

Neurolectins: Biochemical Characterisation and Functions

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ABSTRACT

The presented work deals with the recent achievements in the field of research of neurolectins, their quantitative and qualitative distribution in cerebral cells of different animals, subcellular structures and synaptosomes in relation to embryonic development. Distribution of neurolectin-binding receptors glycoproteins and glycolipids in the same structures, their role in the processes of migration of neurons and other mechanisms are studied as well. Two membrane neurolectins VL-1 and VL-NAGA have been revealed in synaptosomes, which according to our hypothesis are actively involved in absorption and secretion of neurotransmitters. It was established that VL-1, located on the outer membrane of synaptosomes participates in absorption and metabolism of biologically active substances, though VL-NAGA, which is located in the inner side of the membrane, is also involved in their metabolism. It is considered that negative effects of *wheat germ agglutinin* (WGA) lectins, among them neurotoxic, cardiotoxic

and immunotoxic, can be avoided by means of well toasted bread, because in the process of toasting, lectins undergo denaturation and lose their toxic activity. Food should be chewed well, until it becomes saturated with glycoprotein mucine, which is component of saliva, as N-acetyl-D-glucosamine binding and inactivating WGA is presented in its active centre. In conclusion it is said that uptake of food additives offered by D'Adamo, with the ability of binding WGA and other neurolectins, is not recommended for teenagers and pregnant women, as they may cause inhibition of key processes, involved in mechanisms of structural-functional organization of brain.

Key words: neurolectins, brain, sinaptosomes, neurons, glial cells, synaptic vesicles, mitochondrion, nuclear, glycoconjugates, migration, thermoregulation.

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INTRODUCTION

Lectinology as a new direction of science has started to develop intensively. Lectins are selectively and reversibly bound with carbohydrates and are the cause of agglutination in cells and tissues. They actively participate in such vital processes as communication, signaling systems, adhesion, structural organization, etc. Lectins are proteins of low-molecular glycoprotein nature and are distinguished by multifunctional activity. Emerging from the functions of lectins there are great perspectives for their use in medicine and biology^[1-7]. The anticancer, antiallergic and immunomodulatory properties of lectins have already found practical application in clinical medicine for diagnosis and treatment of infectious diseases^[8-19]. Lectins are also effectively used in biochemical and histochemical investigations. It has become possible to isolate and purify glycoproteins and glycolipids via lectine probe of terminal carbohydrates within biomembranes^[20-26].

RESULTS

The first protein with agglutination capacity was discovered in 1888 by a doctorant of Tartu University Peter Herman Stilmark in the seeds and oils of the plant *Ricinus communis* and was called Ricin. As this substance caused agglutination of erythrocytes it became known as hemagglutinin. Unfortunately Ricin appeared to be a potent

toxic substance commonly employed for committing terrorist acts. The first terrorist act was carried out in 1978 on the Waterloo bridge in London where a Bulgarian oppositioner Georgi Markov was killed by pricking an umbrella tip treated with Ricin. This terrorist act became known as “killing with an umbrella”. Later on it appeared that killing with Ricin was due to the inhibition of protein synthesis on the ribosome’s level.

It is well known that the majority of agglutinins show particular sensitivity towards hydrogen ions, extractive solutions, detergents, sugars and inorganic ions. It is notable that agglutinin extracted from the rat brain glial cells with 0.5 mM EDTA has specific affinity towards glucose, mannose and inositol, whereas the agglutinin extracted with potassium phosphate buffer (PPB) was specific for galactose and inositol. Lectin isolated from the rat brain with 5 mM EDTA was found to be sensitive to glucose, fructose, ribose and mannose. It is noteworthy that their pH optimum ranges from 7.0 to 8.0.

Since the discovery of proteins with agglutination ability hemagglutinins in animal organisms are referred to lectins^[27], we call neurolectins those ones which are separated from nervous tissues^[28].

The first neurolectin was isolated from the electric organ of *Electrophorus electricus* by Teichberg et al.(1975). It agglutinates trypsin-treated rabbit erythrocytes and is specifically inhibited by disaccharides containing non reducing terminal beta-D-galactosyl residues^[29]. It was called electrolectin. Electrolectin was partially membrane-bound, but it was also found in soluble fractions of electric organ homogenates. The neurolectin was purified by affinity chromatography on cross-linked and disulfated agarose (ECD-Sepharose) as a protein of molecular weight 33 kDa. Electrolectin in large amounts was found in the microsomal fraction of electric organ, obtained via 70 min 100000 g centrifugation. Electrolectin amount made up 11% of total proteins. It has been calculated that electrolectin’s amount per 1 kg electric organ is 400 mg, that is rather high index, bearing in mind that the amount of one of the most essential enzymes acetylcholinesterase in 1 kg of nervous tissue does exceed 50-100 mg, whereas the number of acetylcholine receptors fluctuates within 5-10 mg/kg^[30]. Recently it has been found out that galactose-specific lectin is extensively distributed in the animal nerve tissues and subcellular fractions^[31-39].

This discovery has virtually prompted an extensive investigation of nervous system neurolectins and technologies for their isolation, purification and perspective to study their structure and function.

A keen interest has been arisen by the fact that some lectins possess the lymphocyte mitose activating ability. The first discovery of this kind was made on the example of the kidney beans. It has been shown that phytohemagglutinin, derived from the kidney bean (*Phaseolus vulgaris*) initiates activation of human lymphocytes division^[40,41]. The mitogenic activity manifested other agglutinins as well, namely, similar activity was specific to *Pinat* agglutinin^[40], which was recognized as a revolutionary discovery in immunology.

In 1987 the endogenous two soluble neurolectins CSL1 (Mr = 33,000) and CSL2 (Mr = 31.500) were isolated by Zanetta et al from the cerebellum of young rats by 0.5% triton X-100 solution^[40-42]. On the other hand, by immunoaffinity technique another minor component with Mr = 45.000 molecular mass was isolated from the same tissue, which turned out to be a minor component of the precursor of CSL1 (mainly lysosomal) and CSL2 (mainly cytoplasmic) subunits. The above mentioned neurolectin appeared to be mannose-specific and was effectively extracted with the 0.5 M mannose^[42-44].

In 1999 neurolectin RL-29 was immunohistochemically visualized in the rat spinal neurons. RL-29 immunoreactive (Rh-29 IR) neurons

were found in the lateral parts of Laminae V-VII. RL-29 lectin is a multipolar reactive product revealed in the primary and secondary dendrites and some axons. The results suggest that the neuropathic injury produces a long term transsynaptic change in a subpopulation of the dorsal horn neurons, which is mediated by excitatory amino acid transmitters acting at NMDA receptors. These data show that the majority of RL-29 IR neurons in the parasympathetic nucleus are autonomic preganglionic neurons, thus suggesting that RL-29 can be a useful tool in marking this subpopulation neurons^[45].

Detection of neurolectins at different stages of embryonic development became the subject of our great interest. Firstly, in order to reveal maximal neurolectin activity in an embryonic and adult hen brain, a various compositions of extracting solutions were tested, among them most perspective appeared 0.5 mM EDTA potassium phosphate buffer (PPB), for further purification of which from 0 to 80% saturated ammonium sulfate solution was used. It has been shown that only on 5th day of embryonic development of hen brain neurolectin activity was revealed and it reached its maximum before hatching^[46-48].

Noteworthy results were obtained while studying the neurolectin distribution in the rat brain P₁ and P₂ fractions during 2-16 days before its hatching^[49]. P₁ and P₂ fractions were obtained according to Teichberg et al^[29]. P fractions were dissolved in the 0.5 mM EDTA and homogenized. The Sediment was dissolved in PPB, prepared with sodium chloride and explored lectin activity^[50-52]. It was stated that the rat brain P₁ and P₂ fractions were characterized by agglutinating activity. Much higher activity is predominantly detected on 7th day of embryonal development in P₂ protein fraction. If neurolectin specific activity in dose days in P₁ and P₂ fractions were 79,37 and 132,50 unit, before hatching this index reduces maximally and is no more than 16,04 and 80,62 unit respectively. A contention is expressed that lectin activity reduction can not be accounted by a decrease in neurolectin’s total amount, but by the increase of brain dry mass and volume^[48,51,52]. Similar view has been offered by other authors as well^[36,50-52].

The study was pursued of the neurolectin content in the hen brain at embryonic and postembryonic development. Neurolectin was extracted from 20 day old embryonic chicken brain with solution 5 mM EDTA PPB and following subsequent purification with ammonium sulfate at 80-90% saturation. Neurolectin showed specificity to D-galactose (30 mM), D-lactose (15 mM), N-acetyl-D-glucosamine and was designated as L-LG, consequently. They wear purified by affinity chromatography on lactose-sepharose 4B column. As a result, lectin specific activity enhanced more than 25 times. Using the method of electrophoresis in polyacrylamide gel the molecular mass of neurolectin was 15 kDa. Lectin is a glycoprotein with monosaccharides content of approximately 20%. Neurolectin manifested optimal activity at pH 7.0-8.0^[53]. Neurolectin displays particular sensitivity to Ca²⁺ ions. Neurolectin activity at presence of Ca²⁺ chelate EGTA under 0.2 mM concentration agglutination of rabbit’s trypsinized erythrocytes is maximally inhibited.

