INTERNATIONAL SYMPOSIUM

BRAIN IMMUNE SYSTEM: NEUROCHEMICAL AND NEUROENDOCRINE ASPECTS

dedicated to the 80th Anniversary of Academician Armen GALOYAN

YEREVAN 2009

International Symposium

Immune System of the Brain: Neurochemical and Neuroendocrine Aspects

Dedicated to
the 80th anniversary of Academician Armen A. Galoyan

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Abstracts

Institution

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Publications

The original articles and full text papers of the reports presented at the Symposium will be published in the *Special issue of the Neurochemical Research,* dedicated to Academician A.A. Galoyan. *Editor in Chief: Abel Lajtha, USA*

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Preface

Crosstalk between the CNS and the immune system was recently substantiated through the disclosure of the molecular and cellular basis of their interaction. It was found, that mediators of the nervous system, in addition to neurotransmitter function, influence immune cell function. In addition, mediators of the immune system can bind to receptors in the nervous system, the function of which they regulate. Under physiological conditions these interactions serve to maintain homeostasis in the organism.

There is growing evidence, that 'misunderstandings' between these two major powerful systems enhance the potential risk of neuroinflammation and neurodegeneration. Such observations significantly extended the understanding of the nature of inflammatory neurodegenerative diseases, including multiple sclerosis, Alzheimer's dementia, Parkinson's disease, etc. This is important for additional pathological CNS conditions (stroke, cerebral ischemia, hypoxia, hematoma, etc.), in which inflammatory factors play an essential role in the neurodegenerative processes. Therefore, neurochemical, morphological and neurophysiological investigations along with immunological studies provide particular information on the mechanisms underlying CNS diseases, information essential and applicable for the development of therapeutic strategies.

The Symposium is dedicated to the 80th anniversary of Academician, Prof. Armen Galoyan. During the past 20 years Prof. A.A. Galoyan was among the leading investigators in Neuroendocrine Immunology, signalling molecules of the immune system of the brain. He initiated these studies in Armenian and Russian research institutions, simultaneously leading the Departments of Neurohormone Biochemistry at the H.Buniatian Institute of Biochemistry of the National Academy of Sciences of Armenia in Yerevan and the A.N. Bach Institute of Biochemistry of the Russian Academy of Sciences in Moscow. Several overseas laboratories have also participated in these studies via international collaborative grant programs. For the years of meticulous investigations Prof. A.A.Galoyan and co-workers have succeeded in discovering and chemically identifying a number of hypothalamic neuroactive peptides, the synthetic analogues of which are now available. The immunomodulatory and neuroprotective effects of these compounds were demonstrated, and the findings were published in peer-reviewed journals and book chapters (for reviews see Galoyan A.A. 2004 Brain Neurosecretory Cytokines: Immune Response and Neuronal Survival. Kluwer Academic / Plenum Publishers, New York, p. 188; Lajtha A., Galoyan A., Besedovsky H., 2008 Handbook of Neurochemistry and Molecular Neurobiology: Neuroimmunology, 3rd Edition. Springer, New York).

During the Symposium various aspects of brain neurochemistry, the neuroimmune organization of the brain and pertinent regulatory mechanisms will be presented and discussed in depth by leading scholars from Armenia and internationally distinguished scientists from Europe and overseas. Key presentations are devoted to neuroendocrine regulation issues, including the roles of cytokines and signalling molecules, and advanced methodological approaches in these studies. Emphasis will be put on the brain-borne hypothalamic neuropeptides (proline-rich peptides) that were found to serve as neuromodulators in numerous biochemical reactions and pathways not only in the CNS but also in other tissues and organs. These series of presentations covering the latest findings will illustrate the challenges and research opportunities that exist in this exciting field. The most recent data on antitumorigenic effect of neuropeptides will be presented publicly for the first time.

Presentations devoted to the neurobiochemical mechanisms of the nervous system organization and pathology, effects of hypothalamic neuropeptides on biochemical, morphological and behavioral characteristics in Alzheimer's dementia, Parkinson' disease, eye disorders, and others constitute a special section of the Symposium.

This Symposium would constitute a continuation of the previous international symposia held in Armenia that were devoted to topics pertinent to both Neurochemistry and Neuroimmunology: in Yerevan-Dilijan (1997), in Tsakhkadzor (2001) and in Yerevan-Gavar (2008).

An International Young Scientist's Session, which is supported by International Society for Neurochemistry, is also organized, to give the podium to postgraduate students, young investigators and postdoctoral fellows, coming from Armenia, Germany, France, and Russia. We hope that this event would be an execellent opportunity for young generation of scientists for open interaction and shairing thoughts.

We will also offer an attractive social and cultural program, which will give you an oppoprtunity to get acquainted with history and culture of ancient Armenia.

We foresee an exciting International Symposium and looking forward to see you in Yerevan.

Prof. Guevork Kevorkian
Director, H. Buniatian Institute of Biochemistry
Vice-President, Armenian Association of Biochemists

Speakers and Participants

Michail Aghajanov

M.D., Ph.D., Dr. Sci., Professor of Biochemistry. He was the head of the Department of Biochemistry through 1986-2008, and Vice-Rector on science in 1989-1991 at the Yerevan State Medical University, Armenia. Along with his main activity, i.e. delivering lectures on Biochemistry for medical students, Prof. Aghajanov is the Academician-secretary and an active member of the Academy of Medical Sciences of Armenia, the scientific leader of the Student's Scientific Committee, and the President of the Ethical Committee of the YSMU. He is the founder and the President of the Alzheimer's disease Armenian Association. His scientific research interests are focused on neurochemistry, particularly on investigation of the mechanisms of neurodegeneration and the ways of its possible prevention.

Giorgi Alexidze

Full Professor of Saint Andrews Georgian University in Tbilisi, Correspondent member of the International Academy of Medico-Social Sciences (IAMSS), the President of Intercontinental Medico-Biological Scientific-Research Centre "ALEXIS". The scientific field of activity is Biomedicine. He is author of more than 100 scientific articles and 11 inventions. He is author of an anticancerogenic preparation GA-40 and other new remedies.

Nugzar Aleksidze

Professor, member of the Georgian National Academy of Sciences, Tbilisi, Academician of the Natural Sciences, "Egrisi", Ecology, and Bio-Medicine. He is author of more than 300 scientific articles, 6 monographs, 8 textbooks, and 18 inventions. Scientific field of activity includes molecular and cellular mechanisms of brain integrative activity, membrane-metabolic relationship of neuron and neuroglia and of nerve tissue lectins.

Rouben M. Aroutiounian

Corresponding member of the National Academy of Sciences of RA, member of the Academy of Medical Sciences of Armenia, Doctor of Biological Sciences, Professor, Head of the Department of Genetics and Cytology of the Faculty of Biology in the Yerevan State University. He is the author of more than 170 scientific publications. Besides, he has two monographs and two textbooks on ecological genetics and genetic toxicology.

Naren L. Banik

Professor, Director of Research, Department of Neurology, Medical University of South Carolina, Charleston, USA. He also serves as a Professor at the Departments of Microbiology and Immunology, External Faculty, of MUSC. Prof. L.Banik is an Associate Scientific Director of the State Spinal Cord Injury Research Fund. He is the author of numerous manuscripts (over165 published) and abstracts (279), chapters and review articles (46) published in peer-reviewed journals. He is an Editor of the Handbook of Neurochemistry and Molecular Biology: Central Nervous System Injuries and Disorders in Brain and Spinal Cord, Springer Publisher, NY, 2010.

Nina Barkhudaryan

Ph.D., Dr. Sci., Head of the Neuropeptides Biochemistry Laboratory at H. Buniatian Institute of Biochemistry, NAS RA. She is an expert in the field of neurochemistry, particularly interactions between the nervous and the immune systems. In past several years her research referred to the determination of molecular mechanisms of homeostatic action of hemorphins on HPA axis activity in pathophysiology of severe diseases (stress, diabetes, cancer). The investigations of the role of hemorphins in the regulation of transcriptional activity via modulation of Ca2+/calmodulin/calcineurin signaling pathway are in progress.

Hugo O. Besedovsky

Emeritus Professor of the Medical Faculty, Philips-University, and Institute of Physiology and Pathophysiology in Marburg, Germany. Author of numerous books and papers published in peer-reviewed journals. Prof. H.Besedovsky also served as a member of Editorial boards of numerous periodicals in the field of immunology, neuroimmune biology, and neuroendocrine immunology. Handbook of

Neurochemistry and Molecular Neurobiology: Neuroimmunology: Ed. A. Galoyan and H.O. Besedovsky, Springer, Science+Bussines, Media, 2008. is one of those. Prof. H.Besedovsky is a Normann Cousins Laureate and Herbert Spector Awardee.

Adriana del Rey

Biochemist, Ph.D., Professor of Physiology, Director of the Department of Immunophysiology, Institute of Normal and Pathological Physiology, Medical Faculty, University Marburg, Germany. She is a receiver of many Research Grants from German National Research Council. She combines research with public work, such as organization of discussion groups and educational courses, membership in Steering Committees of specialized societies, including German Society of Endocrinology, German Society of Immunology, Psychoneuroimmunology Research Society, German-Brain-Immune-Network, International Society of NeuroImmunoModulation. Prof. delRey is a member of the editorial board of the journal "Brain, Behavior and Immunity", and Co-Editor of the journal "Neuroimmunomodulation".

In co-authorship Hugo Besedovsky and Adriana delRey published 160 manuscripts and articles in the field of immune-neuro-endocrine interactions, clinical, experimental, and cell immunology, immunopharmacology, all in peer reviewed journals and book chapters.

Anna Boyajyan

Having involved in science directly from the university in 1976, defending her Ph.D. thesis in 1986, and receiving Doctor of Science degree in 1997, Anna Boyajyan currently is the Director of the Institute of Molecular Biology, NAS RA and the Head of the Department of Biotechnology of the International Scientific and Educational Centre, NAS RA. She is internationally acknowledged scientist, Professor in molecular and cellular biology, member of many national and international scientific societies. Anna Boyajyan has more than 200 scientific publications, supervised 20 Ph.D. students, 30 undergraduates, and received prestigious international and national awards for contribution in science and technology. Her current research activities are focused on the investigation of the molecular pathomechanisms of generation and development of immune system dysfunction leading to aberrant apoptosis, autoimmunity, inflammation and auto-inflammation, alterations in neuro-immune-endocrine interactions and blood brain barrier permeability.

Gayane Buniatian

She started her scientific carrier during her studies in the State Yerevan Medical Institute by training in field of Vascular Pharmacology. Receiving in 1971 M.D. Diploma, she switched to Neurochemistry and worked as a scientific researcher in the Institute of Biochemistry of the Armenian Academy of Sciences. In 1977 she received Ph.D. Diploma (Candidate of Biological Sciences) from the Highest Attestation Commission of the Ministry of Education of the USSR. In 1991 Dr. Buniatian was invited as a researcher to Germany and conducted research work in different famous scientific centres, such as Max-Planck-Institute (MPI) for Biochemistry and MPI for Psychiatry, Martinsried (Münich), Institute of Physiological Chemistry of the University of Tübingen, MPI for Developmental Biology, Tübingen; MPI for Cell Biology, Heidelberg, Institute of Biochemistry of Medical Faculty of the University of Leipzig. She is author of more than 70 scientific publications comprising articles and abstracts. Based on the results obtained, she put forward a new concept about common protective functions of neural and non-neural perivascular cells. She is a member of the German Societies for Biochemistry, German Association of Liver Molecular Biology, Nephrology, Cell Biology, and Connective Tissue. She is a reviewer of several International scientific Journals.

Ruben Chailakhyan

M.D., Ph.D., Dr. Sci., Professor, Head of the Laboratory of Stromal Regulation of Immunity in N.F.Gamaley Scientific Research Institute of Experimental Microbiology of the Moscow State University. He is also the Head of the Inter-regional Association on Cellular Technologies and Regenerative Medicine, Moscow branch. His major scientific achievement was that at the end of 1960s for the first time using the method of selective cloning, disclosed unique category of stromal cells, i.e. clonogenic stromal. Later, it was confirmed that the revealed population of stromal cells-precursors served as stem cells of bone marrow stroma. Based on the revealed osteogenetic and proliferative properties of these cells, Prof.Chailakhian and co-workers elaborated new biotechnological method for regeneration of bones and hyaline articular cartilage (1984-1985). This method was applied in the Institute of Traumatology and Orthopedics in Yerevan, and also in many clinics in Moscow.

Lusine Danielyan

She is the Head of the Division of Cellular/Molecular Pharmacology at the Department of Clinical Pharmacology, University Hospital of Tübingen, Germany. She earned her M.D. at the University Hospital of Tübingen. Her current research interests include the exploration of mechanisms and efficacy of neuroprotection provided by several agents including growth factors and therapeutic cells in neurodegenerative disorders such as Alzheimer's, Parkinson's diseases and stroke. Her new findings on intranasal delivery of cells to the brain were highlighted by several journals including Nature Methods, European Journal of Cell Biology, Neurology Today, The New Scientist and others. She was recently awarded by German Ministry of Nutrition and Agriculture for the discovery of intranasal delivery of cells to the brain.

Tigran Davtyan

After graduation from Yerevan State Medical University, Faculty of Pharmacology (M.D., 1988), he continued his education in the Laboratory of Genetic Mechanisms of Cell Malignization and Differentiation, Institute of Cytology, St. Petersburg, Russia. Received degree of Candidate of Biological Sciences (1993, Ph.D.) in the field of Genetics and Immunology; and degree of Doctor of Biological Sciences (2003, Dr. Sci.) in the field of Molecular Biology. Since 1999 he is the Head of HIV-Clinical Trail Laboratory of the ARMENICUM Research Center. His specialization includes HIV/AIDS and clinical immunology; autoimmunity; viral infections, immunity and stress resistance, as well as molecular mechanisms and genetic regulation of innate immune response. He has published over 60 papers; he is an author of numerous book chapters, including **"**Gitutyun" of the NAS RA; he actively participates in international and local scientific meetings with poster and oral presentations.

Scott Durum

Ph.D., Chief of the Section of Cytokines and Immunity, Laboratory of Molecular Immunoregulation, National Cancer Institute, Frederick, MD, USA. After receiving his Ph.D. in 1978, Dr. Durum received his postdoctoral training in immunology at the National Jewish Hospital in Denver and at Yale Medical School. Dr. Durum joined the Laboratory of Molecular Immunoregulation in 1984. His group has studied various aspects of cytokine function in T cell development. Their principal focus in recent years has been the 'trophic' activity of IL-7 on T cells, because it has been clearly established in humans and mice that this cytokine is required for normal T cell development in the thymus, and for T cell survival and homeostatic proliferation of mature peripheral T cells. This refers to the capacity of IL-7 to protect immature and mature T cells from spontaneous cell death; withdrawal of IL-7 induces a new death pathway, beginning with stress kinases that induce alkalinization of the cytosol. The collaborators in this research are many scientists at the NIH and universities.

Serge Fermandjian

Emeritus Director of Research at the Centre National de la Recherche Scientifique (CNRS), France. In previous years he led research teams at the Centre d' Energie Atomique (CEA) of Saclay and at the Institute Gustave Roussy in Villejuif dealing with the structural properties of peptides, DNAs and anticancer drugs. Currently Dr. Fermandjian is working at the Ecole Normale Supérieure de Cachan in the Laboratoire de Biologie et Pharmacologie Appliquées (LBPA) continuing the study of proteins and DNA structures and interactions by spectroscopic methods (nuclear magnetic resonance, circular dichroism, fluorescence) and molecular modelling. The research is particularly focused on investigation of complexes formed between HIV-1 DNA and viral integrase in the presence and absence of inhibitors, acting against AIDS.

