THE NEW ARMENIAN
MEDICAL JOURNAL
Vol. 3 (2009), N 1, 6 - 28
www.ysmu.am

NEW MECHANISMS OF NEUROHORMONAL REGULATION UNDER CONDITIONS OF NEURODEGENERATION

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Abstract

It was revealed that a synthetic analogue of hypothalamic neurohormone, proline-rich polypeptide (PRP), possesses a regulatory activity on development of neurochemical, neurohormonal, immunomodulatory and morphofunctional impairments occurring in models of brain neurodegenerative disorders typical for Alzheimer's disease.

These data were obtained in models of aluminum neurotoxicosis and via intracerebroventricular injection of the aggregated form of beta-amyloid peptide.

In a model of aluminum neurotoxicosis, it was obviously shown that different doses of PRP produced effect on synthesis of immunocytokines (IL-1, IL-2, and IL-6), prolactin, and insulin-like growth factor-1 (IGF-1), which can be produced *in situ* in hippocampus and brain cortex directly or indirectly participating in the processes of atypical neuronal amyloid protein formation during neurodegenerative diseases.

The regulatory effect of PRP was shown on levels of pro- and antioxidant metalloproteins of blood and tissues under the oxidative stress typical for aluminum toxicosis that signifies to the influence of PRP on the processes of lipid peroxidation.

The antioxidant role of PRP to regulate the intensity of lipid peroxidation processes was quite obviously demonstrated.

One of the most significant achievements of the research is detailed characterization of the brain. There was established the ability of PRP to prevent the accumulation of aluminum in the cytoplasm of hippocampus neurons and their penetration through the nuclear membrane.

Data concerning the ability of PRP to prevent the development in the neurons of hippocampal complex, magnocellular nuclei of hypothalamus (SO and PV), neuronal cells of motor and limbic parts of cerebral cortex neurodegenerative changes evoked by microinjection of beta amyloid peptide (25-35) are undoubtful. For the first time in the intact animals and in a model of brain amyloid neurodegeneration neurophysiologic studies have revealed the peculiarities of PRP action at the pre- and post-stimulus impulse current of hippocampus pyramidal neuron during single and frequent excitation of cerebral entorhinal cortex. There were accepted persuasive arguments of functional character proving a developing conception concerning an existence of expressed neuroprotective action of PRP during brain neurodegenerative impairments.

Thus, the results of recent research work allowed us to fulfill the present conception about multiform expressions of high biological activity of PRP and convincingly to demonstrate the existence of its central and peripheral immunomodulating action, the ability to regulate the brain free radical processes, the level of lipid peroxidation and as a whole to prevent and correlate the brain neurodegenerative impairments.

Keywords: Alzheimer's disease, neurodegeneration, aluminum neurotoxicosis, PRP, cytokines, prolactin, IGF, metalloproteins.

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Introduction

One of the fundamental achievements of modern neurochemistry and neuroimmunology is the discovery of a new family of neurohormones, cytokines of hypothalamus, which are synthesized in neurosecretory cells of *N. Supraopticus* and *N. Paraventricularis* (NSO, NPV) and are transported by the peptidergic fibers of hypothalamo-hypophyseal tract into the neurohypophysis [*Galoyan A., 1997*] .

The primary structure of proline-rich polypeptide (PRP) neurohormones, which are transported axonal (and humoral) up to a spinal cord (SC), has been decoded. The investigations have shown that PRP is a product of proteolysis of the neurophysin-vasopressin-associated C-terminal glycoprotein and, along with vasopressin and oxytocin, is transferred from hypothalamus toward the neurohypophysis by the axonal transport.

The discovery of neurosecretion of cells of the hypothalamic magnocellular nuclei (NPV and NSO), formation of vasopressin and oxytocin in them [*Du Vigneaud V., 1954*] underlay a new field of research in the modern molecular neuroendocrinology. The next discovery refers to hypothalamic releasing hormones, the isolation, and study of these peptides [*Guillemin R., 1977; Schally A. et al., 1978*]. The hormones (LH-RH), (GH-RH), somatostatin and others were revealed. The role of the analogue of releasing hormones (LH-RH, etc.) in treatment of cancers of various organs has been established [*Schally A. et al., 2001*].

Further development of mentioned directions has an important value for understanding brain functions and organization of neurohormonal functions as a whole. This is the basis for creation of new endogenic neurohormonal preparations for treatment of nervous, endocrine, immune, and other diseases.

The detailed study of signal molecules of the brain neuroendocrine immune system revealed the occurrence of peptides synthesized in hypothalamus, many of which are produced in the hypothalamus neurosecretory cells. Their primary structure was established [*Galoyan A., 1997*]. In particular, in the hypothalamus neurosecretory cells there was revealed an immunophiline consisting of 107 amino acids and participating in the mechanisms of interleukin biosynthesis (in particular IL-2). The biosynthesis of interleukins IL-lα, IL-1β, IL-2, IL-6, TNF, etc. in the hypothalamus neurosecretory cells and their output in neurohypophysis was simultaneously established [*Galoyan A., 1997; Zilfyan A.V., Soghbatyan L.T., 2000*].

During the last decade, in laboratory of Professor A. Galoyan there was discovered a new family of PRPs including 4 peptides, each of which possesses specific properties referring to various functions of an organism. PRP as having a wide spectrum of biological activity is the most investigated one; in our work it is marked as PRP [*Galoyan A. et al., 2004a;b; Vahradyan H. et al., 2004*]. The wide spectrum of PRP biological properties is considered in the aspect of their metabolic activity, antibacterial and antiviral action, myelogenesis, lymphopoiesis, immunomodulatory action, and neuroprotective as well as neurohormonal properties [*Aghajanov M. et al., 2000; Galoyan A., 2000; Gladkevich A. et al., 2007*].

Numerous data specify the polyfunctionality of PRP and the involvement of mentioned neuropeptides in many biologically significant processes in norm and in case of pathology [*Aghajanov M. et al., 2002; Shakhlamov V. et al., 2002; Galoyan A. et al., 2008*]. Thus, it is established that PRP participates in the expression mechanisms of interleukins (TNF, IL-1, IL-6) in fibroblasts, macrophages, and astrocytes. The investigations performed by A. Galoyan on PRP-mediated inhibition of pro-apoptotic caspase-3 and -9, as well as activation of caspase-2 and -6 [*Galoyan A. et al., 2000b*], were the beginning of studies on universal protective properties of PRP in the neurodegenerative processes.

PRP, being immunomodulator and stimulator of immunocompetent cells activates the formation of IL-1, IL-6 and TNF-α in astrocytes [*Galoyan A., 1997*], exerts the neuroprotective action in case of intoxications evoked by snake poisons, hemisection of a spinal cord [*Galoyan A. et al., 2000a;*

Sarkisyan J. et al., 2003], shows the antibacterial influence *in vivo* and antiviral action *in vitro* [*Aprikyan V., Galoyan A., 2003*]. Finally, PRP is the NGF-like compound stimulating the secretion of those compounds from the neuroglial elements [*Chekhonin V. et al., 2001*].

The problem of modeling neurodegenerative lesions of CNS, to which the Alzheimer's disease (AD) also pertains, is closely connected to extremely actual problem to study both pathogenesis and treatment of this most severe human disease [*Vahradyan H., Vahradyan V., 2003*] Data available on AD is based on results of post-mortem investigations/studies, therefore they provide information only about the finishing, occasionally terminal stages of disease, thus bringing forth a problem of working-out the correct AD experimental models to the utmost approximated to the used pathology in a number of actual problems of neuroscience.

Reproducing an adequate model assumes a discovery of new priority directions in studies of the cellular-and-molecular bases of its pathogenesis and purposeful development of ways for prophylaxis and therapy.

The purpose of research was to study the protective and corrective actions of synthetic analogue of hypothalamic neurohormone, PRP, on mechanisms peculiar to development of neurochemical, neurohormonal, immunomodulatory and morphofunctional disorders arising in case of aluminum neurotoxicosis and neurodegenerative changes, induced by the *β*-amyloid peptide (the AD-model).

The following tasks were formulated according to the aim of the study:

• *under conditions of aluminum neurotoxicosis* to investigate immunomodulatory action of various concentrations of PRP – its ability to influence the production of immunocytokines (IL-1 IL-2, IL-6) and insulin-like growth factor (IGF-1) in various formations of a brain (an integral brain, hippocampus and cerebral cortex), thymus, liver and blood serum of experimental animals;

• *in a model of the aluminum neurotoxicosis* to study the role of neuroendocrine mechanisms and, in particular, that of hypophyseal prolactin in realization of immunomodulating action of optimal and low PRP doses on synthesis of various immunocytokines in an integral brain, hippocampus, thymus, liver and blood serum;

• to characterize integrally the ability of PRP to prevent and correct various manifestations of the oxidative stress developing in case of the acute and chronic aluminum toxicosis as disorders in the system of blood pro- and antioxidant metalloproteins, including a level of cytochromes: b_5, $\sum b_{558}$ I-IV of the serum, $\sum b_{558}$ of erythrocyte membranes, the content and O^{-}_{2}-producing ability of suprol, activity of *Cu, Zn-SOD*, catalase, ceruloplasmin and transferrin;

• to investigate the influence of PRP on the shifts of tissue level of metalloproteins as the enzymes of antiradical protection of a cell, *Cu, Zn-SOD*, catalase, cytochrome *c* and content of malone dialdehyde (MDA) in an integral brain and hippocampus, developing in case of the acute and chronic aluminum neurotoxicosis;

• to study the ability of PRP to prevent the ultrastructural neurodegenerative lesions of different structures of the brain, developing in neurons of hippocampal complex, of motor and limbic zones of cortex and cells of magnocellular nuclei of hypothalamus (supraoptical and paraventricular) in case of aluminum neurotoxicosis and intracerebroventricular introduction of the aggregated form of *β*-amyloid peptide (25-35);

• to study the preventive and corrective PRP action on pre- and post-stimulus characteristics of a pulse stream flow of the pyramidal neuron in *CA2* of hippocampus under conditions of a single and frequency irritation of the entorhinal cortex of a brain of the same hemisphere in norm and in model of neurodegenerative lesion induced by introduction of *β*-amyloid peptide into the lateral ventricles of the brain.

Material and Methods

The experimental models.

According to the tasks put forward at the various stages, the investigations were carried out in different variants of AD experimental modeling. The research was carried out in white male mice

(weight = 30-40 *g*) and pubertal inbred white rats (weight = 180-200 *g*).