Beyer and Barondes discovered two endogenous lactose-inhibitable lectins in embryonic and adult chick tissues. They were determined in the purified preparations from different tissues and were indistinguishable in molecular weight and isoelectric point^[54]. In 1982 in embryonic and adult chicken tissues a mannose-specific neurolectin was 1700-fold purified using APL Sepharosa 4B. Such neurolectin preparation in polyacrylamide gel in the presence of sodium dodecylsulfate divided into major (15 kDa) and minor (13 kDa) molecular mass protein fractions with neurolectin activity. The neurolectins extracted from different tissues from embryonic and

adult chicken seemed to be identical^[55]. Developmentally regulated lectins from chicken muscle, brain and liver had similar chemical and immunological properties^[56].

NEUROLECTINS FROM NEURONS, GLIAL CELLS AND SYNAPTIC VESICLES

Since the discovery of neurolectin activity in the hen and rat brains, we pursued the study of neurolectin quantitative distribution in the enriched fractions with neuronal and glial cells. Neuronal and glial cells enriched fractions were obtained by Rose modified method^[57,58].

Neurolectin was isolated from enriched fraction of neuronal and glial cells with 0.5% triton X-100 solution. After its homogenization, the mixture was centrifuged (10000g/30 min), supernatant was fractionated with ammonium sulfate of different saturation. The residue was dissolved in agglutinating PPB solution and after dialysis in each separate fraction neurolectin activity was determined. Neurolectins' specific activity was respectively 780 (neuron) and 713 (glia) unit. The neurolectins isolated from the enriched fractions with neurons and glial cells manifested diverse affinity for carbohydrates. Namely, neurolectin isolated from a neuron enriched fraction appeared to be specific D-galactose (16.2 mM) and N-acetyl-D-glucosamine (8.1 mM), the neurolectin isolated from glial cells enriched fraction showed specificity for D-fructose (16.2 mM), D-xylose (16.2 mM), N-acetyl-D-galactosamine (9.3 mM) and inositol (4.6 mM). In order to obtain proteins with the highest lectin activity of neuron and glial cells, specific affinity chromatography on the tris-acryl-inositol and tris-acryl-galactose column was used. It was isolated from glial cell enriched fraction inosit-specific (GL-I) and from neuron enriched fraction - galactose-specific (NL-Gal) neurolectins^[47,48,53]. The molecular masses of the derived neurolectins were measured with HPLC system (Millipor-Waters, USA). Their molecular masses appeared respectively GL-I = 11.5 kDa, while - NL-Gal = 13.5 kDa. Both lectins are glycoproteins, their carbohydrate content is respectively 28% and 26%.

It is noteworthy that in NL-Gal neurolectin contain the following aminoacids: leucine, isoleucine, valine, phenylalanine, tryptophan, glutamic acid, threonine, glycine, serine, glutamine, asparagine and arginine, while in GL-I except for the named aminoacids, with exclusion of threonine, proline, tyrosine and asparagine were additionally found. In both neurolectins the presence of SH groups was noted, which was stated by Ellman's method^[59]. Number of SH groups per mg/protein was for NL-Gal 35×10^{-4} M, while for GL-I 15×10^{-4} M. Both neurolectins showed particular sensitivity to Ca^{2+} ions, which was maximally inhibited in the presence of 0.2 mM Ca^{2+} chelate, EGTA. It is notable that the glial cell neurolectins of an embryo, in contrast to adult hen, are represented predominantly in a soluble form, that is generally characteristic for embryonal cells, more probably, the soluble forms of neurolectins are transformed into a bound form in the process of postnatal development^[35,60]. It is worth noting that endogenous neurolectin was found in Schwann cells by other authors, stating that neurolectin takes part in stabilization of compact myelin^[61,62].

Bearing in mind a strategic role of glial cells in the maintenance of trophic function, an attempt has been made to study the effect of aminoacids, neurotransmitters and biologically active substances on hemagglutination of rabbit's trypsinized erythrocytes by neurolectins^[29,48,51], assuming that the results of these experiments would provide an answer on a possible role of neurolectins in their bind, transport and inactivation. With the application of hapten-inhibitory method it has been ascertained that NL-Gal and GL-I

drastically vary in their specificity for amino acids and biologically active substances. Experimentally it has been demonstrated that NL-Gal hemagglutination activity of rabbit's trypsinized erythrocytes is inhibited with arginine (37.5 mM), L-serine (18.7 mM) and L-glutamine (18.7 mM), as to GL-I neurolectin hemagglutination was inhibited in the presence in incubation medium of L-arginine (37.5 mM), L-serine (18.7 mM), L-glutamine (9.4 mM) and tyrosine (37.5 mM). It is important to note that a number of other biologically active substances had also an inhibiting effect on neurolectin GL-I activity at comparatively different concentration: acetylcholine (0.62mM), epinephrine (1.25 mM), glycine (0.31 mM), beta-alanine (0.156 mM), serotonin (0.32 mM), norepinephrine (0.62 mM), dopamine (0.156 mM), thiramine (0.63 mM), hydroxythiramine (0.156 mM), choline bromide (0.08 mM), hydroxythriptamine (1.25 mM), gamma-aminobutirate (> 100 mM) and methoxythiramine (> 100 mM). In inverted commas hemagglutination inhibitor substances concentration is presented^[51].

Proceeding from the presented material, determination of orientation of an active center for GL-I and NL-Gal on cell membranes arise great interest. It was found that native glial cells cause agglutination of trypsinized rabbit erythrocytes. Agglutination of GL-I was completely inhibited by inosite (0.6 mM) while galactose had practically no effect on erythrocyte agglutination, that furnished grounds for a statement that on the glial cell membrane an active center for GL-I is oriented on the external side of membrane in the direction of intercellular space and the active center for NL-Gal towards the cell cytoplasm^[27,47].

The question arises: what role may neurolectins play in a trophic function of glial cells. After showing by hapten-inhibitory method that biologically active substances, neurotransmitters and aminoacids had inhibitory effect on neurolectin agglutination of rabbit's trypsinized erythrocytes, it became reasonable to conclude that neurolectin GL-I may actively participate in the transport of aminoacids and biologically active substances for their metabolism and inactivation. It was shown earlier that under conditions of surplus of neurotransmitters in the neuron-glia-synapse area, there occurs active uptake and detoxication of acetylcholine, serotonin, dopamine and gamma-aminobutyric acid-³H by the rat cerebral cortex, glial cells and synaptosomes^[62-64]. It is worth noting that serotonin uptake by glial cells and their inactivation occurs actively via conjugation with glucuronide and forms a biologically inactive glucuronide-serotonin^[65]. In the same way there occurs dopamin and serotonin uptake by glial cells of the rabbit cerebral cortex^[66,67].

Thus it is clear that neurolectins are mainly presented in nerve cells, conducting pathways, dendritic basal membranes and nerve endings. Interestingly, neurolectin amount and its activity is mainly observable at massive synaptogenesis, which suggests their active participation in the formation of synapses. Therefore it is natural that the study has started on neurolectin's distribution and function in synaptic vesicles. Isolation of synaptic vesicles was accomplished by the method of De Robertis and associates^[68].

In 2002 and 2004 from the synaptic vesicles of hen and bovine brain with triton X-100 solution inosite-specific lectin (BVL-I) was isolated^[70], which proved to be a glycoprotein with 10% carbohydrate total content. Neurolectin showed high affinity for Ca^{2+} ions, in the presence of 0.2 mM concentration EGTA neurolectin activity was maximally inhibited. Of special interest is the fact that BVL-I neurolectin shows specific affinity for phosphatidylinosite and phosphatidylcholine and it is localized on the vesicular membrane outer surface, that was experimentally testified. It was first detected that agglutination of rabbit's trypsinized erythrocytes occurred via

membranes bovine brain native synaptic vesicles, while the synaptic vesicles agglutination was inhibited with inositol^[70]. It suggests that BVL-I is an inositol-specific neurolectin. Neurolectins are enriched in sulfur containing aminoacids whose amount is 21.4×10^4 M per 1 mg protein.

On the other hand neurolectin from synaptic vesicles from the mature hen was separated. For the purpose of maximal extraction of neurolectins proved to be 0.5% triton X-100 on 40 mM PPB (pH 7.4). As a result of the use of this solution the specific inositol (VL-I) and N-acetyl-D-galactosamine (VL-NAGA) neurolectins were isolated and purified on Sepharose 4B N-acetyl-D-glucosamine and inositol immobilized column, neurolectin activity of the extracted protein increased 2-5 times^[27]. Both of them are glycoproteins containing respectively 6% and 13% carbohydrates. Neurolectins are enriched in sulfur containing aminoacids whose amount is 21.4×10^4 M per 1 mg protein.