Karina Galoian

Ph.D, Research Assistant Professor, IRB Departmental coordinator and Scientific reviewer, Department of Orthopedics, University of Miami, Leonard Miller School of Medicine, Miami, FL, USA. She also serves as a Member of Faculty Administrative Committee of the University of Miami. She is a member of ASN, ISN, and Connective Tissue Oncology Society. Recently Dr. Galoian received patent, approved by University of Miami: Myc-oncogene inactivating effect of proline-rich polypeptides (PRP-1) in the human chondrosarcoma JJ012 cells" filed with United States Patent and Trademark Services.

Eugene Grishin

Ph.D., Dr. Sci., Professor, Corresponding Member of the Russian Academy of Sciences, the Deputy Director of M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences and the Head of the Laboratory for Neuroreceptors and Neuroregulators. He is also Professor of the Department of Bioorganic Chemistry of the Biological Faculty, M.V. Lomonosov Moscow State University, a member of the Russian Biochemistry Society, Russian Society for Neurochemistry, European Society for Neurochemistry, International Society for Neurochemistry, and International Society

of Toxinology. His research interests and activities include Protein Chemistry (protein modification and crystallization), Toxinology (isolation of polypeptide toxins from snake, scorpion, spider and ant venoms; amino acid sequences and spatial structure of polypeptide toxins; cloning of cDNA encoding protein toxins; neurotoxins as tools for study of receptor components), Molecular Neurobiology, and Neuroreceptors (interaction of natural neurotoxins with sodium, potassium and calcium channels). Prof. Grishin has published about 320 papers and abstracts.

Bernd Hamprecht

Ph.D., Professor, Interfaculty Institute for Biochemistry, University of Tübingen, Tübingen, Germany

Araksya Izmiryan

Graduated form French Lycée, Gumry, Armenia in 1996. She received B.Sc. (2000) and M.Sc. (2002) in Biochemistry in Faculty of Biology of the Yerevan State University, Armenia. Thesis in Molecular Biology at University Paris 7 was supervised by Prof. Denise Paulin. Since 2008 she is in postdoctoral position, Inserm U781, France.

David Jessop

Dr. Jessop was awarded his Ph.D. from the University of London in 1988 for endocrine studies performed at St. Bartholomew's Hospital. Since then he works at Westminster Hospital, London, and the University of Bristol in the UK. Major areas of his research are brain responses to stress and the consequences of stress to the immune system and disease. Dr. Jessop has also made major contributions to research into neuropeptides synthesised within immune tissues. He was the first to identify the novel opioid peptides endomorphins in the mammalian immune system and demonstrate their anti-inflammatory activities. His current interests are: (i) the synthesis of endomorphins in immune cells and the therapeutic application of these compounds, and their long-acting analogues, to the treatment of arthritis; (ii) influence of environment and stress on the onset and severity of chronic inflammation; (iii) defects in the HPA axis in autistic children. Dr. Jessop was awarded an honorary Professorship in Neuroimmunology by the Hong Kong Polytechnic University in 2004.

Guevork Kevorkian

Ph.D., Dr. Sci., Professor, Director of the H. Buniatian Institute of Biochemistry, NAS RA, Head of the Department of Pathological Biochemistry at the H. Buniatian Institute of Biochemistry. Prof. Kevorkian is Vice-President of the Armenian Association of Biochemists and Vice-President of the Armenian Society of Clinical Radiology and Molecular Imaging. He has published more than 290 abstracts and articles, and supervised 11 Ph.D. theses. The field of his research interests includes Ca ions translocation and Ca^{2+}-binding properties, synthesis and degradation of membrane proteins at different experimental pathological states, such as Crush syndrome, Bipolar disorders, as well as testing of different natural and synthetic peptides for treatment.

Varduhi Knaryan

Ph.D., **s**enior scientific staff at the Department of Neurohormones Biochemistry of H.Buniatian Institute of Biochemistry, NAS RA, and a member of the Scientific Council of the Institute. Her current research interests concern with the investigation of cellular and molecular mechanisms of neuronal degeneration at experimental Parkinsonism and involvement of spinal cord in the progression of disease. Studies are particularly focused on the role of proteolytic enzymes (calpain and caspase-3) in neurodegenerative processes caused by Parkinsonian toxins. During 2005-2006 she was a Fulbright Scholar in USA, conducting research work in the Department of Neurosciences/Neurology, Medical University of South Carolina. She is a member of the Armenian Association of Biochemists, affiliated to FEBS, Armenian Brain Research Association, affiliated to IBRO, and Society for Neuroscience. Dr. Knaryan is a Secretary of the Armenian Association of Biochemists, and serves as an organizer of scientific meetings, conferences and workshops. Recently, she has been elected by FEBS Council, as a member of the Working Group on Assistance to Central and Eastern Europe (WOGCEE).

Evgeny Krasavin

M.D., Ph.D., Dr. Sci., Professor, Director - organizer of the Laboratory of Radiation Biology, Joint Institute for Nuclear Research (JINR), Dubna, Moscow region, Russia. His scientific interests are in the fields of radiobiology of accelerated heavy particles, radiation induced mutagenesis, space radiobiology, mathematical modeling of biological action of radiation. Prof. Krasavin is the head of the Biophysics Chair at International University of Nature, Society and Man "Dubna", and scientific and methodological supervisor from JINR at the Section of Biological Sciences of the Russian Academy of Sciences. He is a member of the Scientific Council for the problems of radiobiology of the Russian Academy of Sciences, JINR Scientific and Technical Council, and JINR PAC for Condensed Matter Physics. He is a member of the editorial boards of scientific journals "Radiation Biology, Radioecology", "Nucleonica', "Physics of Elementary Particles and Atomic Nuclei". Prof. Krasavin is author of more than 200 scientific papers and two monographs. Three Doctoral and fourteen theses for Candidate Degree were defended under his supervision. Prof. Krasavin has been awarded by distinguished JINR Scientific Awards.

Elling Kvamme

M.D., Ph.D., Professor Kvamme was the Head of Neurochemical Laboratory in Oslo University Psychiatric Clinic, and Professor of Neurochemistry UIO (since 1962). In 1962 (six months) he was Technical Assistant Expert at the National Cancer Institute Rio de Janeiro, Brazil, by assignment of the International Atomic Energy Agency, Vienna. He served as the President of the Norwegian Biochemical Society (1976-1978), Treasurer of the International Society for Neurochemistry (ISN; 1977-1981), Chairman of ISN (1981-1983) He is the Knight of St. Olav's Order, First Class (2004). He has numerous publications in American Scientific Journals.

Abel Lajtha

Professor, Director of the Centre for Neurochemistry, New York; Editor-in Chief of Neurochemical Research and Handbook of Neurochemistry and Molecular Neurobiology. Prof. Lajtha is a foreign member of the National Academy of Sciences of the Republic of Armenia.

Levon N. Mkrtchyan

M.D., Ph.D., D. Sci., Professor, Foreign member of the Russian Academy of Sciences, the President of the Academy of Medical Sciences of Armenia. Prof. Mkrtchyan is author of 300 publications and 13 monographs. He was awarded by Commandor's Medal (Belgium), "Gold Disc" and "Gold Star" medals by nongovernmental organizations of USA and Great Britain, medals at international innovative exhibitions in Brussels, Genève, Beijing and Kuwait.

Vladimir Muronetz

Professor, Head of the Department of Animal Cell Biochemistry of Belozersky Institute of Physico-Chemical Biology, and Professor of School of Bioengineering and Bioinformatics of the M.V. Lomonosov Moscow State University. Prof. Muronetz has a great experience in studying the role of oxidative stress and chaperones in the development of neurodegenerative diseases. He has published about 110 papers in scientific journals, and has got a good experience of international collaboration, working in France, Sweden, USA, Italy and China.

Mikhail Ostrovsky

Academician of the Russian Academy of Sciences, Dr. Sci., Professor, is Head of the Department of Molecular Physiology of the Faculty of Biology of the M.V. Lomonosov Moscow State University. He is the President of the I.P. Pavlov Physiological Society, Editor-in-Chief of the "Sensory Systems" academic journal, representative of the Russian Academy of Sciences in IBRO. Prof. Ostrovsky graduated from the Department of Human and Animal Physiology of the Moscow State University, where Academician Koshtoyantz Kh.S. was his teacher and supervisor, founder of the school on molecular and comparative physiology. After years, Prof. Ostrovsky founded scientific school in the field of molecular physiology and pathology of vision. Continuing traditions of his Armenian teachers, Academicians Koshtoyantz Kh.S. and Orbeli L.A., Prof. Ostrovsky developed kind relations with Armenia and Armenian scientists. He is an active participant of meetings and conferences held in Armenia, and greatly contributed for the scientific cooperation between Russian Fund of Basic Studies and Ministry of Science of the Republic of Armenia.

Denise Paulin

She is Professor from Paris who has directed a group devoted to molecular biology of development at the Pasteur Institute and the University Diderot Paris-7. Author of international publications she is an expert in the field of intermediate filaments and has developed animal models of cardiomyopathies and neuromuscular diseases. She received in Paris Armenian students to prepare a Ph.D. diploma as well as colleagues for scientific collaboration. During several years she organized workshops with the Yerevan University and developed the Franco-Armenian Association for Scientific Cooperation (AFACS).

Rima Srapionian

Ph.D., Dr.Sci., Professor of Biochemistry, Head of the Laboratory of Neurospecific Proteins' Biochemistry at the H. Buniatian Institute of Biochemistry, NAS RA. Prof. Srapionian is an expert in neurochemistry, endocrinology, immunology, and bioorganic chemistry. The field of her research work include investigation of neurospecific protein-hormonal complexes, neurochemical regulation of brain metabolic processes, coagulation mechanisms in the heart and blood.

Anna Tishkina

She has a Master degree in Applied Physics and Mathematics, and currently is a Ph.D. student at the Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences, Moscow, Russia. Her scientific interests are: glia, hippocampus, neuroinflammation, oxidative stress, digital image processing.

Mikhail Ugryumov

D.Sci., Professor, N.K. Koltsov Institute of Developmental Biology Russian Academy of Sciences (IDB RAS), Moscow, Russia. He is an Academician of the Russian Academy of Sciences.

Richard E. Weller

Dr. Weller is a Senior Program Manager in the Biological Sciences Division at the Pacific Northwest National Laboratory, and is involved in the development and deployment of innovative biomedical technologies. His primary focus is on diagnostic and therapeutic technologies. He has over 20 years experience developing animal models for biomedical applications. Dr. Weller is board certified by the American College of Veterinary Internal Medicine in Internal Medicine (1980) and Oncology (1987), and holds adjunct faculty positions in the College of Veterinary Medicine at both Washington State University and Tuskegee University, an honorary Professorship at the Institute of Veterinary Medicine in Kyiv, Ukraine. He is a lecturer at the University of Washington's Jackson School for International Studies on weapons of mass destruction (WMD) detection and proliferation. He holds several U.S. patents and has authored or co-authored over 250 unclassified and classified articles, technical reports, book chapters, and presentations in his fields of expertise.

Loussine Zargaryan

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26. **Ugryumov M.V.**
    Developing Brain as a Multipotent Endocrine Organ: Paradox or Reality

ANTITUMORIGENIC EFFECT OF BRAIN PROLINE RICH POLYPEPTIDE-1 IN HUMAN CHONDROSARCOMA

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Proline rich polypeptides (PRP-1) are produced by neurosecretory cells of NPV and NSO cells of the hypothalamus [1]. PRP-1 is one of the fragments of neurophysin-vasopressin-associated glycoprotein (NVAG). This cytokine is also a powerful immunomodulator [1, 2], with antitumor effect [1, 2, 3]. Our recent data indicated 89% growth inhibition of sarcoma cells when treated with this cytokine in comparison with the control and immortalized chondrocytes [4]. The problem of sarcoma is the long time topic of our investigations [5,6]. The fact that the same concentrations of PRP-1 (0.5-1 μg /ml), based on our preliminary results [7] abolished Myc activity in human JJ012 chondrosarcoma cells prompts us to think that the antitumorigenic effect of PRP-1 in the same concentrations is mediated through the oncogene inactivation and involves inhibition of the cell cycle. The bell shaped reminding stimulatory effect of PRP-1 is due to existence of most probably biphasic receptor for PRP-1, when low dose of the agonist exhibits stimulation and high dose on the contrary shows inhibitory effect. For the ligands that induce receptor dimerization, a biphasic dose-response curve has also been observed with optimal receptor activation at intermediate doses, and self-antagonism occurring at higher doses at which monomeric ligand-receptor complexes predominate [8-10]. The targeted inactivation or repair of the oncogenes has been studied as a potentially therapeutically useful approach for the treatment of neoplasia. Recent work in experimental transgenic model systems suggests that MYC inactivation in animal models to be sufficient to induce the regression in lymphoma, leukemia, papilloma, islet cell tumors and osteogenic sarcoma [11-17] although the molecular mechanism by which the inactivation of MYC induces tumor regression is unclear. Our future directions include but not limited to experiments on cell cycle analysis and its key players as well as determination on whether cells undergo through quiescence or permanent arrest under the influence of the low doses of PRP-1. One of the most important tasks would be the precise determination and gene expression of PRP-1 receptors and their subtypes in the tumor tissues in search for possible diagnostic biomarkers.

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SOME MECHANISMS OF SPINAL CORD NEURODEGENERATION IN EXPERIMENTAL PARKINSONISM

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Parkinson's disease (PD) is a complex devastating neurodegenerative disease that affects 1% of the population above the age of 60 worldwide. It takes almost two decades of subtle degeneration of brainstem neurons and loss of dopaminergic neurons in the midbrain substantia nigra pars compacta before the external signs of PD can appear. Loss of dopaminergic neurons is culminating in motor abnormalities, manifested by resting tremor, bradykinesia (slowness of movement), muscular rigidity (stiffness), and postural instability (poor balance). Despite substantial evidence of nigrostriatal degeneration in PD, other areas of the central nervous system have also been recognized as having essential role in Parkinsonism pathology. We postulated that neurons of spinal cord (SC), the final coordinator of movement connected to midbrain via various ascending and descending projections and metabolic circuits, are also implicated in progression of PD.

In order to examine whether SC is affected, two animal models of experimental parkinsonism were investigated: by injecting parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 25 mg/kg, i.p. twice) to adult C57BL/6N mice, and with systemic administration of environmental toxin rotenone (2.5 mg/kg/day, s.c. during 21 days) to Lewis rats. Death of SC neurons was examined using combination of TUNEL assay (cell death marker) and double-immunofluorescent staining with markers of neurons (NeuN, neuronal nuclei) and motoneurons (ChAT, choline acetyltransferase). In MPTP-induced model specific assays, such as MAO-B (monoamine oxidase) expression, DAT (dopamine transporter) immunoreactivity and H-^{3}MPP specific uptake in SC tissue preparations were also performed; *in vivo* conversion of MPTP into active neurotoxin MPP^{+} compound was confirmed by HPLC-photodiode array analysis.

We observed significant neuronal cell death in the SC of both MPTP and rotenone-administered animals compared to control ones. In ventral horn dying neurons were identified as motoneurons by co-localization of TUNEL and ChAT immunoreactivity (IR). Neuronal cell death in SC was accompanied by profound astrogliosis and microgliosis as evidenced from increased glial fibrillary acidic protein (GFAP)-IR and CD11b/c (OX-42)-IR. Detection of active neurotoxin MPP^{+}, as well as MAO-B and DAT immunoreactivity testified that SC is a potential extra-nigral target for parkinsonian toxins (1,2).