• The acute aluminum neurotoxicosis was caused by subcutaneous injection of 0.2 *ml* 10% solution of aluminum chloride to white rats. Likewise, the control animals were administered 0.9% solution of NaCl. Animals were decapitated under the mild ethereal anesthesia. Blood and other organs (brain, hippocampus, and liver) were drawn and placed in various containers on a cold and processed simultaneously. In case of double introduction of aluminum chloride there was revealed a rather low mortality of animals that formed the basis for using the specified dose for AD-modeling in rats. An experimental model of AD in mice was obtained by the single subcutaneous introduction of 0.2 *ml* of 3% aluminum chloride. The animals were divided into the following three groups: Control Group of intact animals, Experimental Group I with the single injection of $AlCl_3$, Experimental Group II– at the 4th day after introduction of $AlCl_3$ animals were exposed to intraperitoneal (*i/p*) administration of PRP at a dose level of 15 and 1.5 *μg*/40 *g* of animal weight. The animals were sacrificed on the 8th or 12th day after injection of $AlCl_3$ (mice and rats, accordingly).

• The chronic aluminum neurotoxicosis in white rats was induced by oral administration (with drinking water) of the 10% solution of aluminum chloride to animals within one and three months;

• The model of amyloidal brain damage. With the purpose to induce the amyloid damage of brain and study the neurotoxic properties of β-amyloid peptide fragment – βA (25-35) in processes of neurodegeneration, 5 series of the investigation were carried out in male rats with body weight of 250-300 *g* under the nembutal narcosis: 1) intact animals; 2) animals injected by the sterile distilled water intracerebroventricularly (*icv*) into the lateral ventricles of brain from both sides on the coordinates of stereotaxic atlas [*Paxinos G., Watson C., 2007*] AP-1 *mm*, L±l.5 *mm*, DV+3.5 *mm* with the speed of 1 *mcl/min* and being kept before the experiment within 45 days; 3) animals injected *icv* by 30 *nmol* of βA (25-35), aggregated according to T. Maurice and A. Privat [*Maurice T., Privat A., 1998*] as 1 *mg/ml* in sterile distilled water at t=37°C within 4 days into the lateral ventricles of brain from both sides with the rate of 1 *mcl/min* and being kept before the experiment within 45 days; 4) animals injected *icv* by aggregated βA (25-35) in a combination with the single intra-abdominal (*i/a*) injection of 15 *μg* of PRP, 24 hours prior to introduction of βA; 5) animals *icv* injected aggregated βA (25-35) in a combination with single intra-peritoneal (*i/p)* injection of 15 *mcg* of PRP on the 6th day after introduction of βA.

The neuromorphological investigations.

The isolated rat brain was fixed during 1-3 days in 10% formalin at 4°C and then washed out overnight in physiological solution containing 15% of saccharose for preserving the structures.

The brain was put in paraplast and frontal sections with the thickness of 10 microns were obtained. The frontal frozen sections (10 microns) and free-floating microtome sections (50 microns) from the various parts of the brain were washed out in 10% and 5% solution of saccharose and then in a physiological solution. With the purpose of revealing the nervous structures, sections were stained with toluidine blue by Rokhman's method. After staining within 15 minutes in 0.1%-solution of toluidine blue prepared in acetate buffer with pH=4.8, the sections were washed out in abundant amount of buffer, then incubated within 10 minutes in 1% solution of potassium ferrocyanide prepared in distilled water. The material was dehydrated in three portions of 70° alcohol and in two portions of xylol, and then put into the *canadian balm*; in sections stained with toluidine blue, the estimation of neurodegeneration was carried out.

Synthesis and identification of a new hypothalamic PRP, consisting of 15-amino acid residues, as well as β-*amyloid neuropeptide (25-35)* was synthesized in the laboratory of Prof. Galoyan by the use of F-moc amino acid residue. The molecular weight and purity degree of peptide were determined with the help of high performance

liquid chromatography (HPLC) and the mass spectrometry analysis (Matrix-assisted laser desorption/ionization – MALDI). The preparation was dissolved in the NaCl solution, sterilized by filtration (the size of pores equal to 0.22 microns) with the subsequent spectrophotometric determination of its content in 1 *ml* solution.

Determination of pro- and antioxidant metalloproteins.

White pubertal rats were used in order to study the neuroprotective influences of PRP on content of pro- and antioxidant action metalloproteins.

Animals were divided into four groups: Control Group (*i/p* injection of 0.2 *ml* of physiological solution on the 1st and 3rd days of the experiment); Experimental Group I (*s/c* introduction of 0.2 *ml* of 10% solution of $A1C1_3$ on the 1st and 3rd days of experiment); the Experimental Group II (*i/p* introduction of 20 *μg* of PRP on the 6th day after $A1C1_3$ injection); Experimental Group III (*i/p* introduction of 20 *μg* of PRP one hour prior to an injection of aluminum chloride). In the following series of experiment the dose-dependent effect of PRP on the content of the mentioned metalloproteins was investigated. The animals were sacrificed on the 12th day of the experiment.

Blood metalloproteins of prooxidant (cytochrome B_5 from the hemolysate, B_{558}I and B_{558}II from blood serum, B_{558}III and B_{558}IV from the erythrocyte membranes and suprol from the blood serum) and antioxidant action (Cu, Zn-SOD and catalase from hemolysate, ceruloplasmin (CP) and transferrin (TF) from the blood serum) were simultaneously obtained according to the method suggested by M. Simonyan [*Simonyan M., 1988*].

After separation of serum and erythrocytes and washing-up of the latter from various corpuscles and plasma with the use of physiological solution, cleaned erythrocytes were hemolyzed in water. The erythrocyte membranes were separated by centrifugation at pH=5.6 and the membrane proteins were solubilized by the 0.5% non-ionic detergent (a triton X-100 and nonidet P-40, Germany). After the dialysis of hemolysate, the serum and fractions of membrane proteins were dialyzed against water and after centrifugation the supernatants were analyzed by the ion-exchange chromatography on cellulose of KA-52 and DE-52 (Whatman, England) and on Sephadex DEAE A-50 (Pharmacia, Sweden). The enriched protein fractions were purified on biogels P-100 and P-150 (Reanal, Hungary). In procedure of obtaining and cleaning metalloproteins the centrifuges K-24, K-70, spectrophotometer "Specord M-40" (Germany) and glass columns with filters (2×20 *cm* and 2×80 *cm*) were used.

Superoxide dismutase (SOD) activity and superoxide-producing activity of suprol were determined in blood serum and rat tissues by nitrotetrazolium method [*Nishikimi M. et al., 1972*] by determination of inhibition percentage or increase (in case of suprol) in formation of formazan at the rate calculated per 1 *ml* of blood serum or 1 *g* tissue, correspondingly.

The catalase activity of fractions obtained from homogenates of brain tissues, hippocampus, liver and erythrocyte membranes was determined by the spectral method or permanganatometry with calculation of the amount of hydrogen peroxide cleavage in presence of the certain amount of fractions for 1 minute at 20°C. The specific activities were determined by calculation per 1 *ml* of serum (for suprol) and 1 *ml* of erythrocytes (for SOD and catalase).

The ferroxidase activity of ceruloplasmin in blood serum of rats was determined by the spectral method [*McGahan M. et al., 1989*]. The values of density of characteristic maximum optical absorptions were used as a quantitative index of blood metalloproteins.

The amount of transferrin was defined by a density value of the maximum optical absorption at 470 *nm*.

The isolation of metalloproteins of antioxidant action (Cu, Zn-COD and catalase) from brain was carried out by M.A. Simonyan's method [*Simonyan M., 1988*], with some modification. The tissues of brain, hippocampus and liver in the certain ratio were homogenated in 0.04 *M* potassium phosphate buffer at pH=7.4. A part of homogenates was left for MDA by the method of

Yu. Vladimirov and A. Archakov [*Vladimirov Yu., Archakov A., 1972*].

Further, the homogenates were homogenized in acetone. The acetonic powders were mixed with 0.04 M potassium phosphate buffer and incubated for one hour. The insoluble residue was removed by centrifugation, and supernatant was dialyzed against water. The dialyzed supernatants of these mixes were subjected to ion-exchange chromatography on cellulose DE-52. SOD and catalase from columns were eluted by the 0.03 *M* and 0.1 *M* potassium phosphate buffer, correspondingly.

The neuroimmunological investigations.

In order to study the PRP-immunomodulating influence on the content of cytokines (IL-1, IL-2, IL-6), prolactin and insulin-like growth factor-1 (IGF-1), the blood serum and supernatants prepared from the internal organs (an integral brain tissue, hippocampus, thymus, and liver) of mice were subjected to immune-enzyme analysis *(ELISA)*. For this purpose, the animals were decapitated and an integral brain, liver, and thymus were taken. After cleaning the brain from *dura mater* and vessels, as well as washing off blood by the cooled isotonic NaCl-solution the material was exposed separately on ice. The cortex of cerebral hemispheres and hippocampus were kept in liquid nitrogen freeze until the research was performed. Then the tissues were weighed and homogenated in the separation medium of 0.9%-solution of NaCl (1:10) in homogenizer "Potter S", with the speed 1500 *rot/min* within 1 minute. The homogenates were centrifuged at 3000 *rot/min* during 10 minutes at 4°C, and then the supernatants were selected.

The cytokines were determined by means of appropriate kits (DRG International Inc., USA) and their amount expressed in *pg/ml*. The content of prolactin was determined using corresponding kits (Microwell™ FSH EIA, USA). The amount of prolactin was expressed in *ng/ml*.

The radioisotope method for determination of insulin-like growth factor-1 (IGF-1).

IGF-1 in a cerebral cortex and blood serum of mice was determined with the help of the radioisotope counter "Gamma" (Russia–Ukraine) by means of IGF-1 I^{125} kit (Amersham Biotech., USA). The radioisotope activity was expressed in pulses per minute. The determined indices were inversely proportional to IGF-1 concentration, i.e. the high activity corresponded to low concentrations of a hormone, and vice versa.