Neurolectin VL-I manifests a high sensitivity to Ca^{2+} and consequently its activity is completely inhibited by a Ca^{2+} chelate of EGTA. Its location and orientation in vesicles was also of great interest. With a view of solving this question it was necessary to ascertain the effect of VL-1 and VL-NAGA on agglutination of trypsinized erythrocytes. It was stated that the native synaptic vesicles agglutinate the rabbit's trypsinized erythrocytes and agglutination gets completely inhibited by a specific hapten-inositol and phosphatidylinositol (0.03 mg/100 μ l)^[31]. It is becoming clear that BML-I and VL-I neurolectins possess essentially the same features and are characterized with the same affinity to haptens namely to inositol, inositolphospholipid and phosphatidylcholine.

Based on these data a hypothesis is offered about participation of VL-1 and VL-NAGA in the secretion of neurotransmitters from the synaptic vesicles. In terms of the offered hypothesis via the neurolectin VL-1 is anchored on the presynaptic membrane as a terminal carbohydrate residue of inositol and inositolphospholipid which is oriented toward the cytoplasm. Simultaneously there occurs merging of presynaptic membrane and vesicles thus forming the vesicular lumen through which there is an active rush of Ca^{2+} ions from the synaptic cleft. Formation of an analogous structure has been shown in electron-microscopic studies. The formation of a presynaptic lumen is accompanied by secretion of neurotransmitters, simultaneously stimulation of receptors activate an enzyme phospholipase C^[71-74]. As a result of action of phospholipase C there occurs cleavage of inositolphospholipid into inositol-1,4,5-triphosphate (ITP) and diacylglycerol. The latter remains within the presynaptic membrane, while ITP in a linked form with vesicles gets chipped off the membrane. Part of ITP in a free state contributes to an increase Ca^{2+} ions concentration in the cytoplasm, but diacylglycerol activates protein kinase C. Consequently, with rising concentration of cytoplasmic Ca^{2+} exit of neurotransmitters from vesicles through the presynaptic lumen is accelerated (Figure. 1). This ends the stage neurotransmitter secretion and there begins the stage of formation of vesicles and their filling with neurotransmitter.

As for the formation of vesicles and closing of their lumen, then according to the offered scheme VL-NAGA and Ca^{2+} ions were involved in it. During the increase of Ca^{2+} ions concentration one can observe inhibition of ITP binding with receptors in the cytoplasm^[73], and simultaneously there occurs disruption vesicular medium of ligand-receptor link (ITP-VL-1). This process is enhanced by neurolectin mediated ATP breakdown and by the accumulation of phosphate in the vicinity of vesicles^[75-77]. It is well known, that the neurolectin-receptor binding is disrupted in acidic medium^[79]. ITP in a free form still enhances release of Ca^{2+} ions and increases its

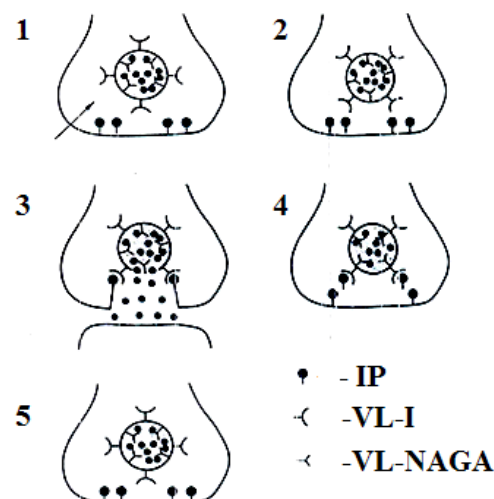


Figure 1 A hypothetical scheme of participation of neurolectins VL-1 and VL-NAGA in the secretion process of neurotransmitters. 1. A synapse in a resting state; 2. Anchoring of vesicular lectin VL-1 of the presynaptic membrane with inositolphospholipid; 3. Formation of presynaptic clefts and secretion of neurotransmitters; 4. Separation of vesicles with a bound inositolphospholipid (IP) and recovery of a presynaptic membrane; 5. Separation of inositoltriphosphate from the synaptic vesicles and transition of a synapse into relaxation state. Conventional designations: IP – inositoltriphosphate, VL-1 – vesicular inositol-specific lectin, VL-NAGA – vesicular N-acetyl-D-glucosamine-specific lectin.

concentration in the cytoplasm, agglutination and merging of ends of vesicular membrane being sharply stimulated with the participation of VL-NAGA. It should be noted that VL-NAGA is more sensitive to Ca^{2+} ions (3 mM), as compared to VL-I (> 100 mM). Ca^{2+} ions promote merging of endings both of vesicular and presynaptic membranes. Thus conclusion is drawn that following release from and loading of vesicles with neurotransmitters is provided with specific neurolectins that merging of vesicular membrane endings. The authors assume that the offered hypothesis is in need of further elaboration of biochemical basis of separate stages of vesicles with neurotransmitters. Though part of neurolectins participation in the process of neurotransmitters secretion and merging of membranes in all likelihood is doubtful.

NEUROLECTINS FROM MITOCHONDRION

Since the neurolectins availability and their quantitative distribution in animals' whole brain and nerve cells had been established, study was pursued on the level of nervous cell subcellular fractions. First, neurolectin was separated from bovine brain mitochondria by specifically selected with 0.5% triton X-100 solution and purified under conditions of 80% ammonium sulfate saturation. It was detected that agglutination by the extracted neurolectin was specifically inhibited by glucose, galactose and N-acetylglucosamine and N-acetyl-galactosamine was named as BML-GluG^[78]. BML-GluG was fractionated on Sephadex G-100 column. Chromatography revealed BML-GluG neurolectin's A and B isoforms which were also characterized by lectin activity. BML-GluG is glycoprotein with carbohydrate content per mg protein being 16.7 μ g/mg protein. The neurolectin appeared to contain disulfide bond. After recovery of disulfide bond with dithiothreitol lectin activity was inhibited. BML-GluG appeared sensitive to Ca^{2+} ions. In the presence of Ca^{2+} chelator EGTA agglutination of trypsinized erythrocytes gets maximally inhibited. Study of its orientation on mitochondrial

membrane became necessary. For this purpose it was established that agglutination of rabbit's trypsinized erythrocytes occurs at native mitochondria, thus with the use of hapten-inhibitory method it was shown that agglutination of rabbit's trypsinized erythrocytes by BML-GluG was maximally inhibited with N-acetyl-D-glucosamine. Thus it is concluded that BML-GluG is oriented on the outside of mitochondrion membrane^[81].

NEUROLECTINS FROM RAT BRAIN NUCLEAR

The data on nervous cell nuclear lectins are rather limited. Therefore special study was made on nuclear neurolectins in the rat brain. In order to establish availability of neurolectin activity in brain nuclei it was first detected that agglutination of rabbit's trypsinized erythrocytes occurred via native nuclear homogenate (Nuc-L). Then homogenate of native nuclear was centrifuged and agglutinated and no agglutinated supernatant was investigated by electrophoresis in 7.5% polyacrylamide gel. In contrast to electropherogram of nonagglutinated nuclear supernatant, several protein fractions are missing in agglutinated nuclear supernatant electropherogram, which indicates for the binding of nuclear lectins with agglutinated erythrocytes and precipitation with them. Based on these experimental results the study of neurolectins' distribution in the brain nuclear has been started. For this purpose different composition buffers were used amongst which the most perspective to be following: 0.5% triton X-100 + 0.9% NaCl + 20 mM potassium phosphate buffer (PPB), pH 5.0^[80-85]. After homogenization of nuclear fraction soluble proteins were removed by PPB, the mixture was dialyzed against hemagglutination buffer and in the mixture lectin activity was determined. Specific activity of nuclear neurolectin was shown to equal 104.4 unit. For further cleansing of neurolectins by method of stepwise salting out by ammonium sulfate at the saturation 20%, 40%, 80% was used. After salting out, proteins were cleared by HPLC (Waters, USA) on the gel filtering tandem columns Protein PAK-300-SW. It was established that at salting out, only at 40% saturation by ammonium sulphate, specific neurolectin activity increased by one and a half times. After further clearance of proteins on rabbit's trypsinized erythrocytes fixed by glutaraldehyde column specific neurolectin activity of nuclei (Nuc-L) increased 5 times and reached 534 unit. It is worth noting that nuclear neurolectins upon gel filtration on columns Protein PAK-300-SW are divided into two protein fractions. Both fractions of proteins are characterized by neurolectin activity. With application of hapten-inhibitory method it was demonstrated that both neurolectins of brain nuclei are N-acetyl-D-glucosamine, D-galactose and L-fructose-specific neurolectins.

DISTRIBUTION OF NEUROLECTIN BINDING PROTEINS IN BRAIN

In this section of article, distribution of neurolectin binding proteins, glycoconjugates were investigated as well. Following detection of lectins in the brain an attempt has been made to study lectin binding glycoproteins in the rat brain nuclear with the use of plant lectins (Diagnosticum, Lvov, Ukraine)^[79].

Homogenate of brain nuclear was centrifuged and sediment was processed by 20 mM PPB (pH 5.5) prepared on 0.9% solution of NaCl with 5 mM phenylmethylsulfonylphthoride as a proteinase inhibitor. After homogenisation it was centrifuged (6000 g, 20 min) and to sediment was added 0.1% solution of triton X-100 prepared on 40 mM PPB (pH 7.4), after mixing and centrifugation the supernatant was dialysed vs PPB (pH 5.0) up to a complete removal

of soluble proteins in PPB and put it at +4 C° temperature for 30 min. After centrifugation soluble proteins were fractionated on the column Protein PAK-300-SW and lectin binding glycoproteins was determined^[80-85].