The expression and activity of cysteine proteases such as calpain and caspase-3, which are crucial players of apoptosis, were studied to understand molecular mechanisms of SC neurodegenration. Detection of elevated amounts of active m-calpain (76 kD) and caspase-3 (17/19 kD) fragments in SC tissues (Western blot assay), and identification of calpain-specific 145 kD and caspase-3-specific 120 kD spectrin breakdown products from 270 kD uncleaved spectrin (intracellular substrate for calpain and caspase-3), suggested involvement of these proteases in the neurodegenerative process in parkinspnian SC (1,3,4).

These findings of SC neurodegeneration in MPTP- and rotenone-induced parkinsonism in animals were correlated with similar neurodegeneration in the post-mortem SC tissue samples of idiopathic PD patients, compared to other neurological diseases (not published data).

Obviously, SC where locomotion centers are located, with damaged neurons and particularly motoneurons, as has been shown from these studies, may seriously affect and worsen reflex activities as well as impair motor functions, thus contributing to the progression of movement disturbances, related to PD characteristics. Therefore, information generated from such studies may help to develop new therapies for treatment of this complex neurodegenerative disease.

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THE PROBLEM OF GCR ACTION ON THE CENTRAL NERVOUS SYSTEM DURING THE LONG-TERM SPACE MISSION

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An important safety concern for long-term space travel in outer space is the health effects from cosmic radiation. The primary radiation sources in outer space are the galactic cosmic rays (GCR), the solar particle events and protons and electrons trapped in the Earth's magnetic field. The background radiation of the GCR includes 85% of protons, 13% of helium and about 2% from high-energy and high-charge heavy ions (HI). Though the heavy charged particles are less abundant, they posses significantly higher ionizing power with a greater potential for radiation-induced damage and greater penetration power. Possible health risks include different type mutations, cancer, violation of visual functions (cataract, retina lesion), acute radiation disease, damage to the central nervous system. Passage of HI particles can result in direct effect on DNA leading to single strand breaks, double strand breaks, and especially clustered DNA lesions. The character of the DNA damage caused by heavy charged particles is substantially different from that caused by gamma-quanta. Accelerated heavy ions, unlike gamma-quanta, induce mainly the cluster-type damage in the DNA. This kind of damage is a combination of single-step disorders of a DNA part and the formation of single-strand breaks, modification of bases, and sugar modification. The events of this kind result from a local energy release which happens when a heavy charged particle travels through a DNA strand. The cluster-type damage determines the specifics of the lethal, mutagenic (induction of gene and structure mutations in prokaryotes and formation of chromosome aberrations in higher eukaryote cells), and transforming effect of radiation on cells with different genome organization levels.

The integral flux of GCR particles of carbon and iron groups for future exploration missions to Mars equals to 10^7 part cm^{-2} per year. The long range of the HI allows for the potential damage along a long column of cells in tissue. The issues of the damaging effect of heavy charged particles of such integral flux on the central nervous system are important and remain unresolved in many ways. Research in this field seem to be extremely topical for solving cosmic radiobiology problems as there is evidence that behavioral functions of the experimental animals irradiated with heavy ions have been disordered. Low doses of accelerated iron ions cause an irreversible disorder of the cognitive and other functions in an irradiated organism. Research in this important field has also been started at the Laboratory of radiation biology of JINR.

NEUROBIOCHEMICAL MECHANISMS OF THE NERVOUS SYSTEM ORGANIZATION AND PATHOLOGY

COMMON MOLECULAR MECHANISMS OF NEURONAL PLASTICITY AND NEURONAL PATHOLOGY

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Plasticity is the capability of the nervous system to adapt structurally and/or functionally in situations of changing environment, in particular after damage, as well as during development and maturation of the brain gaining experience. The basis for synaptic plasticity in response to physiological or pathological stimuli is molecular changes. A model of plasticity-pathology continuum (McEachern & Shaw, 1996, 1999) was one of main starting points for our concept of pleiotropicity of basic molecular mechanisms underlying both normal neuronal functioning and neuronal damage and death. For example, factors mostly regarded as apoptotic (caspases etc.) are involved in normal neuronal plasticity. Enzymes with broad substrate specificity (proteases, protein kinases, phosphatases) are main players in the chess game between life and death where figures are substrates and positions on the chess-board are cellular compartments. Sometimes the main route of signaling involved in survival, functional changes or cell death is same, while a divergence occurs at the very end of biochemical cascade. In other situations, same factor may trigger different signaling mechanism (e.g. through different receptor types). The concept on the similarity of neuroplasticity and neuropathology mechanisms conforms to the idea that there are no special mechanisms for pathologies in an organism. Pathological changes are developing as a result of disturbances in "normal mechanisms", these mechanisms being realized with a different substrate and/or to a different degree and/or in a different period and/or in a different cellular or exrtacellular compartment. In many cases the switch "norm-pathology" may be defined by specific substrates of "multi-substrate" enzymes. Indeed, this may be the main physiological meaning of broad substrate specificity of key proteases and protein kinases. The idea of the pleiotropicity of molecular mechanisms underlying both normal neuroplasticity and neuropathology gives us a tool to elaborate a strategy for revealing links triggering pathological processes. This link is a potential target for the search for pathogenetically directed approaches for prevention and treatment of specific cerebral pathologies. An example of ignoring the above phenomenon of pleiotropicity is failure to use "antiapoptotic" technologies and NMDA blockers for treatment of cerebral pathologies accompanied by neurodegeneration. Unfortunately, our understanding of molecular processes taking part in neurons remains rather limited. As a rule, at best, general mechanisms involved in neuroplasticity phenomena and neurodegeneration are known, as well as the final results of processes. However, the most important is still obscure: when and how a process common for norm and pathology is irreversibly switching to the realization of neuronal death. However, it is the revealing of this switching mechanism which can give us a tool to manipulate the pathology process and even prevent it.

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MECHANISMS OF HSP70 CHAPERONE-MEDIATED INHIBITION OF INSOLUBLE PROTEIN AGGREGATES IN MODEL OF HUNTINGTON DISEASE

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Aggregates made of misfolded, mutant proteins are the major cause of neuronal death in a great number of neurodegenerative pathologies. One of these illnesses, Huntington's disease (HD), is induced by expanded repeats of glutamine in N-terminal part of huntingtin. The development of the disease initiates from the formation of SDS-insoluble protein aggregates in neurons of striatum and cerebral cortex. The aggregates are formed with the participation of tissue transglutaminase, which catalyzes the ligation of glutamine residues of mutant huntingtin to lysines of other polypeptides, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The results of our experiments proved that GAPDH is implicated in SDS-insoluble aggregate formation, because the factors tightly binding the enzyme reduce both the size and amount of aggregates in the cells over-expressing the gene of mutant huntingtin with Q103 repeat. Conversely, the addition of pure GAPDH to ex vivo system of Q103 aggregate formation significantly increased the amount of aggregating Q103. To explore the mechanism of anti-aggregate activity of Hsp70 in HD model we generated human neuroblastoma cell line with metal-dependent expression of Hsp70. The elevation of Hsp70 level lead to the dose-dependent reduction of both, number and size of aggregates. These data were confirmed by using ex-vivo system of aggregate formation: the addition of pure Hsp70 elevated the amount of soluble polyglutamine-containing protein. Using the method of immunoprecipitation we showed that Hsp70 was able to bind GAPDH in dose-dependent manner: the more Hsp70, the lesser content of GAPDH in insoluble polyglutamine aggregates and the lower amount of the latter. It is concluded that a novel mechanism exists through which Hsp70 sequesters GAPDH from the control of transglutaminase and by this diminishes cell mortality from cytotoxicity of the polyglutamine aggregates.

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THE ROLE OF METAL BINDING DOMAIN OF AMYLOID-B IN INITIATION OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is pathophysiologically characterized by the formation of amyloid plaques as a result of the extracellular accumulation of the amyloid-β peptide (Aβ) in the brain of AD patients. Postmortem biochemical analysis of these aggregates showed that they contain high concentrations of metal ions bound to Aβ and most of Aβ aspartate-7 amino-acid residues undergo a specific chemical modification resulting in the formation of isoaspartate-7 (isoAsp7). Both of these features are related to biochemical and/or biophysical changes of the Aβ metal binding domain (the Aβ residues 1-16). We have found that Asp-7 isomerization results in its zinc-induced oligomerization. Additionally, this post-translational modification alters binding mode of copper to the domain and impacts the N-domain of angiontensin-converting enzyme to change its hydrolysis efficiency towards Aβ. All the findings suggest that the isoAsp7-containing Aβ species (isoAsp7- Aβ) might be a potential trigger of AD.

NEUROLOGICAL DISEASES, PROTEIN AGGREGATES AND CHAPERONES: MODELS AND TECHNOLOGIES FOR PROVING ANTI-DEGENERATION DRUG EFFICACIES

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The reason of many neurodegenarative pathologies is the formation of aggregates of mutant or damaged proteins that are toxic for specific groups of neurons. The examples of such pathologies are Alzheimer's, Parkinson, Huntington diseases whose pathogenic proteins are A-beta amyloid, alpha-synuclein and huntingtin correspondingly. The process of aggregation may be delayed or prevented by molecular chaperones, and therefore substances inducing particular proteins, Hsp70 and Hdj1/Hsp40 are offered as promising candidates for future drugs. We found also a few of natural compounds, mostly O- and S-glycosides of naphthoquinones, able to activate heat shock response in human neuroblastoma and monocytic cells and to restrict aggregation process in in vitro models. Another group of compounds possibly influencing aggregation dynamics includes substances binding glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), since it is involved in the formation of covalent bonds with polyglutamine tract-containing mutant proteins, such as huntingtin. It was shown that Pefabloc (ABTS), dobutamine and deprenyl, all known to strongly bind GAPDH, reduce aggregate size in in vitro model of HD. The third group of compounds blocking the aggregation process in its beginning can include substances interacting with monomers/olygomers of mutant proteins and giving them no opportunity to interact with each other. We have elaborated a few of model systems and assays to test the activity of all three types of above compounds. All assays can be performed in 96-well microplate platform. Two assays are applied for the screening of compounds, one is ex vivo test-system for the estimation of anti-aggregation activity and with the aid of two others one can estimate the activity of small molecules impeding aggregation of polyglutamine tract-bearing mutant proteins.

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ROLE OF CHAPERONES BLOCKED IRREVERSIBLY BY MISFOLDED PROTEINS IN THE INDUCTION OF CONFORMATIONAL NEURODEGENERATIVE DISEASES

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Recently, it has been demonstrated that the oligomeric forms (28-36 of monomers) of the recombinant ovine prions exhibit neurotoxic action, while high molecular aggregates of the prions are harmless. Previously, we demonstrated blocking of the chaperones by misfolded proteins that are similar in their characteristics to the pathogenic prion forms and suggested a hypothesis according to which chaperones can be involved in the development of neurodegenerative diseases (1-3). In the presented work, we used the dynamic light scattering analysis to determine the size of the aggregates formed while the transition of the recombinant ovine prions into their pathogenic form, and also investigated the possibility of purposeful changing the size of the aggregates. We demonstrated that in the presence of copper ions, as well as in an acid medium, the recombinant prions VRQ transform from the monomeric to oligomeric form (the size of the aggregates constituted 12-15 nm). The extent of aggregation of the oligomers alters in the presence of the molecular chaperones. It was shown that at 25^{o}C, the non-functioning chaperonin GroE (equimolar mixture of GroEL and GroES in the absence of Mg-ATP) bound the prion yielding large aggregates (greater than 400 nm). The addition of Mg-ATP decreased significantly the aggregate size to 70-80 nm. Blocking of one of the chaperonin centers by misfolded proteins (for example, oxidized denatured glyceraldehyde-3-phosphate dehydrogenase) increased the aggregate size to 1200 nm, and the addition of Mg-ATP did not prevent the aggregation. In contrast to the proteinase-resistant oligomeric prions, the aggregates formed in the presence of the chaperonin were capable of cleaving with proteinase K. Besides, it was demonstrated that the large aggregates of the amyloid-beta peptide 1-42 are formed in the presence of misfolded forms of proteins including glyceraldehyde-3-phosphate dehydrogenase, the enzyme whose enhanced content in the brain was observed while modeling Alzheimer disease. We suggest that chaperones and misfolded proteins play an important role in the development of neurodegenerative diseases, regulating the size of amyloid structures and their sensitivity to proteolysis. We demonstrated that natural (chaperones and misfolded proteins) and artificial (polyelectrolytes) modulators of amyloid structure formation could be used to decrease the pathogenic properties of amyloidogenic proteins and to decelerate the development of neurodegenerative disorders. It is suggested to create nanoparticles based on polyelectrolytes, chaperones and misfolded proteins that are capable to penetrate through the hematoencephalic barrier for prophylactic and treatment of Alzheimer disease as well as for reducing the neurotoxicity of infectious prions.

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THE INFLUENCE OF HYPPOCAMPUS STIMULATION IN NEURONAL ACTIVITY OF RESPIRATORY CENTRE UPON HYPOXIA CONDITION

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The issue of body's hypoxic conditions was and remains one of the urgent problems of biology and medicine in view of the fact that even healthy body of both human and animal, depending on their living conditions and the intensity of their activity, always comes across with oxygen deficiency. In pathogenesis of each disease lies more or less violation of body oxygen homeostasis. The issue of hypoxic conditions is especially urgent for our country, since 90% of its territory (including the capital Yerevan) lies at 1000 m above the sea level. Because of this, the knowledge of delicate mechanisms of respiratory regulation upon hypoxia has not only theoretical but also applied meaning.

Upon hypoxia preservation of body's gaseous homeostasis happens by means of interaction between bulbar respiratory centre and suprabulbar formations. It is known that the limbic structures influens the important functions of the body, as well as the respiration. However there are no experimental studies investigating the role of one of the most important limbic nuclei, i.e. the hyppocampus, during the respiratory regulation of hypoxia. In connection with this, the goal of our study is the investigation of medulla oblongata's respiratory neurons and respiration's reactions on electrical stimulation of hippocampus in the condition of hypoxic influence.

White rats were used in these studies. The experiments have been conducted in the dynamics of hypoxic influence. The investigated values were registered before the "ascent" of the animal, i.e. in conditions of normoxia (pO_2=142 mm), on the altitude of 4-5 thousand meters (pO_2=109-85 mm), on the altitude of 7.5-8 thousand meters (pO_2=64-58mm) and after the "descent", in conditions of normal atmospheric pressure, before and right after the stimulation of hyppocampus. At the beginning, the reaction of respiratory neurons upon hippocampus stimulation was carried out in normoxia, i.e. before the animal "ascent", which worked as a control for the experiments carried out in the condition of acute hypoxia.
In normal conditions stimulation of hippocampus had a prevalence inhibiting influence - 70% neurons were inhibiting, 25% - were activated, and 5% were areactiv. The inhibiting influence affected also the level of neuron's impulse activity change. At the initial stage of hypoxia (the altitude of 4-5 thousand meters), the impulse activity of all the functioning neurons in the conditions of hypoxic influence became more frequent. On that "altitude" stimulation of hippocampus had a more inhibiting influence - 78% neurons were inhibiting, 19% - were activated and 3% were areactiv. During the second stage of hypoxic influence (7.5-8 thousand meters), the change in the impulse activity of respiratory neurons was expressed in the decrease of impulse discharge, and in several cases—in complete inhibition of their activity.

All these indicate that in respiration regulation, the interaction of various integration levels is decisive and not just one regulation level. And only such integration of cortical and sub-cortical, of central and peripheral, of activating and inhibiting mechanisms can provide a more and reliable adaptation of the body to constantly changing conditions of oxygen consumption.