Electronic microscopy studies in case of aluminum neurotoxicosis were carried out by Prof. A. Shakhlamov and Prof. A. Galoyan at the Institute of Human Morphology of the Russian Academy of Medical Science (Moscow). The investigations were carried out in 30 inbred male rats weighing 120 *g* arranging the following series: the I series: 3 rats (control 1) – intact, without injection; the II series: 2 rats (control 2), with daily subcutaneous injection of 0.2 *ml* 10%-solution of $AlCl_3$ within 3 days; the III series: 5 rats preliminary administered 0.5 *ml* of PRP-solution containing 0.1 *mg* of PRP, next day the same animals were injected 0.2 *ml* 10%-$AlCl_3$, on the 3rd day again 0.2 ml of 10%-$AlCl_3$, and on the 4th day 0.2. *ml* 10%-$AlCl_3$ as well.

The animals were sacrificed under the ethereal anesthesia, slices of tissues from hippocampus were fixed by 2.5%-solution on a cold glutaraldehyde on the cocadilate buffer (pH=7.3). After double wash-up within 24 hours the tissue slices were fixed by 1%-solution of osmium tetroxide on cocadilate solution (pH =7.3) during 2 hours, and it was washed out again by the buffer, and then dipped in alcohols of increasing concentrations. The embedding of tissue slices was carried out in an epon-araldite. Ultra-thin slices were studied using electron microscope JEH-100CX with the accelerating voltage 80 *kV*.

Results

1. *Corrective effect of PRP on the content of interleukins, prolactin and insulin-like growth factor-1 in the rat tissues at acute aluminum neurotoxicosis.*

Recently it was established that IL-1, IL-6 and TNF can be the provoking factors, which provide the synthesis of precursor of an amyloid protein in neuronal structures of the brain [*Shigematsu K., McGreer P., 1992; Muller T. et al., 1998; Wen T.*

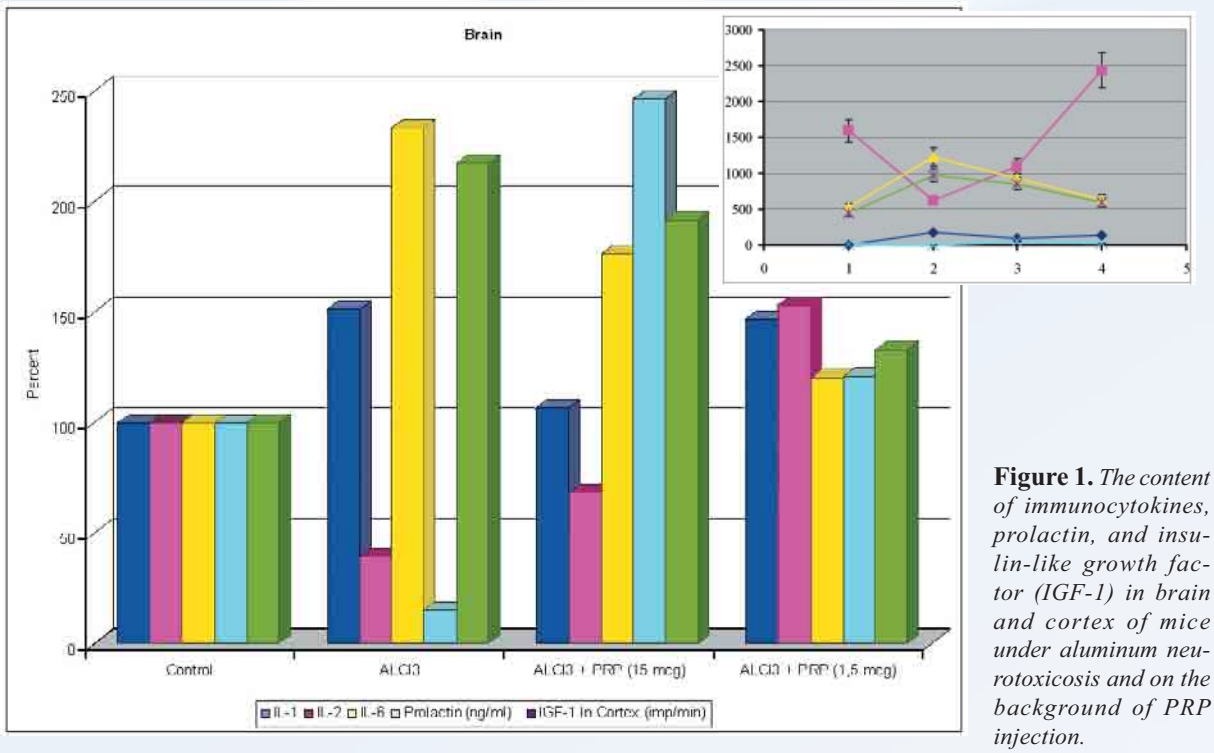


Figure 1. *The content of immunocytokines, prolactin, and insulin-like growth factor (IGF-1) in brain and cortex of mice under aluminum neurotoxicosis and on the background of PRP injection.*

et al., 1998; Zalsman S. et al., 1998]. It was also revealed that the synthesis of immunocytokines in neuronal structures under conditions of physiological activity of an organism is rather precisely balanced due to secretion of growth hormones by an adenohypophysis referring also to prolactin and insulin-like growth factor-1 (IGF-1).

As indicated by results of the performed immune-enzyme analysis, after administration of $A1C1_3$ the trace amounts of IL-1 are determined in the blood serum of mice (Figure 2), while IL-1 was not revealed in control group animals. After the single PRP injection the IL-1 level in blood of mice with aluminum neurotoxicosis raised appreciably (more than twice). In supernatant prepared from the thymus homogenate of the Experimental Group I mice (with aluminum neurotoxicosis), there were determined only the trace amounts of mentioned cytokine, while the PRP injection was accompanied by the tendency directed toward normalization of IL-1 content. However, the indices of IL-1 in animals of the Experimental Group II were 1.3 times lower than the similar parameters in intact animals. In liver of animals with the aluminum toxicosis the contents of IL-1 decreased, while the PRP injection resulted in observable increasing of its level. IL-1 was revealed in trace amounts in brain homogenates of control mice (Figure 1).

In the brain supernatant the non-significant amount of IL-1 was determined in case of aluminum chloride injection. Under administration of PRP the level of IL-1 in a brain tissue lowered almost twice in comparison with the Experimental Group I. IL-1 was not revealed in hippocampus of animals from either control or experimental groups.

The content of IL-2 in blood serum, liver, and brain of mice with aluminum neurotoxicosis sharply decreased (more than two-fold). The administration of 15 *μg* PRP was accompanied by a various degree of IL-2-normalization. In thymus of mice of Experimental Group I, in

comparison with a high level of IL-2 in intact animals, the sharp decrease of its content (more than 2-fold) took place. The PRP administration was accompanied by the increase of IL-2 level in thymus (more than three times in comparison with the Experimental Group I), however its content was not normalized: it was six times lower than the control indices.

The results of IL-6-analysis represent a certain interest. The sharp decrease of its content took place in serum, thymus (Figure 3) and liver of animals of the both experimental groups, however in hippocampus and integral brain (Figure 2) of animals with aluminum neurotoxicosis and under conditions of PRP injection a rather high level of IL-6 was revealed greatly exceeding an initial control level, accordingly more than 2 and 1.5 times.

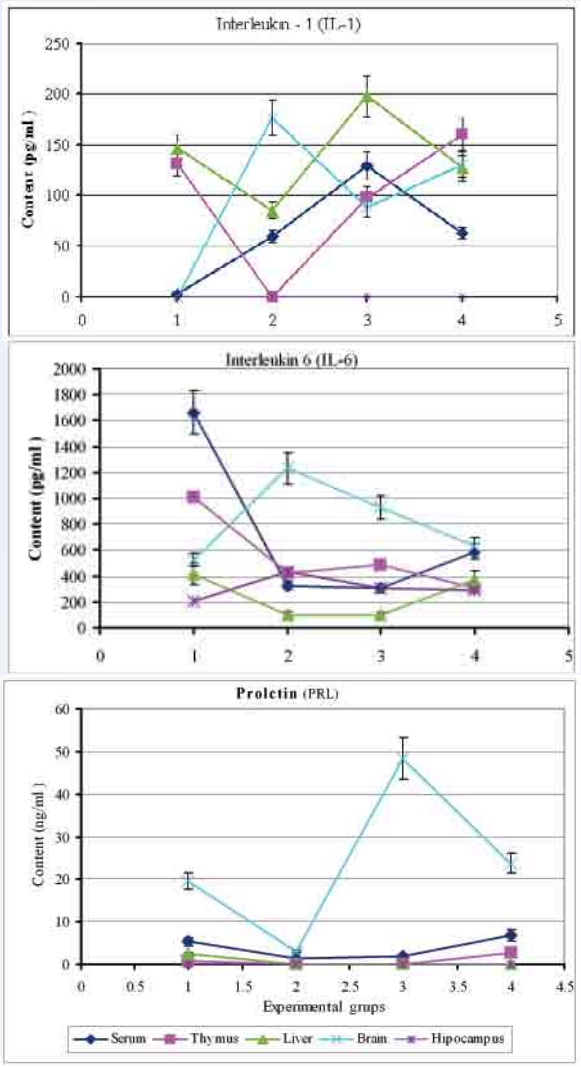


Figure 2. *Quantitative changes of IL-1, IL-6 and prolactin in mice brain, hippocampus and inner organs (thymus, liver and blood serum) at the aluminum neurotoxicosis and on a background of PRP injection.*

It is necessary to note that the adequate effects of endogenously active biological compounds are caused by their low concentrations in blood, liquid media of an organism, since just the same doses are as much as possible approached to the concentrations of biologically active substances, secreted in conditions of physiological functioning of an organism.

As shown by the results of immune-enzyme analysis, PRP at both dose levels (15 and 1.5 μg) rendered similar effects on the level of IL-1 in thymus and liver. In case of administration of PRP small doses, the indices of IL-1 in blood serum were similar to those in a Control Group. It is necessary to note that the injection of precisely low dose of neuropeptide was accompanied by normalization of IL-2 level in thymus. One cannot exclude the possibility that similar concentrations of a preparation are really the most effective ones, since even under conditions of aluminum intoxication they provide an optimal level of IL-2 in the central organ of immunogenesis. For the benefit of this circumstance testified also a fact that in case of injecting rather high doses of PRP there was observed only a tendency directed toward the recovery of its level, in spite of the fact that in the given concrete case there was registered some neuroprotective effect as well.