Lectin binding ability was estimated by the hapten-inhibitory method at minimal concentration of proteins inhibiting lectin hemagglutination after their joint preincubation and expressed in the values of quantitative ratio of lectin and lectin-bound protein (LBP)^[85]. As judged by quantitative indices of ratio of lectin and LBP, both soluble in PPB and extracted by triton X-100, protein fractions exhibit affinity for separate neurolectins and cause inhibition of their hemagglutinating activity. By the highest affinity among the neurolectins the lectin of ordinary bean (PVA) was explored, which exhibits sensitivity to many terminal carbohydrate residues of the rat brain cellular nuclei glycoproteins. Based on these experiments presence of glycoconjugates was evident in protein fractions of cellular nuclei extracting by PPB and triton X-100. If we proceed from the data of quantitative ratio of lectin and LBP, then by the level of affinity for soluble LBP lectins in nuclear are arranged in the following sequence: PVA (*Ordinary bean*)>SNA (*Sambucus nigra*)>PNA (*Arachis hipogaeae*)>STA (*Solanum tuberosum*)>PSL (*Pisum sativum*)>SBA (*Glicine max*)>ConA (*Canavalia ensiformis*)>RCR (*Ricinus communis*). In relation to LBP extracting by triton X-100, the mentioned sequence is disturbed to some degree, though number of lectins with a high affinity for LBP remains unchanged^[22,23,80-85]. This enables us to make a conclusion concerning the presence in the rat brain nuclei glycoprotein fractions of the following terminal carbohydrates: Gal, Man, Glu N-acetyl-D-glucosamine.

Glycoprotein nature also appeared to be the myosin-like nonmuscular contractile proteins neurostenin and gliostenin isolated from the rat brain neuronal and glial cells^[86]. A conception was expressed that gliostenin takes an active part in the glial cell pulsation, with a high affinity for uptake and metabolism of biologically active substances including neurotransmitter^[65-67]. Gliostenin also exhibited Mg²⁺-, Ca²⁺- and HCO₃⁻-ATPase activity^[75-77]. It was stated that neurolectins were shown to regulate their activity in a concentration depending way. Namely, within the concentration ranging from 0.5 µg/ml to 3 µg/ml of neurolectin GI-Gal, HCO₃⁻-ATPas activity increases by about 100%, while at the concentration 10 µg/mL of ATPase activity, compared to control, reduces by 50%. At GI-I neurolectin concentration to 2.0 µg/ml, HCO₃⁻ - ATPase activity increases approximately by 250%, at further increase of concentration (10 µg/ml) the enzyme activity decreases by 75%. It is important to note that by preliminary addition of neurolectin-specific monosaccharides the lectin losses the ATPase regulating (either inhibition or activation) abilities, thus the enzyme activity regulation mechanisms seems to involve the principles of hapten-inhibitory interaction.

Badache and his colleagues have shown that the myelin-associated glycoprotein (MAG) could be ligands for the mannose-binding cerebellar soluble lectin (CSL)^[42,44,90]. Fluorescent and other derivatives of lectins have been used as histochemical stains to study the nature and distribution of carbohydrates on many cell types including neurons. It was reported that the lectin from the legume *Dolichos biflorus* (DBA), selectively stains neuromuscular junctions in several vertebrate species. This result demonstrates the existence of a synapse-specific carbohydrate in neuromuscular junctions^[91].

Glycoproteins from the central nervous system for developmental alterations in their carbohydrate composition by autoradiographic analysis of radioiodinated lectin binding after separation by high-resolution sodium dodecylsulfate-pore gradient slab gel

electrophoresis (SDS-PGE) have been studied. Sixteen lectin-binding components were used in highly purified myelin preparations from 15th-day, 18th-day and adult rat brains. Changes in lectin binding for individual glycoproteins were evaluated semiquantitatively by comparing densitometric scans of the autoradiographs. In lectin binding for individual components there were observed increases and decreases as a consequence of development, as well as the appearance and disappearance of lectin binding to three low-molecular weight components. These developmental changes in lectin binding suggest that increases in glycoprotein density, as well as an elaboration of oligosaccharide branching for individual glycoproteins occur. These observations suggest that during myelin development alterations in myelin-associated glycoproteins occur. Such alterations gave the authors possibility to conclude that regulation of membrane glycoproteins enhances the significance of their potential role in myelin maintenance^[62].

Plant lectins and chemically glycosylated neoglycoproteins have been used to map occurrence of components of this putative recognition system. The labeled endogenous lectins and the lectin-binding ligands can add to the panel of glycohistochemical tools. Experiments were prompted to employ the abundant β -galactoside-specific lectin of human nerve tissues in affinity chromatography and histochemistry to purify and localize its specific glycoprotein ligands. β -galactoside-specific plant lectins from *Ricinus communis* and *Erythrina cristagalli*, notable similarities were especially detectable in the respective profiles of the mammalian and the *Erythrina* lectin. Overall, the introduction of biotinylated mammalian lectins as well as the lectin-binding glycoproteins will aid to critically evaluate the physiological significance of glycobiological interplay between endogenous lectins and distinct carbohydrate parts of cellular glycoconjugates^[38].

Distribution and mobility of Con A and *Ricinus communis* (RCA) receptors on the external surfaces of purkinje cells, hippocampal pyramidal cells and their attached buttons were studied using ferritin-lectin conjugates. Cell fragments and pre- and postsynaptic membranes were labeled with the ferritin-lectin conjugates and the distribution of lectin receptors was determined by electron microscopy. Con A receptors were most concentrated at the junctional membrane, indicating that a mature neuron has a specialized organization of carbohydrates on its outer surface. It was proposed that structures in the postsynaptic density may be transmembrane-linked to postsynaptic receptor and determine topographic distribution and limit diffusion of specialized synaptic molecules^[97].

Tirado and his colleagues have studied the binding ability of several lectins (Con A, DBA, UEA, WGA) in the embryonic development of the chick cerebellum at different stages 18 to 45 days. Differential labeling in the cortical layers of the cerebellum was observed. The authors concluded that different labeling and modifications in the labeling pattern suggest intense variations of the glycoproteins and glycosaminoglycans in the extra-cellular matrix during development of chick brain, which reflects the correlation between brain development and brain structural organization^[55].

Bearing in mind a number of facts on the effect of neurolectins on hemagglutination of the rat brain nervous cellular nuclei^[22], the quantitative content of glycoproteins blocking the nuclear hemagglutination was explored. Con A is fully inhibited by protein fractions isolated from nuclei by 0.1% triton X-100 and PPB. The proteins to be extracted were preliminary purified by chromatography by means of HPLC on the column Protein Pak 300-SW and study was made to identify glycoproteins in cellular nuclei by the hapten-inhibitory method, considering the quantitative ratio of lectin and

lectin-binding proteins at inhibition of hemagglutination activity in the rabbit's trypsinized erythrocytes. In terms of the obtained data neurolectins are arranged in the following sequence: PNA > SNA > STA > PSL > SBA > Con A > RCA. It should be mentioned that ability for inhibition of lectin activity by proteins extracted with PPB is an order lower than by the proteins extracted by triton X-100^[22,23,82,84].

In the next series of experiments an attempt was made to explore lectin-binding proteins in chromatin and in nonhiston proteins of the rat brain cellular nuclei, his prompted to study the effect of chromatin on hemagglutination of rabbit's trypsinized erythrocytes by plant lectins with a view of revealing the existence of lectin binding proteins in chromatin. Primarily it was established that chromatin inhibits hemagglutination of Con A that indicates to the existence in chromatin of hapten N-acetyl-D-glucosamine, the ratio of Con A and lectin binding proteins of chromatin being 0.03 μ g/47 μ g. Consequently, taking into consideration the quantitative ratio of lectin and lectin binding proteins of chromatin during inhibition of hemagglutination activity by chromatin, the plant lectins are arranged in the following sequence: PNA > SNA > STA > PSL > SBA > Con A. Bearing in mind carbohydrate-specificity of the above mentioned lectins it can be concluded that protein fractions of chromatin in the form of terminal carbohydrates contain: glucose, galactose, fructose, N-acetyl-D-glucosamine, lactose and mannose^[23,85,87].

Peanut lectin (PNL), Con A and *Ulex europaeus* lectin 1 (*Ulex*) were chosen to map their binding sites in different regions of formalin fixed and paraffin embedded human CNS tissue. In astrocytes the cytoplasm was stained by PNL. Intracytoplasmic Con A receptors were observed in astrocytes, oligodendrocytes and in some cells of the pituitary gland. *Ulex europaeus* lectin 1 was selectively binding in the pituitary gland. The authors suggest that lectins may be good tools for the evaluation of their target cells in the CNS^[38].

THE INFLUENCE OF NEUROLECTINS IN NERVE CELLS AND NEUROLECTINS TRANSPORT AND MIGRATION

Intensive study is also pursued on the role of neurolectins in nerve cell transport and their purposeful migration mechanisms. A many published papers in this context our attention was attracted by the one on the roles of lectins and glycoproteins in their possible involvement in neurons migration^[92-95].