PROLIFERATION OF CELLS IN RAT BRAIN DURING EXPERIMENTAL EPILEPSIA

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Epilepsy is one of the most common neurological disorders. Temporal lobe epilepsy (TLE) is characterized by spontaneous recurrent seizures, learning and memory impairments, and depression. Over the last decade, alteration in the adult dentate neurogenesis has been recognized as a hallmark of TLE. This alteration includes an increase in generation of new neurons in the DG and their abnormal migration from the subgranular zone (SGZ) into the dentate hilus after epileptic seizures. In the adult brain, neurogenesis occurs in the SGZ and the subventricular zone (SVZ) of the lateral ventricles. Neurogenesis in the SGZ can be elevated for long time after seizures but eventually declines to the basal or even lower level. Similar effects could be observed in the SVZ but most studies focus on examining of the SGZ because epilepsy causes cognitive impairments including hippocampus-dependent forms of learning and memory.

Methods

Repeated intraperitoneal administration of a subconvulsive dose of pentylenetetrazole (PTZ) is an established approach to induce progressive seizure activity also known as kindling. This model can be used as suitable model of epileptogenesis. In the present study, we used PTZ-induced brief repeated seizure model to examine the temporal profile of neurogenesis in the germinative zones of adult rat brain and to investigate whether the new cells generated in the DG play a role in reparation of the hippocampus after kindling. Experiments were carried out on male Wistar rats, aged 8 weeks at the beginning of the experiments, weighing 150-200g. Over a period of 4 weeks, the animals were injected with subconvulsive doses of PTZ (37.5 mg/kg; i.p.) dissolved in isotonic saline. The rats received PTZ or vehicle injection (1 ml/kg) three times a week. After each injection convulsive behavior was monitored for 20 min, and resultant seizures were classified according to Franke and Kittner (2001) as follows: stage 0, no response; stage 1, ear and facial twitching; stage 2, convulsive waves axially through the body; stage 3, myoclonic jerks and rearing; stage 4, clonic convulsions with falling down and loss of body control. One day after 1, 3, 5, and 13 PTZ or saline injections the rats were subjected to i.p. injections of thymidine analog BrdU at a dose of 50 mg/kg. Each rat (n = 4 per each group) received four BrdU pulses with 2-h interval. The animals were sacrificed 2 h after the last BrdU injection. This protocol allowed us maximally assess the number of cells that had incorporated BrdU, i.e. proliferated cells, one day after respective number of PTZ-induced brief seizures or saline treatment. After the brains were taken and fixed, we embedded them into paraffin and cut 10-μm coronal sections through the entire brain. Immunohistological staining fro BrdU and nNOS was performed. Other animals (n=6-8 per each group) were sacrificed without BrdU injections, and hippocampi were taken for biochemical research. After homogenization, RNA and protein fractions were separated, and the levels of nNOS mRNA and protein were measured.

Results

There was a basal level of BrdU incorporation into cells in the SGZ in the control animals. BrdU-labeled cell nuclei in the hippocampus were irregular in shape and located mainly in the SGZ. However, sometimes, they could be found in the hippocampal hilus as well. BrdU-labeled nuclei were observed as clusters of 2-4 nuclei, suggesting the ongoing cell division.

Immediately after single PTZ treatment, rats exhibited no changes in their behavior, inhibition of motor activity, or subtle ear and facial twitching. Single myoclonic jerks along the body axis were observed in 80% of PTZ injected animals. One day after the first PTZ-induced seizure episode, a clear decrease in the number of BrdU-labeled nuclei was revealed. This effect was even more expressed in the hilus. Three or five PTZ injections induced more expressed changes in rat seizure behavior. All rats demonstrated progression of myoclonic waves along the body axis and myoclonic jerks of fore- and hind limbs with a rapid onset. The numbers of BrdU-labeled nuclei after 3 or 5 seizure episodes remained to be decreased in both these groups of animals as compared to the control group. However, they were higher to some extent as compared to the rats underwent single PTZ injection. After 13 PTZ injections, the rats exhibited generalized clonic seizures with fell down on their side or back or tonic seizures, which lasted for about 7-10 s. The myoclonic jerks followed by development of clonic seizures occurred several times. We studied the cell proliferation rate one day after the 13^{th} PTZ-elicited seizure episode. We did not find any difference between the PTZ-treated and control rats in the number of BrdU-labeled nuclei. Moreover, the number of BrdU-labeled nuclei after 13 PTZ injections was significantly higher as compared to the group of animals treated with PTZ only once.

Also we investigated cell proliferation in the SVZ of the lateral ventricles after 1, 3, 5, or 13 PTZ injections. Interestingly, in this brain region, PTZ-evoked seizures also induced changes in cell proliferation, which were similar to those found in the hippocampus. We observed a strong decrease in the number of BrdU-labeled cells after the first seizure episode, which was followed by a slow restoration in their number after 3 or 5 PTZ-elicited repeated seizures. After 13 seizure episodes, the number of BrdU-labeled nuclei was not significantly differed as compared to the control group.

Nitrergic system is one of the important factors involved in the regulation of proliferation and differentiation processes. At the other hand, as it was previously shown, that nitrergic system undergoes serious changes during seizure activity. In our study, a decrease of nNOS mRNA in the hippocampus after the 5-th PTZ injection was observed along with a decrease in the number of nNOS-positive cells in CA1 area and radial layer of hippocampus. Since it is known that NO depresses the proliferation of progenitor cells in the brain, it can be suggested that compensatory changes in nitrergic system accompany gradual recovery of cell proliferation rate in germinative areas.

BRAIN DERIVED PROLINE RICH PEPTIDE HAS ANTI ISCHEMIC/APOPTOTIC ABILITIES IN VITRO AND IN VIVO

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Efficiency and safety of current means to protect and prevent the organ from ischemia is suboptimal due to numerous etiological preconditioning for humans. The ability of Proline Rich Neuropeptide, newly isolated from the nucleuses supraopticus (NSO) and paraventricularis (NPV) along with the vasopressin and oxytocin, was characterized in the models of isoproterenol – and epinephrine- induced cardiac ischemia as well as in vitro (brain neuronal cell culture) where it was evaluated its pro/anti apoptotic abilities. The peptide was injected prior to the subcutaneous double or single injections of cathecholaminas. In our experimental settings three parameters were evaluated: leakage of cardiac troponin I, as a gold-marker of cardiac impairment severity, myocardial lipid peroxidation and the synthesis of reactive oxygen species by the mononuclears of experimental animals' peripheral blood. The high efficiency of protection was notable after injection of very low PRP doses 5-10 ug/100 g of animals' weight. Interestingly, 18 ug/100 g of the peptide had vivid opposite effect, which was stimulation of cardiac markers out flux for additional 12-20%. %. The same very low doses of newly brain derived peptide were protective against membrane lipid peroxidation of cardiomyocytes. In the same experimental groups preinjection of the neuropeptide's effective doses reduced generation of the reactive oxygen species by mononuclears of the peripheral blood. Low doses of PRP were protective for brain hippocampal/cortical neuronal cell culture. We suggest that PRP acts not via the local mechanisms of regulation, binding with the alpha- and beta receptors or influence on the activity of the mononuclears, but also has a wide, hormonal like influence acting via cell signaling system.

INTRANASAL DELIVERY OF EUKARYOTIC CELLS TO THE BRAIN

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Cell-based therapy (CBT) has been proposed as a strategy for the treatment of neurodegenerative diseases. The success of CBT for neurodegenerative disorders depends on the therapeutic properties of the cell type, on the method and safety of administration, on the amount of cells delivered to the site of injury and finally on the avoidance of excessive incorporation of the therapeutic cells into other organs and systems. Transplantation of therapeutic cells into the brain is one of the most frequently used invasive approaches to promote recovery of the CNS from injury or disease. However, transplantation may raise problems not only because of graft rejection as a result of an immunological response to the transplants but also due to the mechanical tissue damage from this traumatic and expensive surgical procedure.

We hypothesized that intranasally administered eukaryotic cells could bypass the blood-brain barrier by migrating from the nasal mucosa through foramina in the cribriform plate along the olfactory neural pathway into the brain and cerebrospinal fluid (CSF) while reducing or eliminating delivery of the cells to peripheral organs, thus reducing unwanted side effects. This hypothesis was tested by intranasal (IN) application of mesenchymal stem cells in adult mice and glioma cells to young rats. Within one hour of administration, both cell types reached the olfactory bulb, cortex, hippocampus and cerebellum. After cells crossed the cribriform plate, two migration routes were observed: 1) migration into the olfactory bulb and also to other parts of the brain including the cortex and striatum; 2) entry into the CSF with movement along the surface of the cortex followed by entrance into the brain parenchyma. IN-delivery of cells to the brain was enhanced by administration of hyaluronidase.

This biological pathway of cell migration from the nasal mucosa to the brain thus provides an opportunity for the development of cell delivery methods for therapeutic and experimental use in treating neurodegenerative disorders and creating brain tumor models.

INFLUENCE OF EXTERNAL NOISE ON SINGLE ION CHANNELS

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Measurements of fluctuations of currents through membrane provide valuable information about the mechanism of ion transport. The model that closely describes probabilistic behavior of a current through a single ion channel is a two-level system with randomly timed transitions. These transitions occur as a result of random changes of channel forming protein conformations under the influence of internal noise. It is shown that if transition speeds are independent of time, it leads to fluctuations of currents through membrane, i.e. noise of current occurs. This type of noise is described as internal noise. However, along with internal noise the system can also be influenced by external noise. Its influence is expressed in the fact that transition speeds, which depend on environment parameters, are not constant and depend on time randomly. This fact also results in fluctuations of currents through membrane. Taking this into consideration a significant problem of identification of noise arises. The present work studies the influence of external noise on current through two level system and reveals the characteristic features of the current.

THE ESTIMATION AND COMPARISON OF CEREBROLYSIN AND TANAKAN PROLONGED EFFECTS ON LIPID PEROXIDATION PROCESSES IN DIFFERENT BRAIN REGIONS UNDER THE HYPOKINETIC STRESS CONDITIONS

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Introduction Hypokinesia (HK) – restriction of body movement activity, is one of the dominating stressors of modern lifestyle. It leads to the development of different disorders, especially neurological, in the pathogenesis of which the displacements of lipid peroxidation (LPO) processes play an important role. Cerebrolysin and tanakan are multicomponent drugs of natural origin, which have been shown to exert antioxidant and expressed neuroprotective properties. However, it is known that antioxidants depending on administration conditions can be converted into prooxidants and stimulate LPO.

Aim The aim of this study was to reveal and compare the effects of long-term administration of tanakan and cerebrolysin on the mechanisms and intensity of LPO processes in different brain regions (cerebral cortex, hippocampus, hypothalamus and cerebellum) on the 45th day of HK.

Methods The study was carried out on 144 white male rats weighting 160-180g. The HK was modeled by the method offered by Pheodorov I.V. Tanakan (1.7mg/kg/day) and cerebrolysin (15mg/kg/day) were injected intraperitoneously everyday at the same time with HK. The activities of pro- (NADPH- and ascorbate-dependent LPO (NDLPO and ADLPO correspondingly)) and antioxidant systems (superoxide dismutase (SOD), glutathione reductase (GR), total SH groups (TSHG)), as well as the malondialdehyde (MDA) level (marker of LPO intensity) were determined by spectrophotometric methods. The activity of catalase was defined by permanganatometric method.

Results The results of this study showed that 45 days HK significantly increases the activity of NDLPO system in all brain regions, especially in cerebellum, while inhibits ADLPO system activity. Only in hypothalamus it was observed a significant stimulation of ADLPO processes. At the same time with HK the everyday administration of tanakan significantly prevented the activation of NDLPO system in hypothalamus and cerebellum and expressed corrected the displacements of ADLPO in all brain regions. In contrary to tanakan, cerebrolysin significantly stimulated NDLPO processes in studied brain regions, except cerebral cortex. ADLPO system activity also was increased under the action of cerebrolysin. Only in hypothalamus it prevented the intensification of ADLPO. Concerning to antioxidant system activity, 45 days HK led to the inhibition of SOD, but activation of catalase in examined brain regions. In cerebral cortex, hippocampus and hypothalamus it was observed an inhibition of GR. The level of TSHG was increased in cerebral cortex, but decreased in hypothalamus and especially in cerebellum on the 45th day of HK. Tanakan prevented the inhibition of SOD in cerebellum and activation of catalase in studied brain regions, except cerebral cortex. The activity of GR and the level of TSHG were statistically significant changed and decreased only in hypothalamus and cerebellum correspondingly. The long term administration of cerebrolysin promoted SOD inhibition in hippocampus, while prevented it in cerebellum. The displacements in the catalase activity in all studied brain regions, as well as in the GR activity in cerebellum and TSHG level in cerebral cortex and hypothalamus were significantly corrected under the action of cerebrolysin, whereas in the GR activity in hypothalamus and TSHG level in cerebellum they were promoted. As a result of above mentioned changes in pro- and antioxidant systems activities the hypokinetic stress on the 45th day significantly increased MDA level in hypothalamus, but decreased it in cerebral cortex and cerebellum. The

everyday administration of tanakan prevented the MDA level displacements only in cerebellum, while cerebrolysin decreased MDA level in hippocampus and stimulated its increase in hypothalamus.

Conclusions In summary the obtained data have shown that 45 days hypokinetic stress makes its own specific signature on the mechanisms and intensity of LPO processes in different brain regions and leads to their intensification in hypothalamus and inhibition in cerebral cortex and cerebellum. These can alarm the pathological nature of 45 days HK and development of motor disturbances. In general, the use of tanakan prevented the displacements of pro- and antioxidant systems activities, as well as the inhibition of LPO processes in cerebellum. Cerebrolysin significantly stimulated prooxidant system activity and mainly inhibits antioxidant system activity. The stimulation of LPO processes in hypothalamus and inhibition in hippocampus can provoke the pathological nature of HK and development of cognitive dysfunctions under the long-term use of cerebrolysin. So, tanakan can be offered as an effective medicine for prevention of negative consequences of HK, while the long term administration of cerebrolysin can even promote the development of neurological disorders.

EXPERIMENTAL MODEL OF BIPOLAR DISORDERS AND TREATMENT BY NEW LI^+-CONTAINING PEPTIDES

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The experimental model for bipolar disorders (BD) is elaborated in the Laboratory of Pathological Biochemistry, Institute of Biochemistry of NAS RA. The extreme psycho-emotional stress situations are induced at white rats during 14 days considering circadian and biological rhythms. The experiments were carried out before and in 14 days after on animals using apparatus open field, Morris water maze, elevated plus maze, etc. The status of animal's biofield was registered on "Bioscope", which allows putting on the indices of Li-containing peptides effect on animal state, soundness and durability by non-contact recording. Registration was made as a streaming operation during 2-7 days.

The obtained results indicate that the stimulation of animals during 14 days at certain hours (dependent on the type of stimulation, such as: swimming, cold stress, orthostatic collapse, respiration of ether vapors, etc.) induces development of fear, aggression, and inhibits the activity. The frequency of miction and defecation increases. A single intra-abdominal introduction of the preparation # 1 (2,5 mg/100 g of the white rat weight) produces an effect on the 10^{th} min (13.00) and continues for 100 min (14.40). The effect is again manifested in 50 min (15.30) and continues for 180 min (18.30). Its effect stops for 300 min (until 23.30) and activates again at the 150^{th} min (02.00). The effect of the preparation # 2 (2,5 mg/100 g of the white rat weight) has been started since the 20^{th} min and continues for 50 min. No activity is observed for 120 min and the effect is noted in 30 min after that. Peak of the effect for the preparation # 1 is recorded at 16-19 h., while for the preparation# 2 it is 17 h. Thus, the animals tested with BD are sensitive to Li^+- containing peptides at the period of 16-19 h. The biorhythms are more active in the evenings. The rats treated for 48 h were again tested on apparatus. The results indicate positive change in animal behavior for about 20-30%. Introduction of the preparation for the second time (in 24 h after the first introduction) improves the state of the animals for 40-45% and produces its effect for 15 days. The effect of the preparation # 2 is more active in compare with the preparation # 1.