Administration of high concentrations of PRP was not accompanied by shifts in content of IL-2 in liver, while the low doses of PRP caused 2-fold increase of IL-2 level in liver, as compared with the Control Group. In this case, the clear tendency was observed to be directed towards normalization of the mentioned index in comparison with the Control Group. It is necessary to emphasize that whereas injection of high concentrations PRP was accompanied

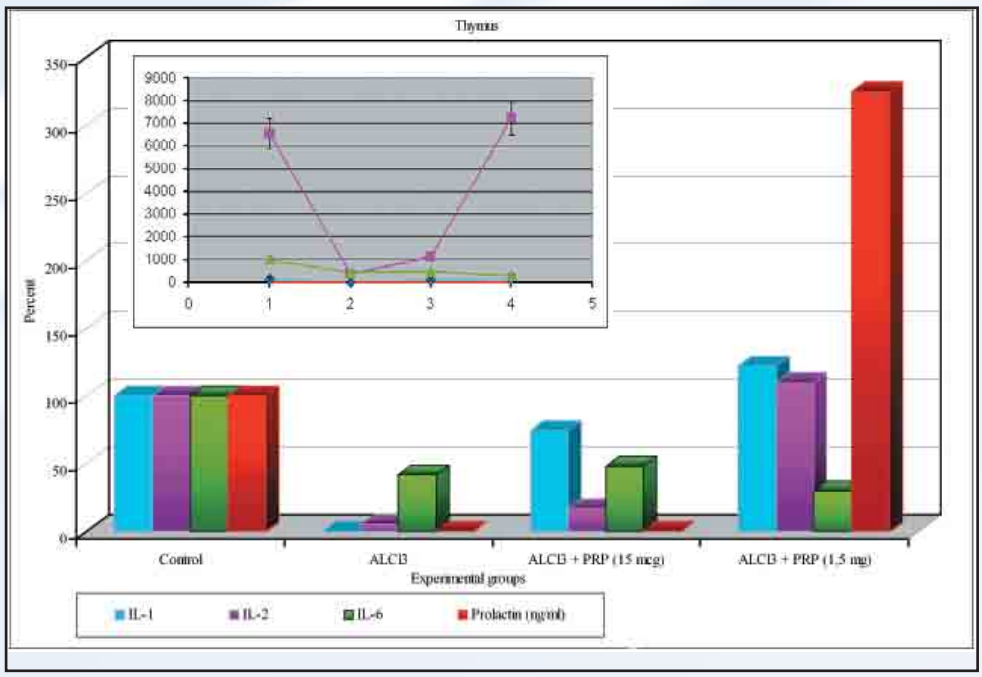


Figure 3. *The content of cytokines (IL-1, IL-2 and IL-6) and prolactin in thymus of mice in case of aluminum neurotoxicosis and on a background of PRP administration.*

by normalization of the IL-2-content in blood serum, the low concentrations of PRP resulted in its significant increase.

Since IL-1 secreted in a brain tissue, represents itself an inductor of synthesis of amyloidal protein precursor in the initial neurons, it is possible to suggest that PRP at the level of neuronal structures partly inhibits the processes of synthesis of the non-typical neuronal proteins. This is testified by a circumstance that IL-1 level in brain tissue decreased 2 times under the conditions of PRP injection. Results of IL-2-content determination also testify to the benefit of its high biological activity. As shown, in all investigated organs the tendency toward the normalization of its content was precisely traced. The possibility that immunomoduling action of PRP is also mediated by stimulation of the IL-2 synthesis in the lymphoid cells of thymus is not excluded (Figure 3). The similar assumption is acceptable as well in relation of neuronal cells, as the similar tendency was observed in the brain tissue as well.

The results of IL-6 content determination present a special interest. Administration of aluminum chloride was accompanied by sharp reduction of its content in blood serum, thymus and liver (Figure 2). PRP in a dose of 15 μg, probably, did not possess a modulating action on IL-6 in all investigated tissues, whereas the injection of low doses of neuropeptide is accompanied by the observed more than 3-fold increase of IL-6 level in liver, as compared with the Experimental Group I.

Therefore, it is exactly possible to suggest that the cells of monocytic-and-phagocytic series, and, first of all, Kupffer's cells of liver can act as a source of IL-6-synthesis. This, in particular, is testified by the absence of modulator effect of a preparation, i.e. the directed synthesis of the same IL-6 in thymus under conditions of aluminum intoxication.

It is necessary to note that in an integral brain and, especially, in hippocampus of model animals the level of IL-6 raised more than twice. Taking into account the circumstance that hyperproduction of *in situ* secreted IL-1 and IL-6 promoted a synthesis of atypical amyloidal proteins of the neuronal cells in case of AD (IL-1 possessed direct stimulating effect, but the effect of IL-6 is realized mediated, by means of inhibition of IGF-1-synthesis), it is possible to conclude with certain confidence that the model used in our

research, as a whole reproduces the most essential immunopathogenetical parts of the development and course of AD in humans.

It is especially necessary to note that PRP injection promoted the observed reduction of both IL-1 and IL-6 levels in brain tissue in comparison with the Experimental Group I, and thus, to a certain extent, testified to the important modulating role of the studied neuropeptide in processes of neuroendocrine regulation of CNS and its immunomodulatory role in conditions of pathology, as well as in concern of inhibiting the cascade of regional immunopathological reactions finally causing the synthesis of abnormal peptides in case of neurodegenerative diseases of CNS, including AD and Parkinson's disease.

One cannot exclude the possibility that directed immunomodulating effect of PRP is realized also by neuroendocrine mechanisms. In this respect, the special place should be given to the hypophyseal prolactin, which, both in CNS and in the organs of immunogenesis has possessed immunomodulatory action causing the directed synthesis of the wide spectrum of cytokines.

The obtained results testified that the immunomodulatory effect of prolactin on periphery was not revealed. The dependence between the low level of prolactin in blood serum, thymus and liver was revealed in all investigated groups. It is especially necessary to emphasize that in brain of mice with aluminum neurotoxicosis the content of prolactin (Figure 1) also decreased (more than 6-fold), however the PRP injection resulted in the significant increase of its content, 2.7 times as much exceeding the control level. Thus, it is possible to assume that PRP possessed the modulating action on the neuroendocrine structures of hypophysis, directly and/or indirectly stimulating the directed synthesis of prolactin.

As obvious from Figure 1, upon injection of $A1C1_3$-solution the content of IGF-1 in the cerebral cortex reduced approximately 2.2 times in comparison with the norm. Under injection of PRP low doses (1.5 μg) the content of IGF-1 considerably rose in comparison with group of animals receiving the aluminum chloride. The tendency to normalization was mainly observed in case of application of small doses of hypothalamic neuropeptide. The results of immune-enzyme analysis revealed no certain changes in content of IGF-1 in blood serum of animals of experimental groups in comparison with the control group. It is not excluded that the revealed PRP-effects in respect of neuroprotective and immunomodulatory properties realization on periphery are mediated by the processes of neurohormonal activation of the CNS, to which indirectly testified the obtained data on changes in IGF-1 level.

2. Effect of PRP on metalloproteins and MDA levels at acute aluminum neurotoxicosis.

The purpose of this series of experiments was simultaneous determination of quantitative shifts of pro- and antioxidant metalloproteins in rat blood, cytochrome B_5, SOD, catalase and MDA in an integral brain, hippocampus and in liver of animals in case of acute aluminum neurotoxicosis (AAN) and under the effect of various doses of PRP medicinal purposes.

In the first series of the study, white pubertal rats (180-200 *g*) were used. Animals are divided into three groups by 10 animals in each: the animals receiving *i/p* 0.2 *ml* of the physiological solution on the first and third day of experiment (Control Group); the animals receiving *s/c* 0.2 *ml* 10% $A1C1_3$ in a similar mode (Experimental Group, EG-1) and the animals receiving both $A1C1_3$ in the mentioned mode and *i/p* 15 μg PRP on the sixth and ninth day of experiment (EG-2).

In the second series of experiment the content of injected physiological solution (K^1), $A1C1_3$ (EG^1-1) and PRP (EG^1-2) was increased 2 times. Similar conditions of the experiment were used in both series.

It was established that in case of aluminum chloride double injection the levels of both prooxidant and antioxidant metalloproteins of blood increased except suprol, the level of which decreased almost twice (Table 1). At the same time in EG-1 the marked increase of serum cytochrome B_{558} and, especially, catalase levels was observed in the soluble fraction of erythrocytes

Table 1.

Relative changes of endogenous levels of metalloproteins (in %) and malone dialdehyde (MDA) in blood and tissues under acute aluminum neurotoxicosis and on the background of PRP at dose of 15 *mcg*

Metallopreteins	EG-1	EG-2
Blood		
Cytochrome B_5	63.3 ± 3.87	25.1 ± 1.16
Σ cytochromes B_{558} from erythrocyte membranes	36.5 ± 1.83	10.1 ± 0.54
Σ cytochromes B_{558} -I and B_{558}-II	88.9 ± 4.24	50.1 ± 3.57
Cytochrome B_{558}-III	71.3 ± 3.97	27.7 ± 1.86
Cytochrome B_{558}-IV	61.1 ± 3.68	32.2 ± 2.56
Suprol	-52.4 ± 3.63	-26.7 ± 1.72
O^-_2-producing activity of suprol	10.0 ± 0.52	no changes
Ceruloplasmin	25.1 ± 2.01	10.0 ± 0.38
Transferrin	67.1 ± 2.75	40.2 ± 2.67
SOD	54.1 ± 3.08	20.9 ± 1.05
Catalase	250 ± 19.05	125.2 ± 13.62
Brain		
SOD	16.5 ± 0.91	9.1 ± 0.56
Catalase	16.8 ± 1.11	5.3 ± 0.26
Malone dialdehyde	11.4 ± 0.6	6.31 ± 0.32
Hippocampus		
SOD	-21.1 ± 1.49	-6.7 ± 0.48
Catalase	-75.0 ± 4.64	-24.0 ± 1.6
Malone dialdehyde	32.2 ± 1.89	10.1 ± 0.37
Liver		
SOD	-32.4 ± 1.13	-16.3 ± 0.77
Catalase	-45.7 ± 2.47	-78.6 ± 4.47
Malone dialdehyde	63.4 ± 2.77	26.6 ± 1.83

Note: $P<0.05$; $n=10$

that testified to increase of hydrogen peroxide level in blood. The increase of serum cytochromes B_{558}-I and B_{558}-II (especially B_{558}-I from the blood serum of rats) was simultaneously observed. The increase of cytochrome B_{558} level testified to changes of microstructure of erythrocytic membranes in case of aluminum neurotoxicosis. In these conditions, PRP rendering the anti-stress effect positively acted in case of AAN, leading the mentioned indices close to norm, though in this case also the level of catalase in blood remained higher than in norm, while in a hepatic tissue it was significantly reduced.