In the CNS postmitotic neurons migrate along astrocytic processes to reach their adult position. The molecular mechanisms of this guided migration are not clearly defined, although some steps have been shown to involve proteases and cell adhesion molecules in this process. It was reported that monovalent antibodies (Fab fragments) raised against an endogenous cerebellar soluble lectin (CSL) completely inhibit neuronal migration in cultures of cerebellar explants at concentrations as low as 50 μ g/mL. A similar inhibition pattern was obtained with Fab fragments prepared against one of the endogenous glycoprotein ligands of CSL, the 31-kDa glycoprotein (this is a membrane-bound glycoprotein specifically occurring in the cerebellum on the surface of immature neurons). It was proposed that this neurolectin interaction supports the adhesion between neurons and the astrocyte guide during the migration of cerebellar immature neurons^[92-94].

The binding and axonal transport of six lectins were studied in the peripheral nervous system of adult mice by an immunocytochemical method. Lectins known to bind preferentially N-acetyl-D-glucosamine or mannose sugars were transported axonally to ventral

horn and dorsal root ganglion neurons. After 96 hr post injection, lectins were bound to neuromuscular junctions, muscle spindles and cutaneous nerves. These differences in axonal transport were seen also when the lectins were applied directly to the proximal and of a transected mixed nerve. It was demonstrated that peripheral axons transport of plant lectins retrogradely from terminal axons to soma and initiate a new direction transport. Furthermore, lectin binding sites are distributed uniformly on the surfaces of neurons. The present study shows that several lectins are transported retrogradely by axons of the peripheral nervous system and that the differences in their distribution between motor and sensory neurons appear to correlate with their carbohydrate affinities^[93,94]. Under retrograde axonal transport conditions targeted binding of plant origin agglutinin to the individual nerve tissue structures is considerably different, namely, binding to the cell ventral horn, large and small dorsal root. For instance, if WGA binds to the mentioned areas in maximal amount, Con A binding to the ventral and dorsal horn is reduced by 25% and to the small dorsal root by 50%, as far as N-acetyl-D-glucosamine specific BSA lectin is concerned, it does not bind at all to the large dorsal root, while its binding to the small dorsal root is 50% reduced.

SUB SERVING OF LECTINS, GLYCOLIPIDS AND PROTEOGLYCANS IN FORMATION OF STRUCTURE AND FUNCTION OF NERVOUS SYSTEM

Now it becomes clear that neurolectins, glycoproteins, glycolipids and proteoglycans actively participate and control the mechanisms of formation of structure and function of the nervous system; dynamically regulate synapse plasticity and interneuronal lectin contacts while forming new neuronal ensembles during the process of learning and memory formation^[9,10,22,67]. Quantitative and qualitative changes in synaptic expression of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)-type glutamate neurotransmitter receptor (AMPA) and specific neurolectins play particularly important role excitatory circuits in the brain and dynamically regulate synaptic plasticity at the embryonic development. Human congenital disorders of ganglioside ligand biosynthesis result in deficit of learning and memory. The discovery that AMPARs and AMPA engage brain gangliosides trafficking provides an unanticipated opportunity to understand the roles of gangliosides in regulating excitatory neurotransmission on the cellular and molecular level.

IMPACT OF NEUROLECTINS AND THEIR ANTIBODIES ON THE INTEGRATIVE ACTIVITY OF NERVOUS SYSTEM

Cerebrospinal fluid of patients with multiple sclerosis contains antibodies to an endogenous mannose-binding protein, the cerebellar soluble lectin CSL, which indicates for the involvement of this lectin in neuropathologies^[95,96]. The involvement of lectins in the processes of memory formation, myelination and stabilization of myelin structure was also studied^[95,97-100].

Natural antibodies to neurolectins are a significant part of the immune system of humans. Antibodies to legume lectins (*Erythrina corallodendron*-ECorL), *peanut agglutinin* (PNA), *soybean agglutinin* (SBA) and *wheat germ agglutinin* (WGA) were discovered in human sera and their binding specificity was examined. The anti-SBA, anti-ECorL and anti-WGA antibodies exhibited high specificity, whereas the anti-PNA antibodies were polyreactive, the antibodies were highly specific for WGA, they also crossreacted slightly toward

some other proteins. The anti-ECorL antibodies bound to native SBA, but the anti-SBA antibodies failed to bind to the native ECorL. Although the anti-SBA and anti-ECorL antibodies both exhibited specificity when interacting with native lectins, they bound to a wider range of denatured lectins, indicating a common or universal epitope which is recognized by many natural antibodies. Interestingly, the natural antibodies did not interfere with the agglutination properties of the lectins. These findings suggest the severe outcomes that might be induced by the antibodies to the lectins available in the food products. It is hardly possible to imagine under conditions of existing knowledge what may be consequences of such interaction between lectins and antibodies, although all this may result in heavy outcomes. For instance, Zanetta and his colleagues have shown that antibodies to CSL cerebrospinal neurolectin are present in the fluid of patients with multiple sclerosis^[99].

In recent years the question of negative effect of wheat germ lectin (WGA) on humans became particularly acute. It is well known that WGA displays neurotoxicity, immunotoxicity and cardiotoxicity. WGA can cross the blood-brain barrier by "adsorptive endocytosis". WGA disorders were detected in the genetic apparatus and in activity of the endocrine system^[67]. WGA may attach to myelin sheath and inhibit nerve growth factor, which is important for the growth, survival of certain target neurons in formation of memory. Unfortunately, there is ample evidence that WGA, as whole wheat, can contribute to significant problems in mental activity too^[101].

At the same time, it is well known that if dietary proteins are degraded during passage through the gut by digestive enzymes in the small bowel, any residual undigested matter is degraded by bacteria in the large intestine. In contrast, dietary lectins resist degradation in the small intestine and are also resistant to breakdown by most gut bacteria, thus, as most plant lectins survive at least in part the passage through the digestive tract in an immunologically and functionally intact form, they can exert their potent negative biological activities *in vivo*^[3]. Via of the effects of natural fermentation the maximum reduction of the lectin fermentation was shown at 72 and 96 h^[61,105]. And this time span is quite sufficient for the lectins to pass into the blood practically unchanged^[3,61], and as a result there are formed antibodies to them which in a complex way negative effect is manifested on the integral activity of brain^[69] and individuals have problems with their psychic, notably increases the risk for the development of atherosclerosis, Alzheimer and Huntington disease. Fortunately, there exist the substances which are binding and detoxicated of lectins and inhibiting their negative action on the organism. Amongst them are glycoproteins and glycolipids with lectin recognizing terminal sugars, of which particular importance is attributed to the nutrient additive proposed by D'Adamo and available in saliva glycoprotein mucin, which is secreted by various animal mucous cells and glands. Of particular interest is the fact that neutralization of lectin activity existing in saliva glycoprotein mucin has an active part whose terminal hapten is N-acetyl-D-glucosamine, which mediates complete binding of WGA and their detoxication. Therefore, it is necessary to continue chewing nourishing product until the food piece is not fully saturated with saliva and not neutralized WGA completely. It is also desirable to eat toasters instead of newly baked bread. In this case occurs denaturation of WGA as protein and as a result there is loss of their biological activity by which injuring effects of agglutinin on the brain will be prevented. For the sake of avoiding negative effect of lectins of plant origin, it is desirable to use food additives offered by D'Adamo considering blood groups of individuals and 15 foods to improve memory and boost brain power^[3,106]. In relation to the

aforesaid, for improvement and maintenance of memory there exist psychobiological data that this is possible to be realized by activation of neuronal ensembles^[68,70], by reading, listening to classic music, physical exercise and public activities. The neuronal ensembles in the brain cannot be left for a long time in hypokinetic state, as a neuron that no longer receives any stimuli loses even more synapses and die after some time^[107]. This is also indicated by Leuner and Shors data, according to which under conditions of hypokinesia plasticity of synapses, and prolonged potentiation dynamics are disturbed and cognitive deficit in long-term memory formation is noticed^[103-105].

NEUROLECTIN pH3(40) AS BRAIN THERMO-REGULATION FACTOR

Among many functions of neurolectins in the nerve tissue great interest was arisen by discovery of neurolectins possessing of thermoregulation ability in the brain^[107]. Quantitative distribution of neurolectins during a year essentially is depending on environmental temperature. The neurolectins were preliminarily extracted by 0.25 M glycine-HCl buffer (pH 3). The extracted protein fraction was treated with ammonium sulfate under 40% saturation and maximal specific activity of neurolectins was found, therefore the neurolectin was named as pH3(40). Neurolectin was appeared N-Acetyl-D-glucosamine specific, that enabled the author to maximally purify the extracted protein fraction by means of affinity chromatography on the tris-acryl-N-acetyl-D-glucosamine column. Chromatography yielded two big and small peaks of proteins with lectin activity and both was maximally inhibited by N-acetyl-D-glucosamine^[107].