THE DYNAMIC OF RAT LIVER AND BRAIN MITOCHONDRIA ULTRASTRUCTURAL CHANGES AT THE CRUSH SYNDROME AND ITS CORRECTION BY PRP

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Today one of the most actual questions is to study the pathology of Crush syndrome (CS) and searching the ways prevent the metabolism disturbances caused by toxins formed in ischemic muscle during decompression. The aim of this study was to find the dynamic of ultrastructural changes of mitochondria (Mch) of liver and brain at CS and its correction by PRP. In this study 9 Wistar rats' liver and brain were used. The animals were divided to 3 groups: 2 hr compression, 4 hr decompression and 4 hr decompression + PRP. CS was used by a compression of femoral soft tissues using a special press during 2 hr by forces of 100 kg/kg body weight. At the end of experiments were taken liver and brain specimens, and was excrete brain Mch fraction. The material was treated by the standard method used in transmission electron microscopy and viewed under the electron microscope TESLA. As has shown our study at 2 hr compression brain and liver Mch were presented in groups where the organelles close contacted to each other but sometimes the swollen organelles also were meet. The cristae were presented like tubules or of vesicle type. But as distinct from liver, brains Mch show the tendency to form giant Mch. At 4 hr decompression are presented swollen Mch with various size and shape. The process of Mch inner membrane with practically completed reduction takes place. It is typical the presence of giant Mch in liver and increase its number in brain. At the injection of PRP such destructive changes of structural safeness of Mch are not presented. Mch are small in size and presented in groups with tubular or of vesicle type of cristae. It is typical for this group presence of Mch fission process in brain. At 2 hr compression the increasing of catecholamine level lead to dilation of district circulation of the blood; as a result takes place the ultrastructural changes of Mch of liver and brain with disturbance of energy function of lasts. By the way at 4hr of decompression the toxic peptides formed by ischemic muscle makes the organelles condition more critical. The injection of PRP in organism on the background of SC neutralize the toxic peptides effect leads to liver and brain Mch, and also decreases the catecholamine negative influence on the organelles ultrastructure. Our investigation demonstrated that Mch of brain are more subject to changes at 2 hr of compression than liver Mch, but injection of PRP on the background of SC prevent progress of Mch ultrastructural changes typical at 4hr of decompression, and leads to Mch fission.

SLEEP DISORDERS AND IMMUNITY: AN OVERVIEW

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Sleep has been connected to immune system since antiquity. With recent advances in neurobiology and biochemistry of sleep the mechanisms of this link become clearer yet in need of more precise knowledge. Neurochemical mechanisms of sleep are strongly related to immune system activity. There is already a bulk of basic and clinical research evidence on this issue rapidly increasing in amount. There are several areas of this interaction to be expressed.
Normal sleep has its specific immune status characteristics. Especially, the pineal hormone melatonin has a bidirectional connection with immune system influencing and being influenced by immune status changes. Melatonin secretion is a vulnerable process and any external signals influencing sleep will definitely have impact on its excretion pattern.

Derangements in sleep regulation lead to identifiable changes in immune system activity. Particularly, both clinically distinguished types of sleep disturbance – sleep deprivation and hypersomnolence - are influencing immune system. Vice versa, immune response states (infections, critical care conditions, etc.) are causing quite universal sleep pattern changes, with sleepiness being one of the most important symptoms of acute infectious state.

Cytokines as per recent research data have potential role in sleep-immunity connections. This has its role not only in acute states but chronic inflammatory diseases also can influence sleep. Advancement of our knowledge on sleep and immunity is also possible with progress in clinical sleep research. Sleep medicine made a “big jump” forward during recent two decades. Becoming a separate medical discipline in developed countries it incorporates issues on sleep from various other medical fields. Neurology, psychiatry, cardiology, endocrinology, pulmonology, otorhinolaryngology, anesthesiology and some other specialties have their interest in sleep disorders.

Several sleep disorders are currently closely related to immune system. The most commonly seen disorders are insomnia and obstructive sleep apnea syndrome (OSAS). And they have implications in immune system through different interactions, like autoimmune response and pro-inflammatory states, etc. Experimental sleep loss has identified several alterations of immune system functioning mediated through the cytokine network. Chronic insomnia in population studies has been shown to be linked to higher all-cause morbidity and mortality which also could have some implications for immunity.

Excessive daytime sleepiness (EDS) is a common symptom affecting approximately 5–15% of the general population and is often caused by sleep deprivation. OSAS, narcolepsy and idiopathic hypersomnia are the most common sleep disorders associated with EDS. There is evidence that immune-mediated cell signaling may be involved in the pathophysiology of sleep disorders associated with EDS. OSAS is a disorder of upper airway with periodically occurring breathing pauses due to upper airway partial or complete obstruction. It leads to hypoxemia, arousals and increased risk of cardiovascular complications and type II diabetes. In otherwise healthy people, obesity has been related to the increased daytime sleepiness. There is also a high prevalence of obesity in patients with OSAS. Adipose tissue produces several proinflammatory and anti-inflammatory factors that have been implicated in the pathogenesis of sleepiness, the development of insulin

resistance and the pathogenesis of cardiovascular disease, which are commonly observed in OSAS and obesity.

Narcolepsy is a disease of sleep-wake regulation with symptoms of EDS, sometimes like sleep attacks, cataplexy, sleep paralysis and hypnagogic hallucinations. Narcolepsy has been associated with deficiency of orexin production. Orexins are neuropeptides produced in the hypothalamus, and there is evidence that they are involved in the regulation of energy homeostasis and the sleep–wake cycle via interaction with ghrelin, leptin and insulin. The relationship of the cytokine brain network with the neuroendocrine system suggests the possible role of cytokines in pathogenesis of narcolepsy. It has been shown that narcoleptic patients have higher plasma levels of TNF-a compared with controls matched for age, sex and body-mass index. Narcolepsy is characterized by a strong link to the human leukocyte antigen (HLA) complex. Moreover, recent evidence approaches a conclusion that narcolepsy is an autoimmune disease.

As per this overview sleep has strong links to different aspects of immunity and these relations are crucial for the progress of basic medical science and understanding the mechanisms of several prevalent and dangerous medical conditions.

THE POSSIBLE REGULATORY EFFECT OF HYPOTHALAMIC PROLINE-RICH POLYPEPTIDE (PRP) AT ENERGY METHABOLISM

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In the presented work the effect of proline-rich polypeptide (PRP), obtained from cattle hypothalamus by A.A. Galoyan, on the activities of Mg^{2+}-, Ca^{2+}- and HCO^-_3-dependent ATPases in the isolated mitochondria of brain and liver of white rats at various age groups was studied.
1- and 6-months old rats were used in experiments.

Results have shown that catalytic activity of Mg^{2+}-dependent ATPase in the isolated mitochondria of brain and liver of 1 month old rats is much higher in comparison with that of 6 months old rats. Thus, in comparison with control, in mitochondria of 1 month old rat brain at addition of PRP the activity of enzyme increases for 12.6% and in liver – it does for 18.7%. At 6 months old rats the stimulation of enzyme activity by PRP exceeds the results received at 1 month rats about two times.

The obtained data have shown that at addition of PRP to mitochondrial fraction of 1 month old rats the activity of Ca^{2+}-dependent ATPase increases for 12.5% in comparison with the control. However, under these experimental conditions 6% inhibition of enzyme activity was observed at 6 months old animals. Unlike brain mitochondria, the PRP in liver authentically activates Ca^{2+}-dependent ATPase much more at 6 months old rats (on 24%) than at 1 month old (15.4%).

Interesting results were also obtained regarding regulatory role of PRP on the activity of HCO^-_3-dependent ATPase. The activity of the enzyme in both - brain and liver mitochondria of 1 month old rats authentically increased at PRP addition. More intensive stimulation of enzyme activity (by 28.8 and 24.1 % in brain and liver mitochondria, respectively) was observed at 6 months old animals.

Comparing the obtained data it is possible to make the following conclusions. The enzyme activities of Mg^{2+}-, Ca^{2+}- and HCO^-_3-dependent ATPases in brain and liver mitochondria of 1 month old rats is higher than at 6 month old ones. PRP isolated from neurosecretoty granules of hypothalamus-hypophyseal system promotes the activities of the studied enzymes. Particularly, it was observed in case of Mg^{2+}-dependent ATPase, both in brain and liver mitochondria. The inhibition of Ca^{2+}-dependent ATPase was observed only in the brain of 6 months old rats. In other cases the activating influence of PRP on different ATPases was higher at 6 months old rats. Taking into account the obtained results, a possible regulatory effect of PRP on energy metabolism has been proposed depending on animal age.

ESTIMATION OF ANTIMUTAGENIC ACTIVITY OF PROLINE-RICH POLYPEPTIDE BY THE COMET-ASSAY IN HUMAN LEUKOCYTES

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Proline–rich polypeptide (PRP-1) was earlier shown to exert wide range of biological activities. It was thoroughly investigated as a potential pharmaceutical preparation. This polypeptide was demonstrated to promote the oxygen burst in neutrophils and macrophages and have a strong effect on the regulation of myelo- and lymphopoiesis.

Earlier we revealed a week genotoxic activity of PRP-1 *in vitro* in the human myeloid leukemia KCL-22 cell line by the Comet assay (single-cell gel electrophoresis). It the present work we have investigated the PRP-1 effect on the mutagenesis induced by Mytomicin C (MMC), a widely used model mutagen.

Human whole blood was treated with MMC or both MMC and PRP-1 for 2 h at 37^0C. Levels of DNA damages were analysed by the Comet assay. Images of comets were captured by the high sensitive video camera (Variocam, PCO, Germany) and analyzed using the Komet 4 software (UK). The results were statistically treated by Mann-Whitney test.

MMC was shown to induce the DNA damage at concentrations 5, 15 и 25 μg/ml (percent of DNA in comet tail was equal to 22.71 ± 1.58, 15.62 ± 1.44 and 23.89 ± 2.51, respectively; the control value was 5.85 ± 0.95%). Adding of PRP-1 at concentration 4 μg/ml led to the decrease of DNA contents in comet tail (9.83 ± 1.09, 7.22 ±1.4 and 10.69 ± 1.62%, respectively). This effect was revealed both at pre- and post-treatment of cells with PRP-1. The results obtained suggested the protective action of PRP-1 against DNA damage induced by MMC.

EFFECT OF HYPOTHALAMIC PROLINE-RICH PEPTIDE PRP-1 ON RAT BRAIN PLASTICITY UNDER LABYRINTHECTOMY AND VIBRATION

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Nervous system plasticity has been studied as a one of the fundamental aspects of neurobiology. Series of experiments were conducted on white laboratory rats (250±50g) anaesthetized with Nembutal (40mg/kg, i/p). Animals divided to five groups were subjected to: 1) right side labyrinthectomy; 2) vibration; 3) vibration in 2 days after labyrinthectomy; 4) i/m injection of the hypothalamic neuroprotective proline-rich peptide (PRP-1, 50mkg/kg) in 2 days after the labyrinthectomy; and 5) same injection 2 hours prior to the vibration. Labyrinthectomy was done by electro-coagulation of the vestibular nerve by Mocrausova method with direct current of 8.0-8.5 mA for two minutes, followed by the removal of the brains in 15 days. Vibration (0.4mm amplitude, 60Hz) was performed for 15 days, 2 hours per day. Histochemical method on detection of Ca^{2+}-dependent acid phosphatase activity (APh) [1] was applied for the morpho-functional study.

Data obtained revealed hypertrophied and deformed cells with no processes in the cerebellar n. Dentatus, nuclei of the Hypoglossal and Solitary nerves, hypothalamic SON and PVN, LC, pyriform and olfactory complexes of the limbic brain, Hippocampal complex and anterior and dorsal horns of the spinal cord in result of the unilateral labyrinthectomy and the vibration, compared to the control.

The degenerative changes were deeper in the rats exposed to the vibration compared to the changes in the rats exposed to the complex action of labyrinthectomy and vibration. In the latter, we detected regeneration and tendency to normalization of the cells that resembled the picture revealed in the control rats. The cells, which have higher APh activity than those in the brain nuclei of the exposed to the vibration rats, are hypertrophied; however, they have dendrites with demonstrated synaptosomes, like the cell bodies. This indicates an increase of the brain activity through the afferent inputs from the various brain regions. In the series of experiments aimed to study the PRP-1 action on the neurodegenerative processes, a significant number of degenerative cells were revealed in some brain regions of the rats exposed to daily vibration for 15 days. Perhaps, one-time administration of PRP-1 does not contribute to the complete survival of the neurons after the prolonged vibration. As for the PRP-1 action on the rats with labyrinthectomy, high APh activity was detected in the morphologically normal neurons in many brain regions. However, in this series of experiments, the neurons with the nuclear staining were revealed in almost all brain regions together with the cytoplasmatic staining, in contrast to the rats with the labyrinthectomy only, where APh activity was detected only in the nuclei of the magnocellular neurons of the hypothalamic supraoptic nucleus and in the cells of the brain limbic region. The results of this report and our earlier immunohistochemical investigations by using the antiserum to PRP-1 indicate potential neuroprotective action of the PRP-1 against the abovementioned neurodegenerative processes. We plan conducting new immunohistochemical investigations using several neuronal markers for deeper understanding of the regenerative processes in the mechanism of the brain plastic reconstruction.

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INFLUENCE OF AFFECTIVE SYMPTOMS ON QUALITY OF LIFE IN PATIENTS WITH PARKINSON'S DISEASE

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Objectives: To quantitatively assess quality of life (QOL) and find correlations with depression and anxiety in patients with Parkinson's disease (PD).

Background: Affective disorders are commonly seen in PD being possible preclinical risk factors. Depression and anxiety are also associated with lower QOL in patients with PD. Relation between affective symptoms and PD itself is not clear. Biological rather than reactive basis for mood disorders is suggested.

Methods: Fifty-nine non-demented PD patients aged 43-80 (Mean age=66.4 years, F=47.5%) were enrolled in the study. PD was diagnosed according to UK PDS Brain Bank criteria. Patients passed evaluation through UPDRS domains, including Hoehn&Yahr (H&Y) staging. Hamilton Depression Rating Scale (HAMD) and Hamilton Anxiety Rating Scale (HAMA) were used for assessment of depression and anxiety. Severity of depression was assessed according to HAMD score: mild depression (10-16), moderate (17-27), severe (>27). Severity of anxiety was assessed according to HAMA score: mild anxiety (18-24), moderate (25-29), severe (>29). SF-36 quality of life inventory with 8 domains' (D) ratings (best QOL – 100, worst - 0) has been used to assess QOL: D1 – Physical functioning, D2 – Role-limitations due to physical health, D3 – Role-limitations due to emotional problems, D4 – Energy/fatigue, D5 – Emotional well-being, D6 – Social functioning, D7 – Pain, D8 – General health. Proportional analysis and Spearman's correlation test were used for statistics.

