UDC 57.017.23+112.7:352.465:151.643

LOCALIZATION AND LEVEL OF CELLULAR PRION AND ACTIVITY OF Ca^{2+}-ATP-ases IN THE RATS' LIVER DEPENDING ON AGE

M. V. Kushkevych, PhD student, *V. V. Vlizlo*, Dr. Sc., Prof., Member of NAAS
m_kushkevych@ukr.net

Institute of Animal Biology of NAAS, Lviv, Ukraine

Prions are infectious proteins causing bovine spongiform encephalopathies of humans and animals. They occur not only as a result of infection but can be sporadically. According to the prion hypothesis, the disease arises due to changes in the conformation of the normal (cellular) prion (PrP^C) molecule at the conditions of direct contact between the two molecules of prions: cellular and pathological (PrP^{Sc}). PrP^C is a sialicglycoproteid consisting of a polypeptide chain containing 253–256 amino acid groups, which arranged in the form of three α-helices and a short β-structure section. Investigation of the PrP^C localization in tissues and organs is important in explaining the mechanism of prion diseases pathogenesis. The PrP^C localization and its level in the liver tissue of rats of different age were studied.

PrP^C was founded in hepatocytes of one month rats' liver by the results of immunohistochemical analysis. In six months animals PrP^C was founded in hepatocytes and sinusoids, in particular von Kupffer cells. The hepatocytes contained the minor amount of cellular prion. By the results of dot blot analysis was studied that protein level increased by 5 % in liver of mature rats, compared to young animals, while in the old rats its level was decreased by 36 % compared to mature rats.

Profile of PrP^C isoforms expression was studied. The diglycosylated form (35–38 kDa) dominated in the ratio hlikoform PrP^C, notglycosylated was represented the smallest amount (19–21 kDa), and partly (mono) glycosylated (23–27 kDa) took an intermediate position. The increase of di-, mono- and nonglycosylated cellular prion forms, respectively, by 68 %, 64 % and 21 % in the six months rats' liver, compared to one month rats, was established. However, the expression of cellular prion isoforms decreased at two times in old animals compared to mature rats.

Since PrP^C is involved in various metabolic processes, particularly in the ions transport through the cell membrane and in the regulation of Ca^{2+}-channels, supporting Ca^{2+}-homeostasis, the relationship between the PrP^C level and ATP-ases activity was suggested. So, the next our task was to investigate the activity and kinetic parameters of Ca^{2+}-ATP-ase of sarco(endoplasmic) reticulum (SERCA), Ca^{2+}-ATP-ase of plasma membrane (PMCA) and Ca^{2+} content in liver tissue of different age rats.

As a result of the studies, a decrease of the enzymes activity with increasing of rats' age was observed. The SERCA and PMCA activity was reduced by 75 % and 81 % in the old rats' liver compared to mature animals. Instead, it was a significant (by 68 %) increase of calcium ions level under this conditions. The kinetic parameters, including initial reaction velocity, maximum amount of reaction product, maximum velocity of enzymatic reaction and Michaelis constant of ATP hydrolysis by SERCA were decreased, respectively, at 2, 5, 6,5 and 3 times, and PMCA at 3, 7, 11 and 6 times in old animals compared to mature animals. The activity of both enzymes was the highest in young and mature animals' liver under the influence of 2.5–3.0 mM ATP in the medium as well as in old animals' liver under the influence of 2.0 mM ATP. The concentration of calcium ions also affected on the enzymes activity. The optimum was at 5 mM of Ca^{2+} for liver enzymes of one and six months rats but in thirty months animals for SERCA was 6 mM, while for PMCA was 7 mM. Optimum protein concentration was 125 mg/ml for both enzymes of young and mature animals while in old animals optimum was 100 mg/ml. ATP hydrolysis reaction by the studied enzymes was less intense and lasted longer, and the product was accumulated in smaller numbers in thirty months animals compared to the one- and six months animals. The enzymes are using smaller amounts of substrate and remain activity for the high concentration of calcium ions in the old animals' tissue.

Correlation analysis between identified parameters in liver tissue was carried out. The direct strong correlation (r=0,766–0,982) between the PrP^C level and both ATP-ases activity was demonstrated.

Thus, the cellular prion is localized in hepatocytes of different age animals. The increasing of animals' age up to six months accompanied by an increase of cellular prion level but during the subsequent growth animals' age under thirty months the PrP^C level decreases, which correlates with a decrease of Ca^{2+}-ATP-ases activity and kinetic parameters.