtual experiment in GLYT2 activity simulation. The described model allows to predict some characteristics of the transporter functioning which can be experimentally proven. This software combined with glycine receptor model can be also used in research laboratories for evaluating concentrations of chloride, sodium and glycine in synaptic cleft and presynaptic terminal in different time points during inhibitory signaling.

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S10.P8

The effect of *Macrovipera lebetina obtusa* viper venom on erythrocyte ghosts membrane ATPase activites

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Macrovipera lebetina obtusa (MLO) is one of the most important poisonous snakes in Armenia. In the venom of this snake a specific toxin was not identified but they form complexes with other non-enzymatic proteins to achieve higher efficiency through synergy. We have studied influence of venom on the erythrocyte ghosts by fluorescent microscopy. Images were collected on an epi-fluorescent microscope FM320-5M (AmScope, USA). The erythrocyte ghosts were visualized with ANS fluorescent probe. The erythrocyte ghosts were deformed after adding the MLO venom. They shrink within 3 min, and pull in. We also studied activities of Na^+, K^+-ATPase and Ca^{2+}-activated Mg^{2+}-dependent ATPase in the absence and in the presence MLO venom. Venom was added into the assay mixture with low, sub-lethal (0.35 mg/kg approx. 0.5 LD 50 for rat) and lethal

concentrations in accordance with LD50. Membranes were incubated with venom 10 min before adding ATP (8 mM). The enzyme activity due to Mg^{2+} alone defined as Mg-ATPase activity is subtracted from that due to Na^+/K^+ and Mg^{2+}, and due to Ca^{2+} and Mg^{2+} to obtain Na/K-ATPase and Ca-ATPase activity respectively. It was shown that Na^+/K^+ ATPase activity in erythrocytes membranes was increased in the presence of the MLO venom (low concentration ~1.81 times, sub-lethal concentration ~3.83 times and lethal concentrations ~4.28 times respectively). Under these conditions $Ca2^+$ ATPase activity was decreased (low concentration ~3.37 times, sub-lethal and lethal concentrations ~17.93 times respectively). We also studied $Mg2^+$ ATPase activity. In this case $Mg2^+$ ATPase activity was not dependent on concentration. These results suggest that ATPase activity is very sensitive to venom components and venom influence leads to possible conformation changes in ATPases.

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S10.P9

Stochastic modelling of neuronal membrane glutamate transporters

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The membrane glutamate transporters are expressed in various tissues but are of the most importance in the brain. Thus, they precisely define termination of excitatory neurotransmission in glutamatergic neurons and prevent brain tissue from glutamate induced excitotoxicity. This transporters belong to solute carrier 1 (SLC1) family and appeared to be secondary active transporters, which use cotransport of three Na^+ ions and one H^+ and countertransport of one K^+ ion as driving force for taking up one glutamate molecule into the cell against its concentration gradient, as accepted for human transporter subtypes. Specified stoichiometry results in a total movement of two positive charges into the cell for each transport cycle, so transmembrane potential can also act as a driving force and transport process called electrogenic. Appropriate glutamatergic neurotransmission is essential for the most aspects of normal central nervous system functioning, such as cognition, memory and learning. Glutamate concentration maintenance under excitotoxic physiological level also plays major role in the CNS development, including synapse induction and elimination, cell migration, differentiation and death. So, function impairing or reduction of membrane glutamate transporters results on many CNS diseases and disorders. In order to clarify kinetic properties of single neuronal glutamate transporter stochastic modeling algorithm was proposed. It consists of several logical blocks and is based on probabilities of elemental steps during the neurotransmitter transport cycle such as substrate binding and translocation across the membrane. As sequence of these elemental steps is still under discussion, our approach is capable of its varying. Virtual computer experiments can also be carried out under different environmental conditions such as sodium, potassium and glutamate concentrations, and pH. The structural properties of the protein are implicit in a probabilities value, which generally derived from equilibrium constants of each elemental reaction or differences of free energies of substrate binding. That's why the whole procedure is less time consuming as many other approaches and affords an opportunity to insight into membrane glutamate transporter functioning mechanism in more detail. Thus it becomes possible to evaluate relevance of evidence of transitional sodium binding site existence.

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S10.P10

Two multi-subunit cation/proton antiporters have major roles in the bacterial pathogen *Staphylococcus aureus*

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Staphylococcus aureus, a major pathogen, has two 7-subunit Mrp-type cation/proton antiporters, Mnh1 and Mnh2, which function as proton motive force-dependent antiporters. Mnh1 was earlier shown to catalyze Na^+ (Li^+)/H^+ antiport in membrane vesicle assays using antiporter-deficient *E. coli* strain KNabc. Mnh2 was noted later and has not been analyzed for catalytic capacity or physiological roles. We show that Mnh2 exhibits both Na^+ (Li^+)/H^+ and K^+/H^+ antiport in vesicle assays, with greatest activity at pH ≥ 8.5; higher than the Mnh1 optimum of ~pH 7.5. No mnh1 gene disruptions were found in a recent screen by Fey et al. for non-essential *S. aureus* genes, using strain JE2, whose progenitor strain is *S. aureus* CA-MRSA USA300 LA clone (LAC). This raised the possibility that Mnh1 is essential, but we found a possible confounding issue, i.e., a transposition in LAC and JE2 that inactivates Mnh2, and concomitantly produces deficits at high K^+ and/or elevated pH relative to reference strain *S. aureus* FPR3757. We used *S. aureus* SH1000 and Newman strains, which have identical antiporter sequences but different lineages, to test whether viability depends on at least one functional Mnh antiporter. In SH1000, the double mutant is viable but is completely inhibited by low sodium concentrations, and its sensitivity to high osmolarity and pH are increased beyond those of each single mutant. In *S. aureus* Newman, only single deletions could be made, but neither Mnh1 nor Mnh2 are "essential". The Δmnh1 strain exhibits large deficits in salt- and alkali-tolerance, and is highly attenuated in vivo in a murine model of bloodstream infection. In contrast, the ΔmnhA2 strain exhibits deficits in osmotolerance in vitro, but no detectable phenotype in the bloodstream infection model. Together, these findings support the notion that the Mnh1 and Mnh2 antiporters of *S. aureus* are critical for the physiology of this organism and at least Mnh1 contributes to the pathogenesis process.

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Ion channel path of cellular transduction

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A better understanding of the selective responses of the ion channels to the multiple concurrent influential parameters of both internal and external environments, can address, complete and refine the existing ion channels models. Widely distributed class of P-type ATPase sodium pump is responsible for the active transport of a variety of cations across cell membranes. The main basic function of the sodium pump is to maintain the N^+ and K^+ gradients across the plasma membranes. Thus, membrane potential, nutrient uptake, intracellular volume and pH are regulated by the proper function of the sodium pump. Consequently, the wide varieties of ion channels are related to their functional source of energy, opportunistically harvesting all available stimuli. One of these