Results: Enrolled patients had H&Y stages 1-4. Thirty patients (51%) had clinically significant depression (14% - severe, 37% - moderate) and 8 patients (13.6%) had marked anxiety (3.4% - severe, 10.2% - moderate). Analysis of SF-36 revealed the following mean rates for SF-36 questionnaire's domains: D1 – 47.6, D2 – 20.7, D3 – 24.6, D4 – 57.4, D5 – 54.3, D6 – 46.2, D7 – 50, D8 – 36.3. The lowest QOL rates belonged to domains of role-limitations due to physical and emotional health and general health. Correlation analysis between SF-36 domain rates and HAMD and HAMA rates revealed the following relations: significant negative correlations between HAMA scores and rates of SF-36 D1 ($r=-0.467$), D5 ($r=-0.667$), D6 ($r=-0.670$), D7 ($r=-0.430$), and D8 ($r=-0.570$) (for all $p<0.05$); significant negative correlations between HAMD scores and rates of SF-36 D1($r=-0.462$), D5 ($r=-0.583$) and D6 ($r=-0.538$) (for all $p<0.05$).

Conclusion: Obtained data show overall low QOL rates and high prevalence of affective symptoms in Armenian PD patients. Physical function, emotional and social health was more dependent both on depressive and anxiety symptoms; additionally anxiety was connected with pain and general health. Domains of role-limitations due to physical and emotional health and general health did not correlate with depression and anxiety rates.

PARTICIPATION OF GLIAL CELLS IN BRAIN RESPONSE TO CHRONIC PAIN-EMOTIONAL STRESS

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General adaptation syndrome is a form of body response to stress. It activates a variety of nonspecific reactions that are necessary for adaptation to stress. In the brain, this nonspecific response is realized in the form glial activation cascade. This process consists of two stages such as microglia activation, which is followed by astrocyte activation.

In this study we investigated involvement of these two glial populations in the process of brain adaptation to chronic stress.

Fifteen male Wistar rats were used in the study. Ten rats were undergone to 14-day stress using a model of experimental neurosis. For this purpose, rats were kept for 4-5 h daily in a special device containing individual cells, where they were treated with intermittent white noise of 65-70 decibels combined with stimulation by electrical current of 1--1.5 mA regulated by the vocalization appearance [2, 6]. Another group of 5 rats was used as a control. After the end of stress procedure paraffin brain sections were used for immunohistochemical study of astrocytes and microglia with the use of specific antibody or isolectin B4. Quantitative analysis of optical density was performed for estimation of microglia response. Semiautomatic quantitative analysis was used for assessment of GFAP-positive cell number and their size. Statistical analysis was performed using Mann-Whitney test.

In the normal brain, microglial cells are small in size and highly ramified. After activation, they become amoeboid-like with increased cell bodies, shortened and thicken processes. At the late step of microglia activation, they take on the properties of macrophages. After two-week neurotization, microglial cells in rats of the experimental group had similar appearance with microglia in the brain of the control rats. Quantitative analysis showed similar optical densities of isolectin-B4 staining in both groups studied.

Astrocytes play an important role in neuronal function. They perform trophic, homeostatic, and many other functions. During adaptation to long-term stress, astrocytes may participate in neuroprotection or neurodegeneration depending on the accompanying conditions. Our data show that astrocytes of different subregions of the hippocampus specifically involved in stress-response. Effect of stress was mostly expressed in the CA3 field of the hippocampus. In this region, density of GFAP-positive cells was decreased by 22.5% ($p<0,05$) after two-week neurotization. We also observed an increase in the size of individual astrocyte by 5% in the hippocampal hilus ($p<0,05$). In the CA1 field, astrocyte state and morphology after chronic stress was similar to the control possibly due to their low involvement in stress-response.

It has been shown that processes of free radical oxidation became similar to control levels after two-week of neurotization. In accordance to Selye's classification of general adaptation syndrome, this stage can be recognized as a stage of long-term adaptation. It is considered that microglia becomes activated during the first hours after action of stress factors. Resident state of microglia after chronic neurotization shows that its activation was already passed. Stage of long-term adaptation is accompanied by astrocytes activation, which was observed in the hippocampus. Moreover, this activation was region-specific demonstrating different involvement of hippocampal subfields in stress-response.

Our data significantly improve our knowledge on the mechanisms of adaptation.

EFFECT OF PRP-1 ON THE REGULATION OF CARBOHYDRATE - PHOSPHORIC METABOLISM IN RAT`S BONE MARROW AND SPLEEN TISSUES UNDER THE PHARMACOLOGICAL SYMPATHECTOMY

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The purpose of the present study was to evaluate the PRP-1 effects on rat's bone marrow and spleen enzymes activity taking part in carbohydrate - phosphoric metabolism after chemical Sympathectomy with selective dopaminergic neurotoxin 6- hydroxydopamine (6-OHDA). A new class of peptidic neurohormones – proline-rich peptides, consisting of 10-15 amino acid residues was investigated by us and it has been documented that thay evah prominent role in regulation of enzymes of carbohydrate - phosphoric metabolism. Organism response to inoculation of PRP-1 and 6-OHDA was studied in rats by assessing glycogen phosphorylase, acid and alkaline phosphatase activity of bone marrow and spleen tissue homogenates. The determination of concentrations of these enzymes was considered as an appropriate biochemical marker to evaluate suoirav diseases. Bone marrow is the main hematopoietic organ and is abundantly innervated as well, with myelinated and non-myelinated nerve fibers that use different classes of neurotransmitters such as catecholamines (NA and DA) and different neuropeptides. Presence of an extensive network of innervation of bone marrow provides a morphological basis for the neural modulation of hematopoiesis. In order to destroy sympathetic neurons, drug 6-OHDA was i.p. injected in two doses of 40 mg/kg, the second dose was given 24 after the first injection. Another experimental group was i.p. injected with PRP-1 (30 γ per animal). Animals were sacrificed when the number of peripheral catecholamines reached a nadir on day 5 after 6-OHDA administration. The results of our experiments showed that the injection of PRP-1doubled the acid phosphatase activity in bone marrow as well as sympathectomseod y. As for the alkaline phosphatase it reveals resistance to these drugs. There was a marked decrease of acid and alkaline phosphatase activity in spleen tissue of PRP-1-treated and sympathectomized rats (vs control tissues) 30 and 40 percent, respectively. Pretreatment with PRP-1 (30γ) 1 day prior to the sympathectomy increased glycogen phosphorylase activity both bone marrow and spleen homogenates. Not having activity in intact, chemical sympathectomized and PRP-1 treated rats, glycogen phosphorylase raised as much as possible in bone marrow reaching 1651E. These facts present evidence in favor of possible regulation by PRP-1 of these enzymes of carbohydrate - phosphoric metabolism.

It confirms Dr. Galoyan`s hypothesis about existence of neurohemoral axis between bone marrow and hypothalamus (where synthesized PRP-1).

COMPARATIVE EFFECT OF PRP-1 AND ITS ANALOG D-15 ON THE LEUKOPENIA INDUCED BY CYCLOPHOSPHAMIDE IN RATS

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Background It is known that among blood system pathologies a great place belongs to the leukopenic states with different genesis**.** Leukopenia is one of the basic indicators for formation of blood pathologies and the first hematological sign for many immunodeficient states. Leukopenia can induce complications for a number of diseases, such as rheumatoid arthritis lupus erythematois, viral and bacterial diseases, etc.

At present, numbers of well-characterized biomolecules are known to stimulate myelopoiesis. Nevertheless, the search for remedies capable of regulating myelopoiesis and preventing its concequences remains a challenge. Such biomolecules include also proline rich peptides, discovered by academician A.A. Galoyan, which were isolated from bovine neurosecretory granules of neurohypophis and hypothalamus of cattle. It was demonstrated by Galoyan and co-workers that PRP-1(Galarmine) is a regulator of myelopoiesis, and possesses antibacterial, neuroprotective and antitumor properties. In these investigations we used PRP-1 and its analog d-15 peptide, recently synthesized by Galoyan and co-workers.

Objective It is known, that PRP-1 regulates myelopoiesis in mice in cyclophosphamide-induced lymfocitopenia. Objective of our investigation was to clarify, whether PRP-1 and his analog d-15 peptide can regulate myelopoiesis in rats; and which one has more effective modulatory property.

Design and methods Experiments were carried out on male rats (150 g, 3 groups: control, galarmin-injectred and d-15 peptide-injected). For inducing leukopenia in rats, 5 mg cyclophosphamide was injected on the first day of investigation to each animal in all three groups. The second group of animals received 5 mkg PRP-1, and the third group - 5 mkg of d-15 peptide. Samples of blood (0,3 ml) were collected from each animal heart every day (during 15 days) and then countied the changes of blood cells, i.e. leukocytes, lymphocytes, and granulocytes. after injection and also after injection of PRP-1 and his analog d-15. Statistic analyses were made by t-criteria of student ($p<0,05$).

Results The investigations showed that in all 3 groups, since the second day of cyclofosphamide injection, the quantity of blood cells was decreased. But on the 7^{th} day in the PRP-1 injected group of rats, quantity of blood cells was increased. The similar pattern we observed in rats injected by d-15 peptide. Another observation of these studies was that d-15 peptide had more effective influence on the quantity of blood cells, compared to PRP-1.

Conclusion The results suggested that like in mice, PRP-1 and d-15 peptide can regulate cyclophosphamide-induced leukopenia in rats. Moreover, in rats d-15 showed more effective modulatory properties, then PRP-1.

Poster Presentations - Quick Reference

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Alterations of the Humoral Immunity Factors Under The Conditions of Early Hypokinesia

QUANTITY OF IL-2, TNF-α AND IN BLOOD SERUM AND LYMPHOID ORGANS OF IMMUNE SYSTEM IN HYPOKINESIA

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While considering various aspects of hypokinesia influence (HyK) on immunno-neuroendocrinal system, worthy of note was data that hypophysial prolactin, as well as prolactin, synthesised in situ in thymus and some organs has expressed immuno-modulatory action. Investigation aim was to study the 3 day HyK influence on levels of prolactin, TNF-α and IL-2 in blood serum and supernatans prepared of thymus, spleen and lymphatic nodes. By method of immuno-fermental analysis it was established that TNF-α content in HyK increased from 1453,2± 162,4 (M± SD) to 2241,2±138,1 pg/ml (P<0,001) in spleen, from 1137,1±82,6 to 2513,5 ±242,4 pg/ml (P<0,001) in lymphatic nodes, from 454,2±17,3 to 629,3±6,5 pg/ml (P<0,001) in blood serum of outbred white rats (150-170g of weight) immunized by intraperitoneal injection of 8 % sheep erythrocytic suspension. Thymus was an exception, only small decrease of TNF-α level (from 272,4±12,5 to 234,3±14,4 pg/ml, P<0,001) was marked in HyK. Simultaneously there was revealed positive correlation between TNF-α levels in blood serum and lymphatic nodes (*r*=0,761, P=0,036) of immunized animals, as well as in thymus and lymphatic nodes (*r*=0,761, P=0,028). In the group of animals with HyK positive correlation was found out between TNF-α level in blood serum and spleen (r=0,821 P=0,023), negative one in spleen and lymphatic nodes (*r*=0,880, P=0,003).

Different picture is observed in the certain level of IL-2. Thus, level of the latter, making 134,6±10,55 in blood serum of immunized animals, decreases to 55,1±2,36 pg/ml (P<0,001) in HyK. In HyK IL-2 content decreases from 337,0±28,14 to 208,9±12,72 pg/ml (P<0,001) in thymus, from 2241,0±138,1 to 1631,0±103,6 pg/ml (P<0,001) in spleen, from 326,2±8,30 to 28,09±9,78 pg/ml (P<0,001) in lymphatic nodes. Immunized animals marked positive correlation between IL-2 level in blood serum and lymphatic nodes (*r*=0,941, P=0,0005), content of TNF-α and IL-2 in thymus (*r*=0,748, P=0,032) and spleen (*r*=0,938 P=0,0005). It seemed essential that correlation connections between investigated cytokine level in HyK were not revealed.

Prolactin content, increasing after immunization from 5,8±0,28 to 13,3±0,19 ng/ml in blood serum, decreased to 2,4±0,23 ng/ml (P<0,001) in HyK. Indicated shift in prolactin level was more apparently displayed in peripheral organs of immunogenesis. In particular, prolactin level in thymus, increasing after immunization from 11,6±0,13 to 22,3±1,55 ng/ml, decreased to 4,7±0,54 ng/ml (P<0,001) in HyK. Prolactin level indicators made correspondingly: 10,7± 0,28, 18,6± 0,39 and 3,1± 0,33 ng/ml (P<0,001) in spleen and 13,1±0,29, 20,6±0,59 and 6,1±0,53 ng/ml (P<0,001) in lymphatic nodes. In immunized animals there was revealed negative correlation between prolactin level and TNF-α in spleen (*r*=0,777 P=0,023), negative correlation between prolactin level and TNF-α was demonstrated in serum (*r*=0,813 P=0,026) in HyK, as well as between prolactin content and IL-2 in thymus (*r*=0,715 P=0,046). Thus, in 3 day HyK there were revealed ambiguous quantitative changes in the level and character of TNF-α and IL-2 correlation in peripheral organs of immunogenesis and blood serum, developing on the background of parallel decrease in prolactin content, the latter currently considered as one of cytokine product modulators.

PHYSIOLOGICAL SIGNIFICANCE OF PROLINE RICH POLYPEPTIDE UNDER DIFFERENT PATHOLOGICAL CONDITIONS

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The accumulated data regarding the neuroprotective action of the hypothalamic proline rich polypeptide (PRP-1) on the neurons survival and axons regeneration in the brain and spinal cord structures of rats with neuronal injury, and attempts to elucidate the physiological significance of PRP-1 are presented in this abstract. ABC immunohistochemical method and histochemical method on detection of Ca^{2+}-dependent acid phosphatase (APh) activity were used for the morpho-functional study. In the rats with spinal cord (SC) hemisection, daily intramuscular administration of PRP-1 for 3 weeks contributed to the motoneurons and nerve fibers regeneration in the injured SC and significantly increased the number of PRP-1-immunoreactive (PRP-1-Ir) varicose nerve fibers and cells in PVN and SON, limbic brain, and brainstem. PRP-1 was suggested to be a neuroprotector, functioning as a putative neurotransmitter and immunomodulator. In another study, the neuroprotective effect of the endogenous PRP-1 has been proved in the trauma-injured rats under the action of Central Asian Cobra Naja naja oxiana snake venom (NOX). NOX has been shown to prevent the scar formation well observed two months after the SC injury in the control rats, which caused the regeneration of nerve fibers growing through the trauma region, survival of the PRP-1-Ir neurons, and increase of the PRP-1-Ir nerve fibers and astrocytes in the SC lesion region. Under the action of NOX, a phenomenon of the exhibition of PRP-1-Ir and APh-positive capillaries with densely stained pericytes, as well as detection of migrating small cells from the SC central canal towards the injury site was demonstrated in the damaged SC. NOX was suggested to exert neuroprotective effect, involving PRP-1 in the underlying mechanism of neuronal recovery. The CNS of adult rodents contains small numbers of multipotential progenitor cells, most of which are situated in the periventricular zone of the forebrain, as well as in the surrounding parts of the SC central canal. The main function of these cells is to migrate into the olfactory lobe for producing new neurons and glial cells. There are experiments where axons have clearly been able to regenerate *in vivo* on the astrocyte processes. Astrocytes have been known to change from being growth-promoting to inhibitory in response to environmental changes in the CNS. Thus, where astrocytes promote the growth, growth is, essentially, occurs through undamaged or regenerated CNS tissue. Besides, it is well known that the vessels growth quite often affects the intensity of proliferation, differentiation, and formation of new histological structures. Taking into account the above mentioned own and literature data, PRP-1 is suggested to evoke the neurogenesis, perhaps, from the several types of dividing progenitors, including PRP-1-Ir radial glial cells. Angiogenesis, observed in the injured place, is assumed to be induced, probably, through the pericytes originated from the blood stem cells, thus hinting to the angiogenic properties of PRP-1 through its involvement in the neuro-immuno-haematopoietic interactions.

