

UDC 57.017.23+112.7:352.465:151.643

CELLULAR PRION LEVEL AND ATPases ACTIVITIES IN THE JEJUNUM OF DIFFERENT AGE WISTAR LINE RATS

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Prion infections are lethal diseases of the central nervous system in the humans and animals, the causative agent of which is the abnormal (infectious) prion (PrP^{Sc} , Sc — scrapie from). However, cellular prion (PrP^{C} , C — cellular from) is a substrate for the PrP^{Sc} conversion. It is located on the outer surface of the cell membrane and involved in the maintenance of Ca^{2+} -homeostasis and other metabolic processes. Pathological prion infection occurs after eating of infected meat products of feed. Because of the sporadic diseases occur in older age persons, we investigated the PrP^{C} level in the jejunum of rats of different ages.

The aim of this study was to determine the ontogenetic changes of PrP^{C} level and activities of Na^+/K^+ - and Ca^{2+} -ATPases in the rats' jejunum and calculate the correlation relationship between these parameters.

Manipulation with the animals were carried out under the law principles of Ukraine. Research was carried out on the males of laboratory rats *Rattus norvegicus* var. *alba*, Wistar line. The animals aged one, six and thirty months were decapitated under ether anesthesia, the jejunum was selected for this research. A western blotting analysis of the jejunum was carried out. For that, the proteins were fractionated by electrophoresis in 12 % gradient polyacrylamide gels (PAGE). The electro blotting of proteins on PVDF-membrane was carried (Millipore, USA). The membranes were incubated with monoclonal primary antibodies (Antibody mAB6H4; Prionics, Switzerland), and secondary polyclonal goat anti-mouse antibodies which are conjugated with alkaline phosphatase (Sigma, Germany). Detection of the immune complexes was carried out using a substrate for alkaline phosphatase CDP-Star (Tropix, UK). The activity of Na^+/K^+ -ATPase was calculated by the difference between the total activity and ouabain insensitive activity, which was determined in medium with 1 mM of ouabain (Sigma, Germany). The activity of plasma membrane Ca^{2+} -ATPase (PMCA) was calculated by the difference between activities that determined in the medium with 1 mM of thapsigargin (selective inhibitor of $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase) (Sigma, Germany) in the presence and absence of Ca^{2+} . The activity of sarco (endo) plasmic reticulum Ca^{2+} -ATPase (SERCA) was calculated by the difference between the activities that were determined in medium with calcium ions in the absence and presence of thapsigargin. The measure of enzyme activity was inorganic phosphate (P_i) concentration. The study of enzymatic reactions kinetic properties was carried out in a standard incubation medium which was modified by the physical and chemical characteristics or certain components composition (incubation time, concentration of protein, ATP, Na^+ , K^+ , Ca^{2+}). The level of sodium and potassium ions in tissues was determined using the commercial kits (*Felicity diagnostics*, Ukraine) and the level of total calcium was determined using atomic absorption spectrophotometer C-115M.

Three glycoforms of PrP^{C} were detected: the diglycosylated (35–38 kDa), partially (mono) glycosylated (23–27 kDa) and nonglycosylated (19–21 kDa). Its forms levels in the one-month rats' jejunum were 23, 13 and 13 standard units, respectively. The diglycosylated PrP^{C} form level was increased by 66 % in the six months rats' tissue compared to one month age rats. Its level was decreased by 44 % in old rats' jejunum compared to mature animals. The mono- and nonglycosylated PrP^{C} forms levels were increased by 56 and 38 % in the six months rats' tissue, respectively, and were almost unchanged in the thirty months rats' tissue.

The Na^+/K^+ -ATPase, SERCA and PMCA activities decreasing by 84, 89 and 26 %, respectively, in the thirty months animals' tissue was demonstrated. The sodium and potassium levels were unchanged, instead total calcium level was increased by 78 % compared to the six months animals' jejunum.

Kinetic characteristics of ATP hydrolysis by the investigated enzymes were also changing with animals' age increasing. The values of initial reaction velocity (V_0) and maximum amount of reaction product (P_{max}) were decreased by 58–83 and 34–91 %, respectively, the value of reaction time (τ) was increased by 34–49 %. However, V_0 value for PMCA was unchanged and τ for Na^+/K^+ -ATPase decreased for 51 %. The maximum velocity of the enzymatic reaction (V_{max}) value under these conditions was lower in 2–28 times, while Michaelis constant (K_m) value was lower by 30–92 % which indicates a growing affinity to the enzyme substrate (ATP). In the thirty months animals' jejunum the Ca^{2+} -ATPases remain its activities under high calcium ions concentration in the medium. It should be noted that the ions concentration value optimum for the Na^+/K^+ -ATPase is shifting towards in the increase of Na^+ level which is consistent with a slight increase of these ions in the tissues in total.

By the results of correlation analysis, a direct strong correlation between the PrP^{C} level and PMCA activity was carried out ($r=0.729$ – 0.780) as well as between the activities of these enzymes with each other ($r=0.800$ – 1) and between the ATPases activities and ions level but an inverse correlation between Na^+ and Ca^{2+} was calculated. However, the weak correlation ($r=0.202$ – 0.250) between the PrP^{C} level and the other enzymes activities was observed.

PrP^{C} level is the smallest in young animals' jejunum, it increases in six months animals and decreases in thirty months animals. The activities of ATPases as well as the kinetic characteristics of ATP hydrolysis decrease depending on the animals' age increasing. But value of τ was increased. There is a correlation between the ontogenetic changes of PrP^{C} level and transport enzymes activity in the different age animals' jejunum.