Double elevation of administered compounds caused slightly different changes in the rat blood endogenous metalloproteins in the EG'-1 (Table 2), where the level of total fraction of the erythrocytic membrane cytochrome B_{558} raised by 75%, though the general level of cytochromes B_{558}III and B_{558}IV in EG-1 and EG^1-1 were nearly the same. The activity of antioxidant enzymes SOD and catalase, as well as content of MDA in blood and brain in case of an acute aluminum neurotoxicosis had identical direction, whereas in liver they differed a little.

During the increase of prooxidant metallo-

Table 2.

Relative changes of endogenous levels of metalloproteins (in %) and malone dialdehyde (MDA) in blood and tissues under acute aluminum neurotoxicosis and on the background of PRP at dose of 30 *mcg*

Metalloproteins	EG-1'	EG-2'
Blood		
Cytochrome B_5	70.2 ± 4.97	46.4 ± 2.24
Σ cytochromes B_{558} from erythrocyte membranes	73.4 ± 4.78	26.3 ± 1.83
Σ cytochromes B_{558} -I and B_{558}-II	75.1 ± 3.77	27.5 ± 1.04
Cytochrome B_{558}-III	42.2 ± 2.62	48.8 ± 2.66
Cytochrome B_{558}-IV	80.7 ± 3.81	-23.1 ± 0.59
Suprol	-52.9 ± 2.42	-24.6 ± 1.5
O^-_2 producing activity of suprol	3.3 ± 0.14	15.0 ± 0.96
Ceruloplasmin	66.7 ± 2.19	61.6 ± 3.36
Transferrin	113.0 ± 8.24	108.0 ± 5.98
SOD	29.5 ± 2.41	8.8 ± 0.45
Catalase	95.4 ± 7.76	74.5 ± 3.94
Brain		
SOD	no changes	no changes
Catalase	no changes	no changes
Malone dialdehyde	23.2 ± 1.45	-11.0 ± 0.38
Hippocampus		
SOD	no changes	no changes
Catalase	no changes	no changes
Malone dialdehyde	33.6 ± 2.23	25.1 ± 1.12
Liver		
SOD	no changesй	no changes
Catalase	-23.1 ± 0.84	-38.5 ± 2.41
Malone dialdehyde	33.4 ± 2.43	16.6 ± 1.1

Note: P<0.05; n=10

protein levels (in case of AAN) an organism, apparently, secrets the appropriate level of antioxidant metalloproteins, i.e. it tries to resist to development of oxidative stress. As a result of the carried out experiments it was established that PRP in a therapeutic mode as a whole renders the corrective action on the process of normalization of a level of metalloproteins and MDA in blood, integral brain, hippocampus and liver. Herewith, the degree of normalisation effect does not depend proportionally on the amounts of injected aluminum chloride and PRP.

Protective and corrective actions of PRP on content of metalloproteins and MDA.

The purpose of this series of experiments was to study at the neurodegeneration model the regulating influence of PRP on the content of pro- and antioxidant metalloproteins in different modes (prophylactic and medicinal ones).

White inbred rats were divided into the following groups: by 10 rats in each: the I group received *s/c* twice (at the first and third days) 0.2 *ml* of 10% solution of aluminum chloride, the II group – 20 *μg* of PRP 1 *h* prior to the first injection of aluminum chloride, the III group – 20 *μg* of PRP 6 days after the second injection of aluminum

Table 3.

Comparative changes in the endogenous levels of metalloproteins (in %) and MDA in rat blood and tissues in case of the acute aluminum neurotoxicosis and under the action of PRP in different modes (prophylactic and therapeutic ones).

Metalloproteins	EG-I	EG-II	EG-III
Blood			
Cytochrome B_5	63.4 ± 4.28	53.3 ± 2.8	48.1 ± 2.79
Σ of Cytochromes B_{558} from the membranes of erythrocytes	36.5 ± 1.39	28.6 ± 2.06	15.7 ± 1.2
Σ of Cytochromes B_{558}-I and B_{558}-II.	88.9 ± 4.59	73.6 ± 5.81	56.4 ± 3.46
Cytochrome B_{558}-III	71.3 ± 4.37	55.9 ± 2.82	44.8 ± 3.26
Cytochrome B_{558}-IV	61.1 ± 3.79	49.9 ± 2.80	38.7 ± 1.63
Suprol	-52.4 ± 2.47	-39.6±1.35	-32.8 ± 1.64
O^-_2-producing activity of suprol	24.5 ± 0.86	10.1 ± 0.45	12.2 ± 0.60
Ceruloplasmin	25.1 ± 1.6	19.1±1.29	16.2 ± 0.89
Transferrin	67.1 ± 4.11	58.3 ± 2.95	47.5± 1.68
SOD	54.1 ± 3.67	41.3 ± 2.05	36.4 ± 2.22
Catalase	350.8 ± 27.89	287.5± 12.89	205.4 ± 15.4
Brain			
SOD	16.5 ± 0.97	14.2 ± 1.2	9.1 ± 0.54
Catalase	5.1 ± 0.37	4.9 ± 0.24	5.5 ± 0.29
Malone dialdehyde	11.4 ± 0.78	9.5 ± 0.61	5.1 ± 0.38
Hippocampus			
SOD	-21.1 ± 0.89	-16.3 ± 1.32	-12.1 ± 0.66
Catalase	-75.1 ± 4.49	-61.9 ± 25.07	-38.7 ± 2.67
Malone dialdehyde	32.3 ± 1.23	21.4 ± 1.25	17.5 ± 0.69

Note: $P<0.05$; $n = 6$

chloride. Control animals received 3 times 0.2 *ml* of 0.9%-solution of NaCl. It was established that double injection of 10% solutions of aluminum chloride caused an increase of both pro- and antioxidant metalloproteins content in blood of rats.

Suprol makes an exception, the amount of which was sharply reduced. The content of antioxidant metalloproteins was increased simultaneously, among which the catalase activity grew 3.5 times (Table 3). Unlike the blood, the reduction of SOD and catalase activity was observed in hippocampus (Figure 5). As to the brain, the special changes in activity of these enzymes were not established in case of aluminum neurotoxicosis, however a certain tendency to their increase was observed.

The preliminary (prophylactic) injection of the peptide (II group) in the certain degree prevented the development of metabolic disorders, which were found out in the I group of rats. The levels of both blood pro- and antioxidant metalloproteins were reduced. Similar changes were observed in an integral brain, as well as in hippocampus. PRP appeared more efficient in research in case of its application with a therapeutic purpose (III group).

Administration of PRP after 6 days following the last injection of aluminum chloride rendered more expressed normalizing effect on a lipid peroxidation (LPO) level and the content of metalloproteins both in blood and tissues (Figure 5).

Thus, PRP in the presented experiment manifested itself as a regulator with a broad action spectrum.

3. PRP as a neuroprotector modulating a degree of oxidative damage of tissues in case of the chronic aluminum neurotoxicosis

The purpose of this series of experiments was determination of quantitative characteristics of oxidative damage of components of blood and other tissues of rats at the 3-month chronic aluminum neurotoxicosis (CAN) and under the influence of neuroactive proline-rich synthetic peptide.

CAN was caused by per oral introduction of the 10% $AlCl_3$ solution during three months. The animals were distributed into three groups: the first group (control one) received 0.2 *ml* of a physiological solution *i/a* each 15 days; the second group received with the drinking water the 10%-chloride of aluminum and 0.2 *ml* of physiological solution *i/a* in each 15 days; the third group of animals instead of physiological solution was

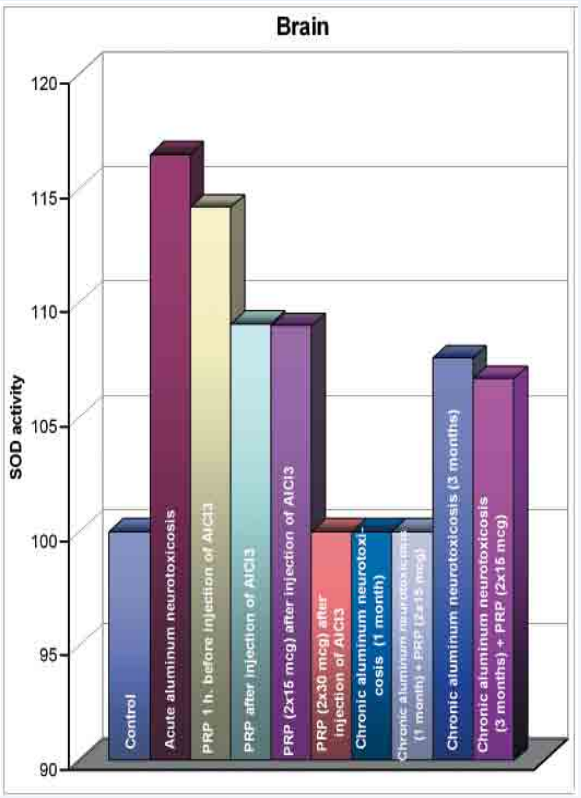


Figure 4. *The effect of PRP on SOD activity in rat brain in case of an aluminum neurotoxicosis.*

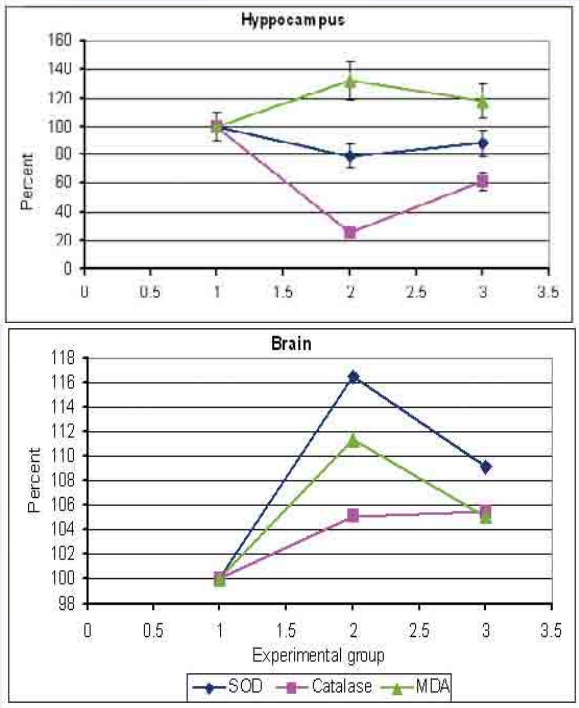


Figure 5. *Quantitative shifts of antioxidant metalloproteins (SOD and catalase) and MDA in brain and hippocampus in case of acute aluminum neurotoxicosis.*

administered 20 *μg* of PRP in 0.2 *ml* of physiological solution – on a background of the chronic aluminum neurotoxicosis.