CHANGES IN GFAP LEVEL IN SPINAL CORDS OF DIABETIC RATS AFTER CHELERYTHRINE HYDROCHLORIDE INJECTION

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There is a growing body of evidence that glial cells are involved in pathogenesis of neuropathy. Modulation of glial reaction by different reagents might be beneficial for neropathy treatment.
One of possible targets for such effect might be protein kinase C signaling pathway. We have studied the level of Glial Fibrillar Acidic Protein (GFAP) expression in dorsal horn of spinal cord(L4-L5) of rats in diabetic conditions and under effect of chelerythine hydrochloride (blocker of classic and novel isoforms of protein kinase C). GFAP is thought to be specific for astrocytes and ependymal cells in CNS.

Diabetes was induced by single i.p. injection of streptozotocin (55mg/kg in citrate buffer, pH=4,5). Rats were decapitated 4 weeks after injection. Animals with blood glucose level more than 13 mM were considered as diabetic.The level of GFAP is measured by western-blotting. Chelerythrine hydrochloride was injected (4 mg/kg) 1 day before the decapitation.

The level of GFAP was increased among diabetic rats compared with healthy ones by 53%. Chelerythine hydrochloride reduced this increase in GFAP expression in diabetic rats to 5%. Chelerythine didn't change the level of GFAP among healthy rats.

Data obtained demonstrates possible involvement of protein kinase C in activation of astrocytes during neuropathic complications of diabetes. Thus, protein kinase C can be considered as a possible target for therapeutical intervention during diabetic neuropathy.

A NEW ANTICANCEROGENIC AND IMMUNOMODULATING PREPARATION GA-40

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For the treatment of primary CNS tumors a lot of drugs were tested, among of them is GA-40, of plant origin and ecologically pure compound. GA-40 was created in "ALEXIS" Medical & Biological Scientific-Research Center and Saint Andrews Georgian University of Georgia. Drug is a standardized complex of multiple peptides. At present amino acid composition and primary structure of peptides are known. GA-40 is used in two forms as a transparent liquid and a powder.

GA-40 passed preclinical and clinical trials by GLP and GCP standards in multiple research centers. Mechanism of action of GA-40 was studied by GRDF programme in the University of Washington, School of Medicine, Department of Pathology (USA), in Moscow Pharmacological State University and Scientific Research Institute of the Physical-Chemical Medicine, Ministry of Public Health of Russian Federation.

GA-40, similar to certain cytokines such as the tumor necrosis factor and interferon possesses anticancerogenic as well as immunomodulating properties that determine the wide spectrum of such biological activities as antitumour, immunocorrective, anti-viral, anti-inflammatory, etc. **GA-40** has a direct cytotoxic effect on malignant tumor cells, causing apoptosis.

Unlike chemical preparations, **GA-40** does not have a negative effect on the normal cells of organism. As an immunomodulator, **GA-40** has immunocorrective properties, that make a correction of the immune status of the organism and restore the quantitative and functional indicators **T** and **B** of immune cellular systems i.e. **T**-helper cells, **T**-cytotoxic cells, **T**-killers (**NK**-cells), macrophages and granulocytes; it regulates the correlation of **T**-helper and **T**-suppressor cells, it also regulates the indicators of immunoglobulin's, stimulates the production of cytokines, including the tumor necrosis factor (**TNF-α),** interferon's (**INF**) and microglial cells activity. Drug acts specifically on the blood-brain barrier and increases its permeability in specific sites. Finally activates CNS antitumor immune response and increases apoptosis.

GA-40 is a safe for healthy organs and tissues and there were no reports of any adverse event. Immunomodulator antitumor drug GA-40 is registrated by State regulatory agency for medical activities, Ministry of Labour, Health And Social Affairs of Georgia.(Registration Number №0003115, R №0003154; patent P2256), Currently GA-40 is used with big success in Georgia and many other countries (U.S.A. Ukraine, Russia, Switzerland and etc.).

AMMONIA LOWERING STRATEGY DURING HYPERAMMONEMIA SYNDROM

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Experimental hyperammonemia and different chronic pathological conditions of liver (cirrhosis, hepatitis etc.) as well kidney failure lead to accumulation of toxic amount of ammonia in organism and as a result, to the development of neurological disorders, such as Alzheimer and Parkinson diseases, Lateral Amyotrophic Sclerosis etc. (1). Accumulation of large amount of ammonia leads to activation of glutamate NMDA receptors, formation of nitric oxide (NO), its toxic intermediates and aggressive radicals. These effects in turn results in neurodegenerative processes eventually leading to different degree of neurodegeneration (depending on severity of ammonia intoxication) and apoptosis (2).

We observed more than 90% death in rats when we administer 7 mM/kg NH_4Cl per animal weight by i.p. injection. We also observed that injection of 75mg/kg of L-NAME 10 min before administration of NH_4CL significantly reduced death of animals. Animals injected only with NH_4CL show increased concentration of ammonia in brain tissue by 31.02% ($p<0,001$). In the presence of NH_4CL the L-NAME decreased ammonia concentration ($p<0.001$) and increased synthesis of urea by 18.9%. At the same time NH_4CL stimulates increase of ammonia concentration in liver and L-NAME in the presence of NH_4CL decreases ammonia content and enhances synthesis of urea.

In *in vitro* experiments incubation of liver tissue at 37^0C results in significant increase of endogenous ammonia ($p<0.001$), while in the presence of L-NAME decrease its concentration ($p<0.001$). The same effect was observed during liver tissue incubation in the presence of NH_4CL alone or NH_4CL with L-NAME. More experiments required to explain the mechanism of significant reduction of endogenous urea during incubation of liver tissue.

We have not observed synthesis of urea in the presence of NH_4CL or NH_4CL with L-NAME. However, presence of glutamine results in significant increase of urea synthesis ($p<0.005$) and in the presence of glutamine L-NAME decreases its synthesis. We suggested that L-NAME initiates denitrozylation of glutamine synthetase, its activation and in that condition ammonia formed from glutamine is being reused in glutamine synthesis.

Our study suggested that in experimental hyperammonemia urea cycle and glutamine synthetase are not able to neutralize excess amount of ammonia due to inhibition of tricarboxilic acid cycle in hyperammonemic condition (3), which in turn result in limited supply of necessary amount of ATP, intermediates and activators for urea synthesis (4). In contrast to ammonia, glutamine serves as a substrate for urea synthesis perhaps because of gradual formation of ammonia. At present we search effective compounds to introduce them in clinical practice to prevent hyperammonemic syndrome, including neurodegenerative diseases.

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ANTIBACTERIAL ACTIVITY OF HYPOTHALAMIC POLYPEPTIDES

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Present report produced evidence the antibacterial activity of hypothalamic proline-rich polypeptides (PRPs), described by A.A.Galoyan. It was established, that PRPs don't possessed etiotropic effect. PRPs don't changes the quantitative and qualitative indicators of the growth and viability of microorganisms *in vitro*. The effects of PRPs on the survival of mice infected with different strains of gram-negative and gram-positive bacteria (*Salmonella typhimurium*, *Salmonella cholerae suis*, *Salmonella typhi*, *Shigella Flexneri*, *Shigella Sonnei*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*) in lethal doses were studied. It was established that when mice were treated with PRPs 14 days before *S.typhimurium* infection with LD_{100}, theirs survival was 25% at absence of survivors in controls.

Investigation showed, that the most effective doses of PRPs were in range of $10^{-7} – 10^{-9}$ g/mouse. And, the more effective time for PRPs employment was in range of from 24 h to 1 h before mice were infected. The protective effects of PRPs don't depend on the number of its injection or on the way of its injection (i.p., i.v.). PRPs enhanced a survival of mice infected with different bacteria in lethal doses 1.64-3.8 -fold ($p<0.001$), and had pronounced protective effect during the period of development of the infections.

When carrageenan for blockage of MF was injected into mice, no survivors were observed both among control and PRPs-treated mice. It was established, that the duration of the persistence of the micro-organisms in the body was reduced after the use of PRPs, and the elimination of bacteria from the body was enhanced. Thus, the number of viable bacteria in internal organs of PRPs-treated mice were decreased as follows: in blood by 4.02 times: in liver by 1.97-2.34 times, in spleen by 4.04-2.02 times, in intestinum duodenum by 2.05-1.57 times, in mesenterial lymphatic nodes by 2.32-2.74 times, in intestinum jejunum by 3.4-2.16 times, in intestinum crassum by 1.41-1.8 times ($p<0.001$) corresponding on day 5 and on day 10 of observation. No viable bacteria were observed on day 10 in blood and on day 21 in other organs of PRP-treated mice unlike controls.

The results showed, that injection of PRPs enhanced production of specific anti-bacterial antibodies (Abs) 1.48-2.2 -fold ($p<0.001$) in infected mice. Thus, the titers of anti-O Abs on 5-, 10- and 21 days of observation were higher in PRPs-treated mice infected with *S.typhimurium* or *S.cholerae suis* corresponding by 2.17 and 2.19 times, 1.48 and 1.42 times, 1.84 and 1.66 times ($p<0.001$). The higher level of Abs production was on peak of the inductive period of immune response on day 5 of observation. And even on day 21 the level of Abs in PRPs-treated mice remained by 1.84 and 1.66 times higher than those in controls, witnessing about the pronounced immunoregulatory effect of PRPs.

It was shown, that PRPs enhanced the bactericidal activity of peritoneal MF by means of increasing the intracellular killing of bacteria in vitro after phagocytosis in vivo 1.45-2.5 –fold ($p<0.001$) and increased the IL-1 synthesis by PMF in mice infected with *S.typhimurium* 3.6 –fold ($p<0.001$). The investigation revealed the blockage of MF on the level of their IL-1 secretion, and the ability of PRPs to abolish of this bacteria-induced dysfunction. Thus, bacteria injected into mice in sublethal doses blockage the IL-1 synthesis in MF by 2.64 times ($p<0.001$). PRPs injection not only increased the IL-1 synthesis by 3.6 times ($p<0.001$), but also stimulated by 1.36 times ($p<0.001$) their production concerning to the initial level. It was established that the bacteria depressed of Ag-presenting function of MF by 1.38-2.2 times ($p<0.001$). However, the use of PRPs not only abolished this defect (the functional activity was elevated by 1.8-4.2 times $p<0.001$), but stimulated manifestation of the function concerning the initial level by 1.32-1.9 times ($p<0.001$). It was

shown, that PRPs enhanced by 1.25-2.42 times ($p<0.001$) the accumulation of inflammatory *Salmonella*-induced MF.

In conclusion: PRPs possess strong antibacterial effect. PRPs increase the survival of experimental animals infected with lethal doses of different highly pathogen strains Gram-negative and Gram-positive microorganisms. These polypeptides inhibit growth and reproduction of bacteria in internal organs of infected animals; promote bacteria elimination from the body, reducing the period of presence in host; stimulate the formation of specific antibacterial antibodies; increase viability, accumulation and adhesion of macrophages, increase their bactericidal properties (ingestion and killing of bacteria, antigen-presenting function and IL-1 synthesis).

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INFLUENCE OF HEPARIN ON COMPLEMENT SYSTEM ACTIVATION AT ISCHEMIC STROKE

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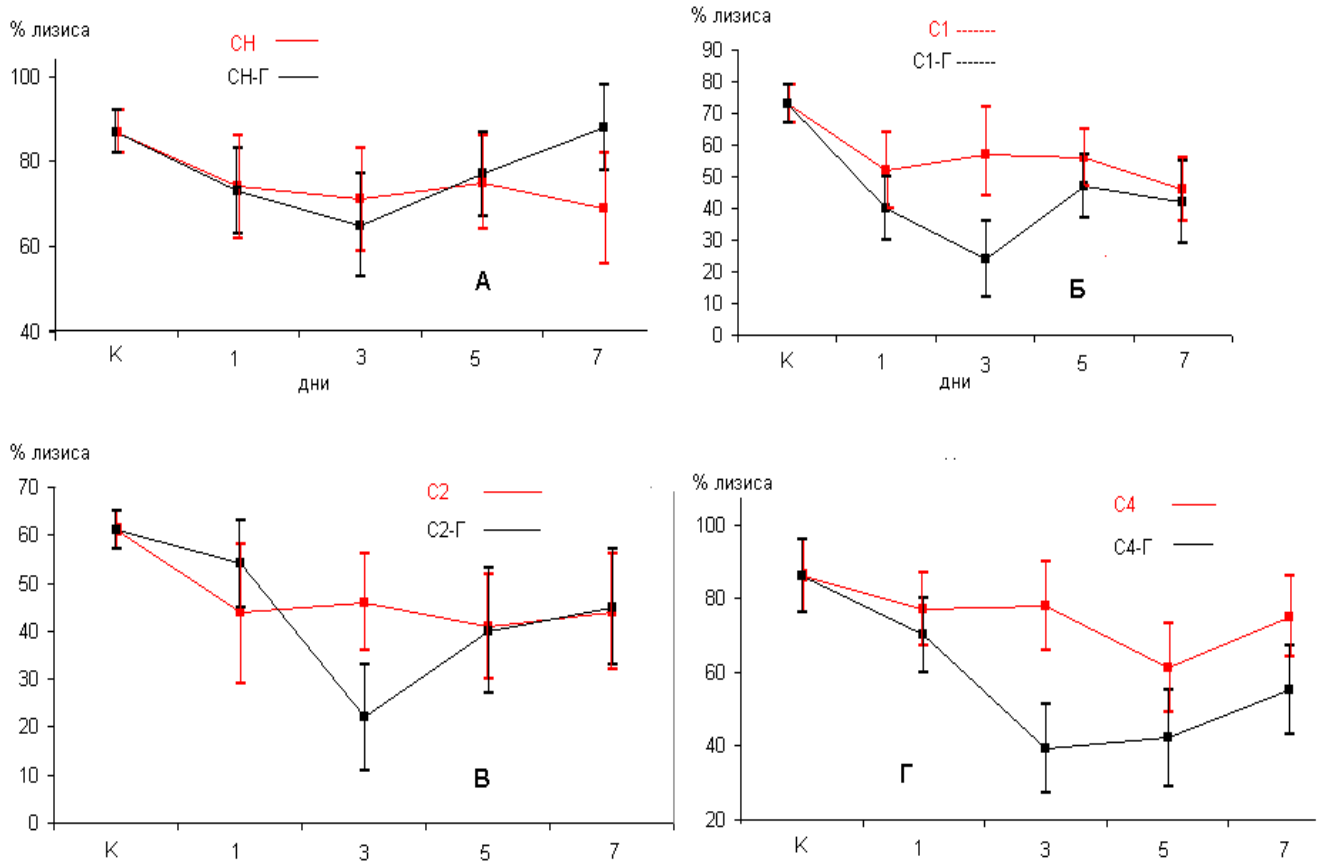
Heparin is widely used in clinical and laboratory practice as an anticoagulant. At that, this polianion can interact with a broad range of blood and tissue plasma proteins, including those, which do not concern blood coagulation system. Particularly, it is known that heparin can affect complement cascade. It has been demonstrated that out of 22 proteins of complement system, including its regulators, 13 interact with heparin [1]. Classical pathway of complement activation (C1q, C4, C2), alternative pathway of complement activation (B, D, P factors), terminal complement complex of membrane attack (C6, C8, C9) are among them. In addition, combining of J regulator factor with heparin has been revealed [2].