In order to study the temperature dependence of brain neurolectin pH3(40), their were placed rat outdoors during a year. Lectin activity study showed that specific activity of neurolectin pH3(40) strongly depended on the environmental temperature, i.e. on the season. It seems that endothermic animals, rats readily adapt to low temperature by the stimulating synthesis of pH3(40) lectin. In May the specific activity of neurolectin pH3(40) in the cerebral cortex were 4 units, in December it experienced more than 37-fold increase. We believe that neurolectin pH3(40) is involved in brain thermoregulation^[107].

CONCLUSION

From the presented data it has become evident that lectins on the one hand actively participate in the structural-functional organization of nervous system, and on the other hand, they can made problems in health of the living organisms and evoke serious harm. That's why one must know and take into account perspective of using nutrient additives offered by D'Adamo for the avoiding a negative effect of plant lectins on the living organisms. It should also be considered that the food additive deflect proposed by D'Adamo is not reasonable during pregnancy. By action of deflect on development of fetus can be disturbed resulting from binding of the animal neurolectins which actively participate in the formation of nervous system. The only principle for the self-protection from plant lectins remains well chewing of the received food until it gets saturated with glycoprotein mucin available in the saliva. As a result there occurs complete binding and detoxication of lectins. Also it ought to be considered that products containing WGA should be well toasted for its denaturation, for complete avoidance of their negative effect.

REFERENCES

- Sharon N. Lectins: carbohydrate-specific reagents and biological recognition molecules. *J Biol. Chem.* 2007; **282**; 2753-2764. [PMID: 17145746]
- Pusztai A. Dietary lectins are metabolic signals for the gut and modulate immune and hormone functions. *Eur. J. Clin. Natur.* 1993; **47**: 691-699. [PMID: 8269884]
- Rudiger H, Siebert HC, Solis D, Jimenez-Barbero J, Romero A, von der Lieth CW, Diaz-Marino T, Gabius HJ. Medicinal chemistry based on the sugar code: fundamentals of lectinology and xperimental strategies with lectins as targets. *Curr. Med. Chem.* 2000; **7**: 389-416. [PMID: 0702616]
- Sharma A, Sharma S, Khuller GK. Lectin-functionalized poly (lactide-co-glycolipide) nanoparticles as oral/aerosolized antitubercular drug carriers for treatment of tuberculosis. *Journal of Antimicrobial Chemotherapy.* 2004; **54**: 761-766. [PMID: 15329364]; [DOI: 10.1093/jac/dkh411]
- Sharon, N. Carbohydrates as future anti-adhesion drugs for infectious diseases. *Biochim. Biophys. Acta.* 2006; **1760**: 527-537. [PMID: 16564136]; [DOI: 10.1016/j.bbagen.2005.12.008]
- Shemer J, LeRoith D. The interaction of brain insulin receptors with wheat germ agglutinin. *Neuropeptides* 1987; **9**: 1-8. [PMID: 3561720]
- Thompson LU. Potential health benefits and problems associated with antinutrients in foods. *Food Research International*, 1993; **26**: 131-149. [DOI: 10.1016/0963-9969(93)90069-U]
- Abdullaev FI, de Mejia EG. Antitumor effect of plant lectins. *Nat. Toxins.* 1997; **5**: 157-163. [PMID: 9407559]; [DOI: 10.1002/1522-7189(1997)5: 4<157:: AID-NT6>3.0.CO; 2-L]
- Aleksidze G, Aleksidze N. New Anticancerogenic and immunomodulating preparation GA-40. *Intern. Simp. Immune System of the Brain: Neurochemical and neuroendocrin aspects. Republic of Armenia, Yerevan*, October. 2009; 6-8.
- Beuth J, Ko HL, Pulverer G, Uhlenbruck G, Pichlmaier H. Importance of lectins for the prevention of bacterial infections and cancer metastases. *Glycoconjugate Journal.* 1995; **12**: 1-6. [PMID: 7795408]
- Cordain L, Toohey L, Smith MJ, Hickey MS. Modulation of immune function by dietary lectins in rheumatoid arthritis. *British Journal of Nutrition.* 2000; **83**: 207-217, [PMID: 10884708]
- Cruz-Sanchez FF, Rossi ML, Rodriguez-Prados S, Cusi V, Coakham HB. Histology and histopathology Immunohistological signposts in central nervous system tumor's with neuronal differentiation. *Histol. Histopath.* 1991; **6**: 503-508. [PMID: 1839510]
- Dumbadze N, Pirtskhalaishvili D, Aleksidze N, Aleksidze G. Anticancerogenic Activity of the Mannose-Specific Lectin Isolated from Rhizomes of the Georgian Endemic Mountain Plant *Polygonatum obtusifolium* (C.Koch) Misch. ex Gross. *Bull. Georg. Acad. Sci.* 2016; **10**: 56-60. in Russian
- Hamid R, Masood A. Dietary lectins as disease causing toxicants. *Pakistan Journal of Nutrition.* 2009; **8**(3): 293-303.
- Mayer EA, Craske M, Naliboff BD. Depression, Anxiety, and the gastrointestinal system. *Journal of Clinical Psychiatry.* 2001; **62**: Suppl.8; 28-36. [PMID: 12108819]
- Mody R, Joshi S, Chaney W. Use of lectins as diagnostic and therapeutic tools for cancer. *Journal of Pharmacological and Toxicological Methods.* 1995; **33**: 1-10. [PMID: 7727802]
- Raz A, Meromsky L, Carmi P, Karakash R, Lotan D, Lotan R. Monoclonal antibodies to endogenous galactose-specific tumor cell lectins. *EMBO J.* 1984; **3**: 2979-2983. [PMID: 6084591]
- Schumacher U, Higgs D, Loizidou M, Pickering R, Leatham A, Taylor I. Helix pomatia agglutinin binding is a useful prognostic indicator in colorectal carcinoma. *Cancer.* 1994; **74**: 3104-3107, [PMID: 7982174]
- Schwechheimer K, Zhou L. HMB45: a specific marker for melanoma metastases in the central nervous system. *Virchows Arch.* 1995; **426** (4): 351-353, [PMID: 7599786]
- Aleksidze N, Tansell N. Influence of environmental and social stress factors on distribution of serotonin in the brain