As a result of the 3-month CAN (Figure 4), the content of pro- and antioxidant metalloproteins, as well as the level of lipid peroxidation in the investigated rat tissues changed in different ways ambiguously. The reduction of the serum cytochromes $B_{558}I + B_{558}II$ and suprol in blood occured on a background of the increased level of cytochromes B_5, B_{558} III, B_{558} IV, though the O_2^--producing activity of suprol increased. The activity of the SOD-soluble fraction of erythrocytes decreased that was compensated by the significant increase of catalase activity. It is obvious that the total increase of antioxidant metalloproteins level in case of CAN due to the activation of catalase more than three times surpassed the content of blood pro-oxidant metalloproteins. In case of CAN this fact testified to disorder of physiological balance between the producing and utilizing O_2^-metalloproteins.

The low level of superoxide radicals in the

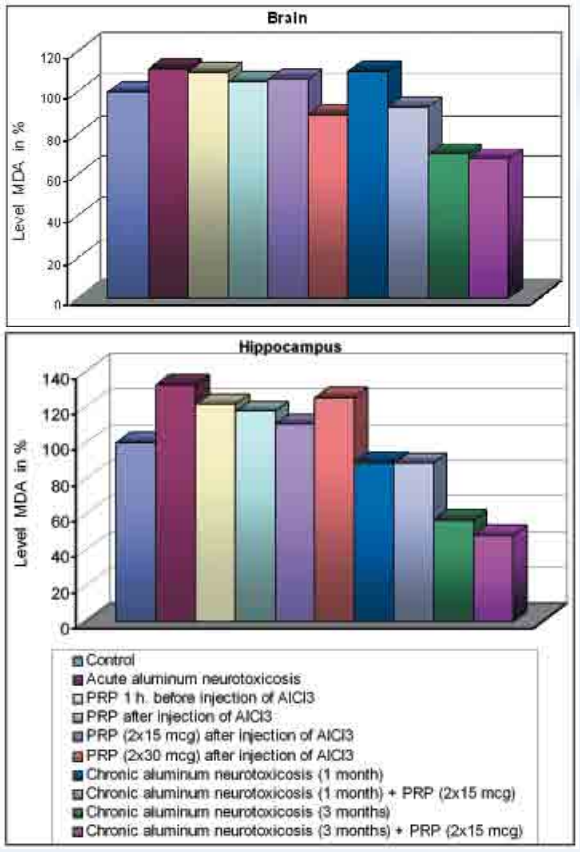


Figure 6. *The influence of PRP on the MDA-level in rat brain and hippocampus at aluminum neurotoxicosis*

blood can promote the delay of the redox processes proceeding with participation of O_2^-. On the other hand, the marked increase of catalase activity in case of CAN testified to an increase of H_2O_2-level, which, probably, was formed in the other way, but not for the account of enzymatic dismutation of O^-_2.

In case of CAN the Cu, Zn-SOD activity in the tissues of an integral brain, hippocampus, liver, and heart was not subjected to the appreciable changes. As to catalase, its activity reduced in liver, while in the other organs, and in the first place in an integral brain, it increased. The content of cytochrome *c* slightly raised in liver, being reduced both in the heart and brain tissues that testified to the certain disorders of a normal process of mitochondrial oxidation in case of CAN. The content of MDA, being reduced in liver, heart, and hippocampus, in an integral brain, on the contrary, grew (Figure 6). Taking into account hyperactivity of catalase in an integral brain, it is not excluded that the level of H_2O_2 rises in the other way: not due to lipid peroxidation.

Under the influence of PRP on a background of CAN, the main blood indices approached the norm, except ceruloplasmin and transferrin, the values of which were rather lower in comparison with the control. It is necessary to note that under PRP action the MDA content in brain tissue sharply decreased, achieving a level below the control one.

One-month CAN in white rats was caused by the oral submission of 10% solution of aluminum chloride with drinking water within 30 days. The animals were divided into three groups: the animals received 0.2 *ml* of physiological solution subcutaneously, in each 15 days of experiment (control group); animals in the experimental group EG-1 received 10% $AlCl_3$ with drinking water, simultaneously with 0.2 *ml* of physiological solution in each 15 days. Animals of EG-2 group received $AlCl_3$ just in the same regimen as in EG-1, but instead of physiological solution they received 20 *μg* of PRP (in 0.2 *ml* of physiological solution) also two times.

In comparison with control indices, in EG-1 in majority of cases there was observed the decrease of levels of cytochrome B_5, cytochromes B_{558} I and B_{558} II, and suprol, – at the average by 25-54%, as well as levels of ceruloplasmin and transferrin, though the SOD-level practically did not change. On this background, the level of cytochrome B_{558} increased, and both the level of catalase and O_2^--producing activity of suprol remained within the limits of the control. In this group, as a result of one-month CAN, the decrease not only of SOD level and catalase, but also that of lipid peroxidation level in the hepatic tissue homogenates was observed. Herewith, if an activity of SOD and catalase fractions in an integral brain did not change, then the MDA level grew insignificantly (Figure 6). In hippocampus the level of SOD decreased insignifi-

cantly and the catalase level did not change; there was also observed a certain reduction of MDA level (Figure 6).

The decrease of blood levels of cytochrome prooxidant metalloproteins B_5, B_{558} I and B_{55} II, as well as suprol level was to some extent compensated by the increase of erythrocytic membrane cytochrome B_{558} levels. Cytochromes B_{558}I-IV were capable to produce O_2^- by means of transfering NADPH-electron to the molecular oxygen, as it was peculiar to the other known cytochrome B_{558} localized, for example, in membranes of phagocytized leukocytes.

The levels of cytochrome B_5, suprol, ceruloplasmin, catalase tended to norm in blood under the influence of PRP in EG-2, though the levels of cytochromes B_{558} I and B_{558} II continued to fall, and the levels of erythrocytic membrane cytochrome B_{558} were raised. The levels of SOD, catalase and O^-_2-producing activity of suprol practically did not change. PRP in liver brought to the certain increase of catalase activity and normalization of SOD level, at the same time the MDA level significantly fell. The level of SOD and catalase in the brain tissue in EG-2 did not change, whereas the content of MDA was slightly decreased. The SOD-activity in hippocampus in the same group raised, at the same time the changes in activity of catalase were not observed, and the MDA level continued to be reduced (Figure 6).

In case of one-month CAN the disorder of physiological balance in activity of key antioxidant enzymes and lipid peroxidation in liver, brain and hippocampus actually occurred in accordance with the change of levels of anti- and prooxidant system of blood. In particular, the notable changes of levels of antioxidant system and LPO were not observed in these tissues. Probably, it was connected with some decrease of O_2^- level, which, in turn, could demonstrate toxicity with regard to the mentioned biosystems and initiate a LPO process of biosystems.

Probably, one of the mechanisms of PRP action was correction of pro-oxidant metalloproteins-cytochromes B_{558}I-IV levels, which were the NADPH-dependent O_2^--producing systems located in blood serum (cytochromes B_{558} I and B_{558} II) and in erythrocyte membranes. It is essential that B_{558} cytochromes of the similar type, which were located in membranes of B- and T-lymphocytes and phagocytized leukocytes neutralized the antigens producing O^-_2 (and their derivatives) that was considered as one of mechanisms of the immune protection of an organism. Besides, serum cytochromes B_{558} I and B_{558} II demonstrated a high resistance in respect of H_2O_2; moreover, they protected blood biosystems from the damaging effects of H_2O_2. Probably, the immunomodulating effect of PRP was caused also by the regulation of cytochrome B_{558} levels in case of CAN.

It is possible to make conclusion that in case of the chronic aluminum neurotoxicosis there is a certain disbalance between the content of metalloproteins and regulators of metabolism of oxygen active forms, which to the certain degree is probably regulated by the antioxidant mechanism of PRP action.

4. *Ultrastructural changes in neurons in case of aluminum neurotoxicosis: effects of PRP.*

In 1980, H. Wisniewski and co-workers demonstrated by means of electronic microscopy that the presence of spiral neurofilament pair cells in brain neurons is typical for AD. G. Polyakova and V. Shakhlamov [*Polyakova G., Shakhlamov V., 1990*] in culture of neurons of neurinoma of the Gasser's ganglion (NGGR-1) investigated the action of aluminum chloride in animals in case of experimental aluminum neurotoxicosis. Further studies were performed by V. Shakhlamov and co-workers [*Shakhlamov V. et al., 2000*] who studied the chromosomal apparatus and chromatids. Data of these investigations showed that the genetic apparatus of nervous cells in presence of aluminum chloride underwent no changes. It only broke the assembly of a spindle fusion and cytoplasmic complex of microtubules. Aluminum penetrated into cells (nervous and epithelial ones) through the ionic channels in plasmolemma. This phenomenon was named cytothesaurismosis, and penetration of Al-ions through nucleus pores into the nucleoplasm became known as nucleothesau-

rismosis [*Avtsyn A. et al., 1991*].

It was established that fluorine prevents an increase of aluminum amount in the organism of experimental animals [*Kortus J., Mayer J., 1969*] According to data of scientific literature, in case of aluminum influence on a cell there occurs a combination of different types of transferrin (TF) C_1 and C_2 with a certain loss of ability to capture and transfer the iron ion. The last circumstance, in turn, causes an intensification of free radical oxidative processes with participation of oxygen active forms (OAF) in various bioformations of an organism, including a brain.

The oxidative damage of lipids, carbohydrates, proteins, and DNA results in inflammatory processes and death of neurons in case of AD [*Kryzhanovsky G., 1995*]. Changes of OAF level in neurons under the influence of aluminum is accompanied by the appropriate histologic changes of these biosystems. The complex study on histologic changes of a nervous tissue, as well as shifts of the levels of metalloproteins (which are regulators of OAF metabolism in case of aluminum neurotoxicosis), as well as the condition of lipid peroxidation will expand the possibility of revealing molecular mechanisms of aluminum neurotoxicosis as a possible model of neurodegeneration.

The purpose of the present research was to visualize the localization of aluminum molecules in different mammalian organs (hippocampus, liver, kidneys, small intestine, and stomach) and under the influence of PRP at the ultrastructural level.

According to the electronic-and-microscopic research data, in 1 *hour* after *i/a* introduction of 0.2 *ml* sterile 10% $AlCl_3$ solution to the experimental animals, the neurons with a diffuse distribution of several particles of aluminum were observed in the cytoplasm of hippocampus neurons. The neurons with small granules of the granular electron dense particles were simultaneously found out, and in some of them, besides these bodies, the diffuse penetrations of fine-grained particles through a nuclear membrane were found out in cytoplasm as well (Figure 7 B).

A fact is worthy of note: the complete impregnation of some myelin nerve fibers with aluminum-containing granules was observed (Figure 7 C).

After preliminary PRP introduction to animals, neither neurons with an edema of cytoplasm and nucleus, nor neurons containing the electron dense granules and large inclusions in cytoplasm, as well as just in the nucleus itself were found in hippocampus (Figure 7E).

The obtained data testify that aluminum is capable to penetrate into cytoplasm and nuclei of cells, where it comes into contact with DNA and RNA, and in case of PRP preliminary introduction $AlCl_3$ is not revealed in hippocampus neurons, pyramidal cells of a cortex of cerebrum, as well as in epitheliocytes.

It is known that on an internal surface of cell membranes the guanosine triphosphate (GTP)-bound proteins are located carrying out a role of conjugating factors in the system of transferring information “incoming” to the endocellular targets from the growth factors (peptides) and hormones. Herewith, the balance between the lipid peroxidation and the activity of antioxidant systems is broken that is characteristic for the oxidative stress.

Thus, it was possible to visualize some morphological manifestations of oxidative stress in rat hippocampus neurons in case of aluminum neurotoxicosis and with the use of electron microscopy method simultaneously to confirm the neuroprotective action of PRP in case of the aluminum lesion of neuronal and epithelial structures.

5. Protective and corrective effect of PRP at the β-amyloid peptide induced morphological and neurophysiological changes in different structures of brain.

The purpose of presented research was revealing morphological and electrophysiological changes in different series of experiments in norm, under the conditions of intoxication by β-amyloid peptide (25-35) without the use of PRP, via its single injection 24-hours prior to intoxication and six days after intoxication.

As a result of carried out pathomorphological analysis, it was established that in groups of

sham-operated animals in area of hippocampus neurons had a normal appearance in the brain slices. In the cerebral cortex and in the hippocampus field CA1 the injections of isotonic solution of NaCl and sterile water caused small damages only in the area of direct introduction of the injection needle. Only in one case there was observed the significant neurodegeneration in the superior branch of cogged fascia that was evidently caused by introduction of water into the hippocampus.

A. Galoyan demonstrated that PRP got tightly combined with amyloid peptide (25-35) in the aqueous medium. At his suggestion, we have

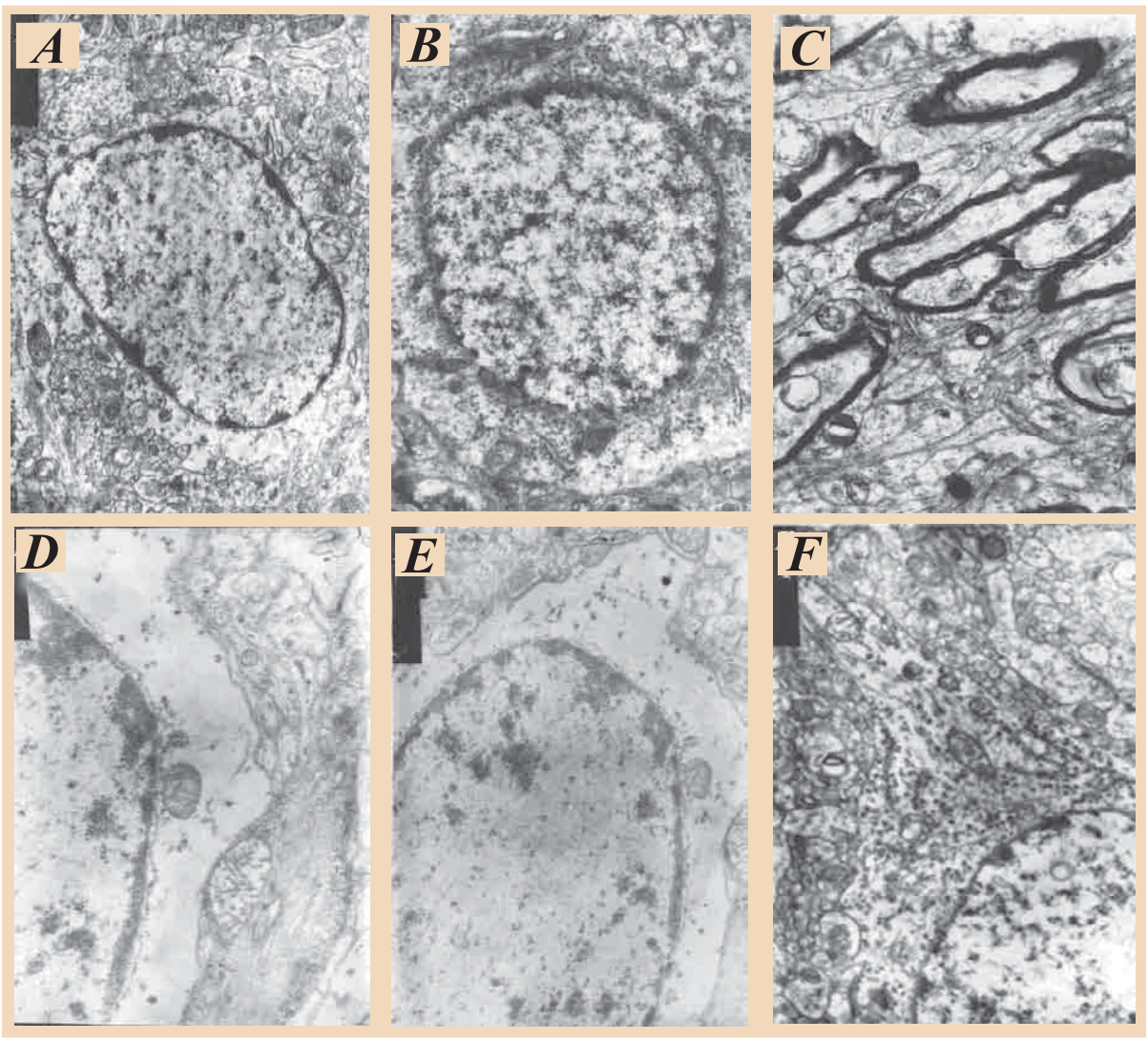


Figure 7. *The rat hippocampus neuron:*
A) Control; × 10000.
B) The rat hippocampus after triple injection of aluminum chloride, diffuse penetration of aluminum molecules through nuclear membranes; × 10000.
C) The rat hippocampus after triple introduction of aluminum chloride, aluminum molecules in myelin membranes of nerve fibers; × 10000.
D) The edematous neuron in 1 h after introduction of $AlCl_3$; × 20000.
E) Part of a nervous cell with an edematous cytoplasm and a nucleus; × 14000.
F) The rat hippocampus neuron after preliminary administration of PRP and triple injection of aluminum chloride; × 14000.

started the study of PRP action on the brain structures and its role in prevention of neurodegenerative processes.

In brain slices of animals administered βA (25-35), there was observed a significant neurodegeneration, mostly expressed in the place of direct hit of the needle, as well as in the nearby areas. In the damaged part of the CA1 field of hippocampus, we observed only the wrinkled cellular nuclei or the damaged cells. The neurodegeneration was accompanied by the expressed reaction of neuroglia.

The paraventricular nucleus cells of the rat hypothalamus under various experimental conditions are shown in Figure 8: A) paraventricular nucleus (PVN) cells of the intact rat hypothalamus; B) hypertrophied or wrinkled, degenerated PVN-cells of a rat subjected to injection by β-amyloid peptide; C) the hypothalamus PVN-cells of rat receiving a single PRP injection before introduction of βA (25-35). As it is seen, the sizes and the form of cells coincide with those parameters in norm (C).

The morphological research demonstrating the hippocampus cells of the right-brain of control rats (subjected to injection by the sterile water) was carried out as well. Almost all gyri of the hippocampal complex CA1, the part of CA2 (the superior gyrus), CA4, and lateral gyri of the cogged fascia were well illustrated. The significant degeneration of cells of almost all areas of hippocampal complex (the right-brain) was revealed in rats with an injection of βA (25-35). The remaining cells were wrinkled and well highlighted in light pericellular space. As obvious from results of the performed research, a single PRP introduction to rats before an injection of βA (25-35) rendered no appreciable effect on the neurodegenerative processes in the hippocampal complex cells.