- and development of killer and self-killer organisms. *New Developments in Serotonin Research*. (Editor: Ming D. Li.) Nova Science Publisher, ISBN. 2015; 51-65.
21. Merkle RK, Cummings RD. Lectin affinity chromatography of glycopeptides. *Methods Enzymol.* 1987; **138**: 232-259, [PMID: 3600324]
 22. Akhalkatsi RG, Aleksidze NG. Agglutination of the brain cores of rats with concanavalin A. *Neurochemistry (In Russian)*. 1995; **12**: 51-54, in Russian
 23. Akhalkatsi RG, Bolotashvili TV, Aleksidze GI, Aleksidze NG. The fractionation of lectin-binding proteins of the rats brain isolated cell nuclei. *Neurochemistry* 1997; **14**: 289-294, in Russian
 24. Nicolson GL, Singer SJ. Ferritin-conjugated plant agglutinins as specific saccharide stains for electron microscopy: application to saccharides bound to cell membranes. *Proc. Natl. Acad. Sci. USA*. 1971; **68**: 942-945. [PMID: 4102564]
 25. Peumans WJ, Van damme EJ. Plant lectins: specific tools for the identification, isolation and characterization of o-linked glycans. *Crit. Rev. Biochem.* 1998; **33**: 209-258, [PMID: 9766639]
 26. Pfenninger KH, Meylie-Pfenninger MF. Lectin labelling of sprouting neurons. 1. Regional distribution of surface glycoconjugates. *J. Cell Biology.* 1981; **89**: 536-546, [PMID: 7251664]
 27. Boyd WC, Shapleigh E. Specific Precipitating activity of plant agglutinins (Lectins). *Science*. 1954; **119**: 419. [PMID: 17842730]; [DOI: 10.1126/science.119.3091.419]
 28. Aleksidze N, Leksishvili T. Identification of neurolectins in neurons and glial cells enriched fractions of the chickens brain. Caucas. Symposium on medical and biological sciences, Tbilisi, 1999; 15-18, in Russian
 29. Teichberg V, Silman I, Beitsch DD, Resheff G. A β -D-galactoside binding protein from electric organ tissue of *Electrophorus electricus*. *Proc. Natl. Acad. Sci. USA*. 1975; **72**: 1383-1387, [PMID: 1055413]
 30. Tchernychev B, Wilchek M. Natural human antibodies to dietary lectins. *FEBS Letters*. 1996; **397**: 139-142, [PMID: 8955334]
 31. Aleksidze NG, Koshoridze NI. Participation of inositol- and N-acetyl-D-glucosamine-binding lectins of synaptic vesicles in the secretion of neurotransmitters. *Neurochemistry*, 2000; **17**: 104-108, in Russian
 32. Lekishvili T, Koshoridze N, Aleksidze G, Aleksidze N. Biochemical characterization of brain glial cells lectin. *Bull. Acad. Sci. Georg.* 1999; **159**: 337-339, in Russian
 33. Surguladze N, Koshoridze N, Aleksidze N. The inositol-specific lectin from synaptic vesicle membranes. *Bull. Georg. Acad. Sci.* 2004; **169**: 574-576, in Russian
 34. Kawasaki T, Ashwell G. Isolation and characterization of an avian hepatic binding protein specific for N-acetyl-D-glucosamine terminated glycoproteins. *J. Biol. Chem.* 1977; **252**: 6536-6543, [PMID: 893426]
 35. Barondes SH, Castronovo V, Cooper DN, Cummings, RD, Drickamer K, Feizi, T, Gitt MA. Galectins: A family of animal beta-galactoside-binding lectins. *Cell*. 1994; **76**: 597-598, [PMID: 8124704]
 36. Barondes SH, Douglas NW, Cooper S, Michael A, Gitt HL, Leffer H. Galectins structure and function of a large family of animal lectins. *J. Biol. Chem.* 1994; 269: 20807-20810, [PMID: 8063692]
 37. Gabius HJ. Detection and functions of mammalian lectins – with emphasis on membrane lectins. *Biochem. Biophys. Acta.* 1991; **1071**: 1-18, [PMID: 2004115]
 38. Park M, Chung K. Endogenous lectin (RL-29) expression of the autonomic preganglionic neurons in the rat spinal cord. *The Anatomical Record*. 1999; **254**: 53-60, [PMID: 9892417]
 39. Schwechheimer K, Weiss G, Schnabel P, Möller P. Lectin target cells in human central nervous system and the pituitary gland. *Histochemistry*. 1984; **80**: 165-169, [PMID: 6370930]
 40. Novogrodsky A, Lotan R, Ravid, A, Sharon N. Peanut agglutinin, a new mitogen that binds to galactosyl sites exposed after neuraminidase treatment. *J. Immunol.* 1975; **115**: 1243-1248. [PMID: 1176775]
 41. Nowell PC. Phytohemagglutinin: An Initiator of mitosis in cultures of normal human leukocytes. *Cancer Res.* 1960; **20**: 462-466, [PMID: 14427849]
 42. Kuchler S, Herbein G, Sarlieve LL, Vincendon G, Zanetta JP. An endogenous lectin CSL interacts with glycoprotein components in peripheral nervous system myelin. *Cell Molec. Biology.* 1989; **35**: 581-596, [PMID: 2611842]
 43. Zanetta JP, Meyer A, Kuchler S, Vincendon G. Isolation and immunochemical study of a soluble cerebellar lectin delineating its structure and function. *J. Neurochem.* 1987; **49**: 1250-1257, [PMID: 2442309]
 44. Zanetta JP, Wantyghem J, Kuchler-Boop S, Badache A, Aubery M. Human lymphocyte activation is associated with the early and high-level expression of the endogenous lectin CSL at the cell surface. *Biochem. J.* 1995; **311**: 629-636, [PMID: 9451031]
 45. Garrison CJ, Willis WD, Carlton SM. The endogenous lectin RL-29 is transsynaptically induced in dorsal horn neurons following peripheral neuropathy in the rat. *Brain Res.* 1993; **620**: 64-71, [PMID: 8402200]
 46. Zanetta, JP, Kuchler S, Lehmann S, Badache A, Maschke S, Marschal P, Dufourcq P, Vincendon G. Cerebellar Lectins. *International Review of Cytology.* 1992; **135**: 123-154, [PMID: 1618606]
 47. Koshoridze N, Balavadze M, Aleksidze N, Lekishvili T. Hens brain lectins at different stages of embryo development. *Bull. Acad. Sci. Georg.* 1997; **156**: 297-299, in Russian
 48. Koshoridze N, Aleksidze N, Garishvili T, Lekishvili T. Quantitative alteration of galactose-lactose specific lectins at different stages of hens brain embryonic development. *Bull. Acad. Sci. Georg.* 1998; **158**: 134-137, in Russian
 49. Zanetta JP, Kuchler S, Marschal P, Zaepfel, M, Meyer A, Badache A, Reeber A, Lehmann S, Vincendon G. Role of an endogenous mannosyl-lectin in myelination and stabilization of myelin structure. *Cellular and Molecular Biology of Myelination. The NATO ASI Series.* 1987; **43**: 433-450. [DOI: 10.1007/978-3-642-83968-9_30]
 50. Aleksidze NG, Koshoridze NI, Lekishvili TN, Aleksidze G. Lactose specific lectin from the brain of chickens: excretion and biochemical characteristics. *J. Evol. Biochem. Physiol. Bull. Acad. Sci. Russia.* 1999; **35**: 154-155, [PMID: 10519204, in Russian.
 51. Lekishvili TN. Biochemical characteristics of glial lectins from brain and their role. Proceedings of Tbilisi State University, Tbilisi, 1999; 60-62. in Russian
 52. Akhalkatsi RG, Aleksidze NG. Lectin-like proteins of the rat brain. *Bull. Georgian Acad. Sci.* 1987; **128**: 597-599, in Russian
 53. Koshoridze N, Balavadze M, Menabde K, Chipashvili M, Aleksidze N. Chick brain L-LGlectin and its impact on the membrane Na^+ , K^+ , HCO_3^- , Ca^{2+} -ATPases and CHE activity. *Proc. Georgian Sci. Biol. Ser.* 1999; **25(4-6)**: 4-6. in Russian
 54. Beyer EC, Barondes SH. Quantitation of two endogenous lactose-inhibitable lectins in embryonic and adult chicken tissues. *J. Cell Biol.* 1982; **92**: 23-27. [PMID: 70556800]
 55. Kobiler D, Beyer EC, Barondes SH. Developmentally regulated lectins from chick muscle, brain, and liver have similar chemical and immunological properties. *Developmental Biology.* 1978; **64**: 265-272. [PMID: 680346]
 56. Kobiler D, Barondes SH. Lectin activity from embryonic chick brain, heart and liver: changes with development. *Dev. Biol.* 1977; **60**: 326-330, [PMID: 902929]
 57. Rose S. Preparation of enriched fractions from cerebral cortex containing metabolically active neuronal and glial cells. *Biochem. J.* 1967; **102**: 33-43. [PMID: 4291562]
 58. Aleksidze N, Akhalkatsi R, Balavadze M. Comparative Study of AChE and BHE activity of the brain cortex, enriched fractions of glial and neuronal cells. *Bull. Acad. Sci. GSSR* 1974; 56-60, in