The results of electrophysiological research were presented on frequency violation of the spike activity of single hippocampus neurons in case of a single and frequency stimulation of the entorhinal cortex (EC) of the same hemisphere of a brain in norm, in case of intoxication by the βA

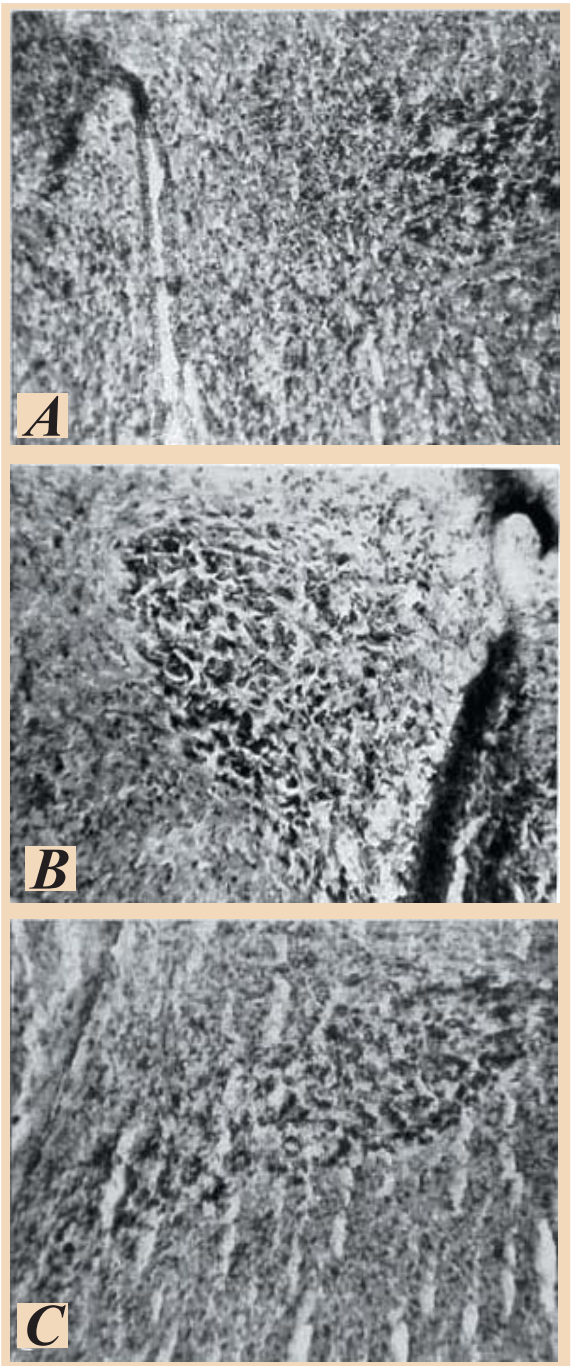
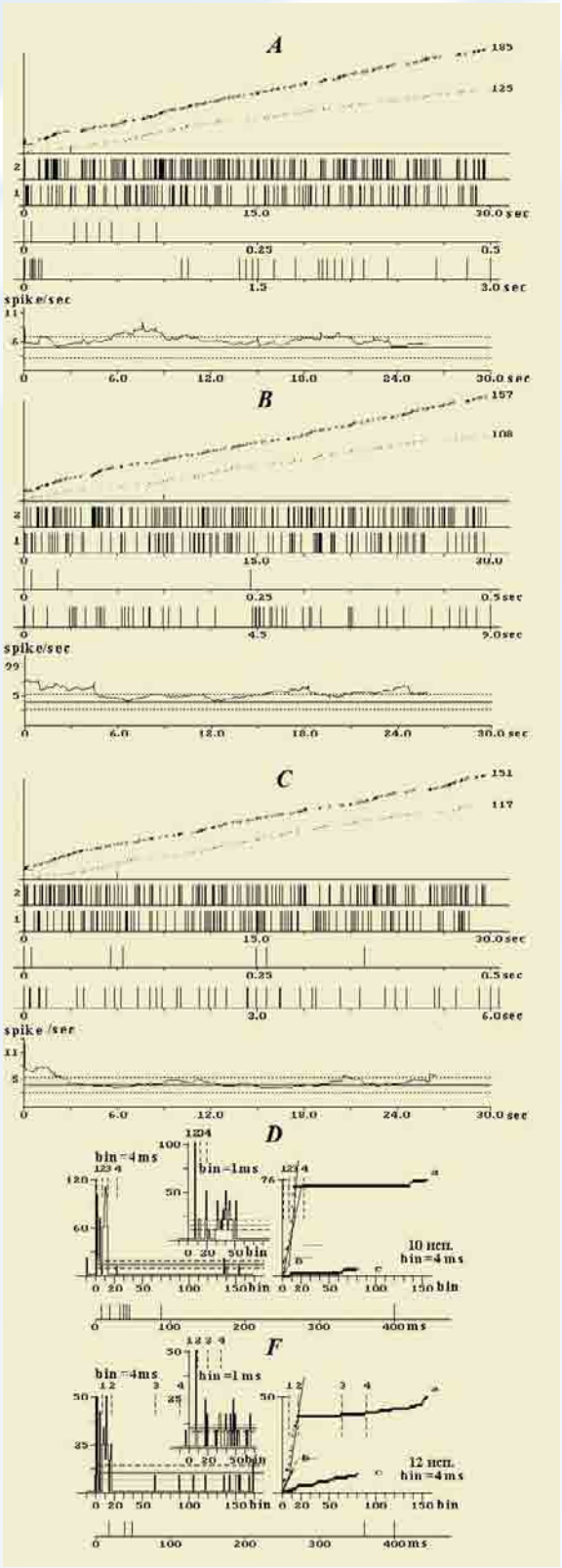


Figure 8. *The paraventricular nucleus cells of hypothalamus.*
A) The paraventricular nucleus cells of hypothalamus of intact rat; Oc. ×10. Obj. ×10.
B) The degenerated cells of paraventricular nucleus of rat subjected to βA injection; Oc. ×10. Obj. ×10.
C) The cells of the same nucleus of hypothalamus of the rat with a single PRP injection before βA injection; Oc. ×10. Obj. ×10.



(25-35), on the 6th day after intoxication by βA.

It was revealed that in the intact animals the hippocampus neurons on the tetanic stimulation of EC response by tetanic (for the period of stimulation) and post-tetanic potentiation, in some cases accompanied by the post-tetanic depression of various duration.

Under the conditions of intoxication by βA, an obvious discrepancy, in comparison with the norm, both in level and form of activity of the hippocampal neurons was revealed, which testified to scarcity of activity as a whole, and to the absence of certain both tetanic and post-tetanic activity probably connected with the expected deficiency of neuromediator, apparently, of acetylcholine.

Under conditions of PRP administration 1 day prior to an intoxication by βA via EC-stimulation with frequency 100 *Hz* during 1 *sec* in the hippocampus neuron after the initial activation up to 150 *ms* there followed the long inhibition up to 1 *s*, then the activity corresponded to background activity (BA) with small excess of its level (A). In case of stimulation by 100 *Hz* during 0.5 *s* the picture corresponded to BA with insignificant excess of an average level (B). The same pattern was observed in case of stimulation with 100 *Hz* during 0.2 *s* (C). In the cumulative histogram in case of single stimulation before the tetanization there appeared an initial activity from 8 to 55 *ms*, then there came the long inhibition up to 550 *ms* (D). The cumulative histogram after tetanization shows the similar picture, however with restoration of activity after 250 *ms* (E) (Figure 9).

In case of PRP single application 1 day prior to an intoxication the certain improvement in post-stimulus manifestations of the hippocampal

Figure 9. *Pre- and post-stimulus characteristics of impulsive flow of pyramidal neuron in CA2 of hippocampus in conditions of the frequency (A-C) irritations of the brain entorhinal cortex with a single PRP injection 24 hours prior to βA intoxication and in six weeks. Tetanic and posttetanic potentiation at the stimulation by the 100 Hz frequency during 1 s (A), 0.5 s (B) and 0.2 s (C). The cumulative histograms on a single irritation brain entorhinal cortex before (D) and after its tetanization (E).*

neuron activity was not revealed, while upon using single PRP administration 6 days after intoxication, an obvious improvement in formation of both tetanic and post-tetanic manifestations of activation and depression of the hippocampal neurons approaching the norm and testifying to the relief of βA intoxication was observed.

Discussion

Proline-rich polypeptide appears to possess corregating and protective effect to limiting the mechanisms of development of neurochemical, neurohormonal, immunomodulating and morphofunctional breaches under aluminum neurotoxicosis and during intracerebroventricular injection of aggregated form of beta-amyloid peptide.

The central immunomodulating effect of PRP was proved, i.e. the ability not unidirectional to stimulate or inhibit the products of various representatives of immunocytokines (interleukins), insulin-like growth factor (IGF-1) and prolactin in case of experimental aluminum neurotoxicosis in integral brain, hippocampus and cerebral cortex.

At aluminum neurotoxicosis the level of IL-1 was elevated, the content of IL-2 was markedly decreased. A significant increase of IL-6 level was revealed; simultaneously its amount apparently increased in hippocampus structures as well. The amount of IGF-1 was found to be increased in cerebral cortex. The injection of lower doses of PRP promoted the decrease of IL-1 level, normalization of IL-2 in brain tissue and decrease of twice-elevated levels of IL-6 content in the integral brain and hippocampus simultaneously reducing the level of IGF-1 in cerebral cortex structures.

The recovery of IL-1 level was marked with the simultaneous increase of strictly lowered levels of IL-2 content on the background of absence of any significant changes of IL-6 content in the thymus in case of PRP injection.

The injection of PRP led to the increase of IL-1 and particularly IL-2 in the liver; the content of IL-6 being strictly decreased in the condition of aluminum neurotoxicosis did not demonstrate any changes during the injection of higher doses (15 μg) of neuropeptide, whereas minimal doses (1.5 μg) of neuropeptide brought forth its certain increase. Due to the action of higher concentrations of PRP in the blood serum under the condition of aluminum neurotoxicosis there was revealed a certain increase in IL-1 and IL-2 levels; the immunomodulating effect of neurohormone referring the content of IL-2 under lower doses of the mentioned neuropeptide was more expressed. The latter elevated extremely decreased levels of IL-6 in animals with experimental neurotoxicosis normalizing the content of IGF-1 in the blood serum.

There was established the ability of PRP to prevent and corregate the disbalanced system of blood pro-and antioxidant metaloproteins developed in the conditions of aluminum neurotoxicosis, to prevent and eliminate various expressions of the *oxidative stress*.

To a significant extent PRP prevents some ultrastructural expressions of neurodegeneration observed in case of aluminum neurotoxicosis, which was expressed in diffused distribution of aluminum particles in cytoplasm of several hippocampal neurons, detection of neurons with small droplets of grained electron-dense bodies along with diffuse penetration of small grained bodies through nuclear membrane.

The ability of PRP to prevent neurodegenerative disorders developed in the neurons of hippocampal complex, magnocellular (*supraoptic and paraventricular*) nuclei of hypothalamus, in the neuronal cells of motor and limbic parts of cerebral cortex induced by intracerebroventricular injection of beta-amyloid peptide (25-35) fragment was established.

Thus, the results of our recent research allowed to qualitatively amend the present conception on multiform expressions of high biological activity of PRP and convincingly demonstrate the existence of its central and periphery immunomodulating action, the ability to regulate the brain free radical processes, the level of lipid peroxidation and, as a whole, to prevent and corregate the brain neurodegenerative impairments.

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