- Russian
59. Ellman GL, Kcourtney YD, Andres V, Feather-Stone RM. Rapid Colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 1961; **7**: 88-95. [PMID: 13726518]
 60. Kuchler S, Perraud F, Sensenbrenner M, Vincendon G, Zanetta JP. An endogenous lectin found in rat astrocyte cultures has a role in cell adhesion but not in cell proliferation. *Glia.* 1989; **2**: 437-445. [PMID: 2531724]
 61. Kuchler S, Lehmann S, Vincendon G, Zanetta JP. Endogenous lectin cerebellar soluble lectin involved in myelination is absent from nonmyelinating schwann cells. *J. Neurochem.* 1992; **58(5)**: 1768-1772. [PMID: 1560231]
 62. Poduslo JF, Harman JL, McFarlin DE. Lectin Receptors in central nervous system myelin. *J. Neurochem.* 1980; **34**: 1733-1744. [PMID: 6966675]
 63. Tirado F, Pena Melián A, Puerta Fonola J. Lectin-binding patterns in the development of the cerebellum. *Anatomy and Embryology.* 1994; **189**: 169-179. [PMID: 8010415]
 64. Lekishvili T, Dolinska M, Aleksidze N, Sonnewald U. Characterization of rat and mouse brain lectins and their possible role. *5th Intern. Conf. on Brain Energy Metabolism. Brain Energy Metabolism in Neurotransmission: Function and Dysfunction. Trondheim, Norway.* 2001; 63.
 65. Aleksidze NG, Bakhanashvili TA, Balavadze MV, Gogvadze NO. On the inactivation of serotonin in glial cells by glucuronic acid conjugation. *Bull. Acad. Sci. GSSR.* 1983; **111**: 621-624, in Russian
 66. Bakhanashvili TA, Maisov NI, Aleksidze NG. Uptake of dopamine-H³ by glial cells and synaptosomes of rabbit cerebral cortex. *Bull. Georg. Acad. Sci. GSSR, Ser. Biol.* 1980; **6**: 273-276, in Russian
 67. Aleksidze N. Biochemical characterization of biologically active lectins and prospects of their uses. *Science and Technology. Georg Natl. Acad. Sci.* 2015; **3**: 80-86. in Russian
 68. De Robertis OE, De Iraldi AP, Rodriguez G, Gomez CJ. On the isolation of nerve endings and synaptic vesicles. *J. Biophys. Biochem. Cytol.* 1961; **9**: 229-235. [PMID: 13720810]
 69. Butz M, Ooyen AA. Simple rule for dendritic spine and axonal button formation can account for cortical reorganization after focal retinal lesions. *PLoS Comput Biol.* 2013; **9(10)**: e1003259. [DOI: 10.1371/journal.pcbi.1003259]; [PMID: 24130472]
 70. Surguladze N, Koshoridze N, Menabde K, Kapanadze K, Aleksidze N. The comparative characterisation of inositol-specific lectins isolated from vesicles of hen and bovine brain. *Proc. Georgian Acad. Sci.* 2002; **28 (5-6)**: 609-615, in Russian.
 71. Beckman ML, Bernstein E, Quick MW. Protein kinase C regulates the interaction between a GABA transporter and syntaxin 1A. *J. Neurosci.* 1998; **18**: 6103-6112. [PMID: 9698305]
 72. Berridge MJ, Irvine RF. Inositol phosphates and cell signalling. *Nature.* 1989; **341**: 197-205. [PMID: 2550825]
 73. Maeda N, Niinobe M, Mikoshiba K. A cerebellar Purkinje cell marker P400 protein is an inositol 1,4,5-trisphosphate (InsP3) receptor protein. Purification and characterization of InsP3 receptor complex. *EMBO J.* 1990; **9**: 61-67. [PMID: 2153079]
 74. Mourey RJ, Verma A, Supattapone S, Snyder SH. Purification and characterization of the inositol 1,4,5-trisphosphate receptor protein from rat vas deferens. *Biochem. J.* 1990; **272**: 383-389. [PMID: 2176461]
 75. Koshoridze N, Aleksidze N. The interaction of lectin KVG (40-80) with the ATP-ase of microsomes of the brain. *Neurochemistry,* 1992; **11**: 49-55, in Russian
 76. Koshoridze NI, Menabde KO, Surguladze NB, Vardiashvili TM, Solomonii RO, Aleksidze NG. Effects of endogenous lectins on HCO₃⁻-ATPase activity of the brain glia cells. *Ukr. Bioch. J.* 1999; **79**: 12-18. [PMID: 17988010], in Russian
 77. Vardiashvili T, Koshoridze N, Menabde K. Aleksidze N. HCO₃⁻-ATPase of bovine brain. *Proc. Georgian Acad. Sci. Ser. A.* 2006; **32**: 465-471, in Russian
 78. Vardiashvili T, Koshoridze N, Menabde K, Surguladze N, Aleksidze N. Mitochondrial glucose-specific lectin BML-Glu and its physico-chemical properties. *Proc Georgian Acad. Sci. Biol. Ser. A.* 2006; **32**: 19-25. In Russian.
 79. Lutsik MD, Panasyuk EN, Antonyuk VA. Methods of searching for lectins and determining their immunochemical sensitivity. *Lviv.* 1980; 520. in Russian
 80. Akhalkatsi RG. Biochemical characteristics of N-acetyl-D-glucosamine of specific lectin and glucoconjugates isolated from cell nuclei of the brain. *TSU, Tbilisi,* 1999; 50. in Russian
 81. Akhalkatsi RG, Bolotashvili TV, Aleksidze NG. The identification of lectin like proteins from the rat brain isolated nuclei. *Bull. Georg. Acad. Sci.* 1999; **159**: 357-360, in Russian
 82. Akhalkatsi RG, Aleksidze NG. Rat cerebral brain cellular nuclei protein fractions as a receptors for plant lectins. *Int. Conf. on Structure and Functions of Biomembrane. India, Calcutta.* 1989; 39.
 83. Akhalkatsi RG, Bolotashvili TV, Aleksidze NG. The nonhiston proteins of chromatin as lectin-binding glycoconjugates. *Bull. Georg. Acad. Sci.* 1998; **158**: 127-129, in Russian
 84. Akhalkatsi RG, Bolotashvili TV, Solomonia R, Aleksidze NG. Identification of concanavalin A binding proteins of rat brain cellular nuclei by gel electrophoresis and blotting. *Bull. Georg. Acad. Sci.* 1996; **154**: 116-118, in Russian
 85. Akhalkatsi RG, Bolotashvili TV, Aleksidze GI, Aleksidze NG. The fractionation of lectin-binding proteins of the rat brain isolated cell nuclei. *Neurochemistry.* 1997; **3**: 289-294. in Russian
 86. Sandalov YG, Glebov RN, Kryzhanovski GN, Chipashvili MD, Beletskaya RP, Aleksidze NG. Actomyosin-like protein isolation from the bovine brain cells and its properties. *Bull. Exp. Biol. Med.* 1977; **8**: 280-282. [PMID: 851630]
 87. Chipashvili MD, Aphadze MG, Beletskaya RP, Aleksidze NG. Understanding biochemical identity of actomyosin-like proteins of neuronal and glial origin. *Bull. Georg. Acad. Sci.* 1980; **100**: 681-684, in Russia
 88. Chipashvili MD, Aphadze MG, Beletskaya RP, Aleksidze NG. Understanding biochemical identity of actomyosin-like proteins of neuronal and glial origin. *Bull. Georg. Acad. Sci.* 1980; **100**: 681-684, in Russian
 89. Davitashvili E, Chipashvili M, Menabde K, Aleksidze N. The glycoprotein nature of rat's brain actomyosin-like protein. *Bull. Georg. Acad. Sci.* 1999; **159 (3)**: 498-502. in Russian
 90. Badache A, Burger D, Villarroja H, Robert Y, Kuchler S, Steck A, Zanetta J. Carbohydrate moieties of myelin-associated glycoprotein, major glycoprotein of the peripheral nervous system myelin and other myelin glycoproteins potentially involved in cell adhesion. *Dev. Neurosci.* 1992; **14 (5, 6)**: 342-350. [PMID: 1284853]
 91. Sanes JJ, Cheney M. Lectin binding reveals a synapse-specific carbohydrate in skeletal muscle. *Nature.* 1982; **300**: 646-647. [PMID: 7144916]
 92. Steindler DA, Deniau JM. Anatomical evidence for collateral branching of substantia nigra neurons: A combined horseradish peroxidase and [³H] wheat germ agglutinin axonal transport study in the rat. *Brain Research.* 1980; **196**: 228-236. [PMID: 6156738]
 93. Borges LF, Sidman RL. Axonal transport of lectins in the peripheral nervous system. *J. Neuroscience.* 1982; **2**: 647-653. [PMID: 6176698]
 94. Lehmann S, Kuchler S, Theveniau M, Vincendon G, Zanetta, JP. An Endogenous Lectin and one of its neuronal glycoprotein ligands are involved in contact guidance of neuron migration. *Proc. Natl. Acad. Sci. USA.* 1990; **87**: 6455-6459. [PMID: 2201031]
 95. Kleim JA, Freeman JH Jr, Bruneau R, Nolan BC, Cooper NR, Zook A, Walters D. Synapse formation is associated with memory storage in the cerebellum. *Proc. Natl. Acad. Sci. USA.* 2002; **99**:

- 13228-13231. [PMID: 12235373]
96. Nachbar M. Lectins in the US diet. Isolation and characterization of a lectin from tomato (*Lycopersicon esculnetum*). *J. Biol. Chem.* 1980; **255**: 2056-2061. [PMID: 7354077]
 97. Zanetta JP, Kuchler S, Marschal P, Zaepfel, M, Meyer A, Badache A, Reeber A, Lehmann S, Vincendon G. Role of an endogenous mannosyl-lectin in myelination and stabilization of myelin structure. *Cellular and Molecular Biology of Myelination. The NATO ASI Series.* 1987; **43**: 433-450. [DOI: 10.1007/978-3-642-83968-9_30]
 98. Zanetta JP, Meyer A, Kuchler S, Vincendon G. Isolation and immunochemical study of a soluble cerebellar lectin delineating its structure and function. *J. Neurochem.* 1987; **49**: 1250-1257, [PMID: 2442309]
 99. Zanetta JP, Warter JM, Lehmann S, Tranchant C, Kuchler S, Vincendon G. Antibodies to cerebellar soluble lectin CSL in multiple sclerosis. *Lancet.* 1990; **335**: 1482-1484. [PMID: 1972431]
 100. Aleksidze N. Molecular and Cellular mechanisms of integrative activity of the brain. *Tbilisi State University. Tbilisi.* 1988; 500, in Russian
 101. Lajolo FM, Genovese MI. Nutritional significance of lectins and enzyme inhibitors from legumes. *J. Agric. Food Chem.* 2002; **50**: 6592-6598. [PMID: 12381157]
 102. Leuner B, Falduto J, Tracey J, Shors T J. Associative Memory formation increases the observation of dendritic spines in the hippocampus. *J. Neurosci.* 2003; **23**: 659- 665. [PMID: 12533625]
 103. Leuner B, Mendolia-Loffredo S, Kozorovitskiy Y, Samburg D, Gould E, Shors TJ. Learning enhances the survival of new neurons beyond the time when the hippocampus is required for memory. *J.Neurosci.* 2004; **24 (34)**: 7477-7481. [PMID: 15329394]
 104. Shors TJ, Matzel LD. Long-term potentiation: what's learning got to do with it. *Behav. Brain Sci.* 1997; **20**: 597-614; 614-55. Review. [PMID: 10097007]
 105. Leuner B, Shors TJ. Synapse Formation and Memory. *Neuroscience Acad. Press.* 2010; 349-355.
 106. D'Adamo PJ. The Non-transfusion Significance of the ABO Blood Groups. *Textbook of Natural Medicine, 2005; Pizzorno and Murray, editors (Elsevier Publications).*
 107. Aleksidze N. The involvement of Lectin pH3(40) in the Termoregulation of the Rat Brain. *Neurochemical Journal.* 2012; **6 (4)**; 265-267.

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