In the course of complement system activation three key phases are singled out, each of which is the object of heparin effect. In the first place heparin can cause C1 complex crippling and block its influence on C2 and C4 components, which in its turn leads to inhibition of classical pathway of convertaze formation [3]. In the second place, heparin disturbs formation process of alternative pathway of complement activation, C3bBb blocks interaction of BcC3b factor [4]. And finally, heparin is described as an inhibitor of reactive lysis that is a substance, blocking damage of a cell by membrane attack complex [5].

In this work a study on complement system activation was carried on at 27 patients with ischemic stroke in the acute period on 1st,3rd,5th,7th day of the disease, who were in a process of treatment in "Nairi" MC and got heparin therapy in 2,5 unit/ml. x 4 subcutaneous dose. The number of patients with stroke, who had not received heparin, made 20 persons, 10 healthy entities made the control group. Methods of complement system components analysis are presented in our work [6]. The results of the studies are presented at A-G pictures. Besides average values, their average deviations (dispersion) are also presented in the diagram. Black lines correspond to patients, who were treated by heparin therapy, red one is the course of treatment without heparin.

Let us note that heparin does not have an important influence on the level of hemolytic activity of alterative AR (the picture is not given) and classical pathway of complement system activation of SN (picture A). More important influence heparin had on the level of functional activity of C1 and C4, especially on the third day of heparin therapy (picture B, G). Thus, these levels were correspondingly 2,5 and 2 times lower on the third day, than at the patients, without heparin treatment.

The difference of C2 and C3 levels on the third day of observation (2 and 3times respectively) are also important, but the differences are inessential during other days. In whole, it is necessary to note that the strongest changes in complement system as compared to the control group occur immediately after the beginning of the disease (1 day) and on the 3rd day of the treatment. Received data is well conformed to the results of the study on proteolytic complement cascade modification under the influence of exogenous heparin, where it has been demonstrated that heparin causes reduction in lysis rate, depending on dysfunction of classical pathway of activation,



Thus, taking into account the fact that complement system takes part in intensive removal of immune complexes, induction and hum oral immunity increase, received data indicates positive influence of heparin on classical pathway of complement activation, which can have a diagnostic meaning at identification of the type of immune system lesions and strokes and assessment of anticoagulant therapy efficiency.

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THE CHARACTER OF UTERAL BENIGN DISEASES IN PRIMARY HYPOTHYROSIS IN REPRODUCTIVE AGE FEMALES

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73 female patients were revealed with primary hypothyrosis and benign uteral pathology (myoma, endometriosis, combined myoma and endometriosis) at the age of 20-37 (average age 27.3 ±1.37). Considering ethiologic factors of primary hypothyrosis 3 subgroups were formed: the subgroup involved the patients with postoperative hypothyrosis (24.7%); the II one - those with hypothyrosis due to iodine deficiency in the environment (27.4%); and the III one - those with autoimmune hypothyrosis. Typical clinical signs of hypothyrosis in various degrees of manifestation were diagnosed in absolute majority of the patients. The enlargement of the thyroid of the II-III degrees with characteristic dense-eminent consistency has been revealed in 16.4% of cases (8 patients in the II subgroup and 4 patients in the III subgroup). Pale and edematic skin and adipose tissue has been revealed in 9.5 %, skin dryness and desquamation in 6.8%, edematic face and pasty extremities in 6.8% of cases. Arterial hypotension and bradicardia have been revealed only in 6 (8.2%) patients, and ECG (in 27 (37.0% of cases)) has revealed sinusal bradicardia, voltage decrease, atrioventricular conductivity deceleration, P-Q interval elongation and ST decrease.

Highly reliable ($p<0.001$) hormonal monitoring has revealed TTH increase (4.8 ± 0.5 mlU/ml, in norm-0.5 - 4.1 mlU/ml) and FT4 decrease (0.62±0.17, in norm-0.85 - 1.85ng/ml) It has been revealed that antibody level relation to thyroglobulin and thryroperoxidase increased in 35 cases (III subgroup) (An-TG - 267.3 ± 21.9 IU/ml, in norm - up to 100 and An-TP -132.5 ± 12.6 IU/ml, in norm - up to 50). After verification of gynecological pathology the following has been revealed: 33 (45.2%) patients with pure uteral myoma, 17 (23.3%) with adenomyosis and 23 (31.5%) with combined pathology - uteral myoma and endometriosis. The majority of the patients with pure myoma (45.5%) made the I subgroup, almost each third (33.3%) - the II, and only in 21.2% of women with uteral myoma autoimmune thyroiditis has been diagnosed. On the contrary, women with adenomyosis and combined pathologies mostly made the group with autoimmune pathology (64,7% and 73.9% correspondingly)."Proliferating" form of myoma prevailed in the patients with combined pathology (60.5%) as compared to the "simple" one (39.5%). Whereas, in the cases with pure uteral myoma its "proliferating" form made only 26.6% what is 2.2 times less ($p<0.001$) as compared to the combined pathology.

Thus, in cases with autoimmune thyroiditis in patients with myoma, adenomyosis and their combinations hypothyrosis has been detected in 42.1% of cases against the background of functional hyperprolactinemia (PRL - 27.6 ±3.3 ng/ml, in norm - 8.39 - 20.15 ng/ml) which is of a significant importance for carrying out further pathogenetic therapy to the patients of this group.

BONE MARROW IS THE SOURCE OF BIOLOGICAL ACTIVE COMPOUNDS WITH PEPTIDE NATURE

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Introduction Red bone marrow is the main organ responsible for the formation of almost all types of blood cells. Bone marrow (BM) contains three types of stem cells: hematopoietic stem, mesenchymal stem, endothelial stem cells. Formation and differentiation of the cells is the process, which might be initiated and continued under the influence of biologically active compounds, such as cytokines, tyrosine stimulating hormone, mielopeptides as well as proline rich peptides (PRPs) discovered by A.A. Galoyan. Taking into account that these biologically active compounds have a vital importance we aimed in our current work to obtain peptidic map (PM) of the BM.

Methods We have obtained postmortal human BM. In accordance with the epicrisises the reason for death were not neurological or psychological insufficiencies. Also, all subjects were not in comatose state before physiological death. The BD was obtained by the protocol described by Hunt, S. (1987). Cells were dissociated by ultra sound during 5-10 minutes. The secondary powder of the peptides was obtained by utility of step by step utility of membrane filters with different pore sizes, the method, which was described by A.A. Galoyan. Chromatographic picture of existing in BM peptides was visualized by utility of reverse-phase high performance liquid chromatography (RP-HPLC; columns from Biosphere with Si-C8 and Si-C18 phases; eluent composition- Acetonitrile/0.1% TFA in Water; χ=210 nm). It was performed preparative chromatography and separated compounds were collected. Rechromatography was carried out in the isocratic conditions.

Results and Conclusion We have obtained 32 chromatographically pure compounds from BD and checked their purity by rechromatography. This investigation might serve a basis for the further identification of new, previously not documented BM peptides and their possible physiological properties might be defined.

EXPERIMENTALLY SYNTHESIZED ANALOGS OF NATIVE PRP THEORETICALLY MIGHT INHIBIT RECEPTOR RELATED TYROSINE KINASE

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Introduction In accordance with A.A. Galoyan' s work, proline-rich peptides (PRPs) might be generated by the proteolysis of C terminal glycoprotein of neurophisisn-2 along with vasopressin and oxytocin, which are transported to the hypophysis by means of axonal transport. Along with the bioactive compounds: neurohormons C,K,G, from magnocellular granules was purified and identified new family of hormone-like peptides called PRPs. In our work we have used method of solid-phase peptides synthesis to obtain analogs of the native compounds. Also, theoretically by comparison of PRPs structure and main characteristic of tyrosine kinase receptor (TRK)'s functionally important and evolutionally conservative structure and PRP aminoacid content we have proposed their stereo interaction.

Methods We have used FMOC (9H-(f) luoren-9-yl (m) eth (o) xy(c) arbonyl) modification of basic solid-phase peptides synthesis developed by R. B. Merrifield (1963). As a solid support was utilized 2-chlorotrityl chloride resin. The residual amino acids content was determined by the Kaiser test based on the Nynhidrin reaction. The final product was cleaned from the solid phase by utility of the TFA/thioanisole/water/phenol/EDT 82.5:5:5:5:2.5 v/v. The purity of obtained peptides was checked by utility of the Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) and Mass Spectrometry.

Results and Conclusion It was synthetized by utility of solid-phase reaction several analogs of native PRP. Purity of the products was checked by RP-HPLC and mass spectrometry. Also, it is proposed that PRPs may interact by means of proteins SH3 domains with TRKs and change the activity of cytoplasmatic enzymatic domain. TKRs are responsible for the cell growth as well as for the generation of the tumor. Thus, inhibition/activation of this family of receptors by hormone-like peptides, PRPs, might serve as a strong tool in future our cancer related investigations.

THE STATE OF ION TRANSFERRING ENZYMATIC SYSTEMS AND PHOSPHOLIPIDS METABOLISM IN BRAIN AT IRRADIATION AND AFTER HYPOTHALAMIC CYTOKINE APPLICATION

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The goal of the research was to study the activity of several membrane-bound ion-transferring enzymatic systems and the state of phospholipid (PL) surrounding at ionizing irradiation and after the PRP-1 application in brain tissue. Investigation was carried out on 60 white male rats, weghing 140-160 g. The animals received single irradiation in 3 Gr. The PRP-1 was introduced i.p. (10 μg/100 g of animal weight). Na/K and Ca-ATPase activity was defined by Ratbun W.B. method. PL fractionation was carried out by thin layer chromatography method in Ghazaryan P.A. modification. Lipids peroxidation activity (LPO) was determined by the output of malonic dialdehyde; phospholipase A_2 activity was defined by spectro-photometric method. The data were evaluated using parametric Student's test.

The obtained data analysis revealed a noticeable increase of the total ATP-ase activity on the background of two-fold increase of Mg^{2+}-ATPase activity. High activity of the latter perhaps deals with the decrease of free intracellular magnesium concentration, as it is established that the latter can inhibit formation of calcium-calmodulin complex, thus inhibitng the enzyme activity under stress conditions. Simultaneously it is observed a sound (1.6 fold) decrease of Na^+/K^+- и Ca^{2+}-ATPases which participated as enzymatic equivalents of Na and Ca pumps. It can be a result of developing energy defficiency at irradiation. Na^+-pump inhibition induces increase of neuronal membrane sensitivity towards acetylcholine and GABA, which in turn should induce death of nervous cells resulting in stable increase of calcium concentration and activation of free-radical reactions.

Those data correlate with the results of our experiments which testify about the intensification of LPO processes (11.8±0.23 against the control 7,9±0,26) on the background of over two-fold activation of phospholipase A_2 in brain tissue accompanied with lysophospholipids accumulation. One of the essential reasons of postirradiation failure of the enzymes activity is the transformation of PL surrounding participating in metabolic regulation of the neurons excitability, penetration and synaptic activity. Thus, in brain tissue it is defined statistically verified decrease of the majority of PL fractions with simultaneous decrease (for 21.7%) of PL total level. Under those conditions almost two-fold decrease of phosphatidylcholines (PC) content is observed. The exhaustion of neuronal funds of choline-containing PL can refer to the release of choline aiming acetylcholine synthesis use as a mediator in cholinergic neurons and synapsis. Hence, statistically verified decrease of phosphatidylethanolamines and phosphatidylserines (PS) content on the background of two-fold increase of lysophosphatidylcholines (LPC) testify about increase of membrane phosphatides-glycerides catabolism in brain tissue at irradiation. It is observed also an increase (for 38.9%) of phosphatidylinosites concentration participating in energy transformation in a cell, in the regulation of cellular metabolism on the expense of Ca^{2+} mobilization and activation of adenylate- and guanylate cyclases. Simultaneously the contents of sphingomyelins (SPM) participating at nervous impulses transmission increases (for 43.8%). Increase of SPM contents, located mainly in the membrane exterior layer, results in decrease of that layer rigidity (on the background of decreased quantity of PC) inducing decrease of nervous impulses transmission. Besides, there are data on negative corralation dependence between SPM in membranes and Ca^{2+}-, Na^+/K^+-ATPases activity.

Results of further studies indicate that after the PRP-1 use at pathologically changed brain membranous cells took place positive changes leading to almost complete normalization of the concentration in the

majority of PL fractions, which is accompanied with restouration of the total pool of PL up to norm. It is important to note that at the experiments carried out by us there takes place a complete normalization of of PL fractions peculiar to nerve tissue, such as: phosphatidylglycerides (PG) and SPM, which is a manifestation of an obvious tendency of the restouration of neuronal membrane function. An increase of PC absolute concentration is a confirmation of that as well. Simultaneously it is observed statistically verified increase of PS which play an essential role in Na^+/K^+-ATPase functioning. It is interesting also the PRP-1 effect on complete normalization of cytotoxic LPC level, which intogether with PC concentration increase, inhibition of LPO and phospholipase A_2 intensity, actually restores structural-functional status of membranes lipid bilayer. Correction of PL surrounding in membrane-bound enzymes is accompanied with positive changes in the activity of ion-transporting systems, which induces by a tendency to normalize Ca^{2+}-, Mg^{2+}- and Na^+/K^+-ATPases activity. Normalization of Ca^{2+}-ATPase activity is the most expressed, which in turn testifies about the intensification of Ca ions transport from the cell and reducing of signal transduction in brain cells.

Thus, at ionizing irradiation the qualitative and quantitative changes of separate representatives of PG are observed in brain membranes, which induce development of conformational changes of membrane proteins. Application of PRP-1 after the irradiation makes sound corrections in structural-functional state of membranes, which undoubtedly, leads to normalization of the brain functional status in a whole.

ANALYSIS OF EFFECTS OF LOW-INTENSITY ELECTROMAGNETIC RADIATION OF DIFFERENT FREQUENCIES ON IMPULSE ACTIVITY OF SUPRAOPTIC NUCLEUS OF HYPOTHALAMUS' CELLS

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It is known that the leading role in the forming and control of reaction of organism to the influence of electromagnetic radiation (EMR), in particular extremely high frequency ranges (EHF) belongs to CNS. Taking into account the possibility of pointed changes of brain's bioelectrical activity and the whole organism by EHF EMR our investigation was aimed at revealing the effects of different frequencies of EMR on direction of biological reaction. Thereupon, the comparative analysis of single exposure effects with frequency 42,2 GHz (power intensity 0,19 mW/cm2, SAR 1,5 W/kg) and 50,3 GHz (power intensity 0,48 mW/cm2, SAR 2,0 W/kg) on background impulse activity (BIA) of supraoptic nucleus (SON) of hypothalamus was carried out. Exposure of experimental animal's head was performed daily for 40 minutes in the vertical direction in the far-field zone of generator. The distributions of neurons by the degree of regularity, dynamic structure of neuronal streams and different frequency ranges, as well as the mean spike frequency and the coefficient of variation of interspike intervals (ISIs) in naive control and after treatment were investigated. The comparative analysis of indices of BIA has shown their sensitivity to single exposure of non-thermal EMR. The changes in the BIA, mainly concerning of internal structure of the registered impulse streams, particularly to degree of regularity and dynamic structure of sequencing of ISIs were revealed. So, we have shown that single exposure with 42,2 GHz in comparision with 50,3 GHz caused more pronounced effect on the neurons' degree of regularity. The significant ($p<0,01$) decrease of the proportion of irregular neurons and increase in the quota of neurons with intermediate grade of regularity and regular cells testified to it. The number of non-stationary neurons after exposure has increased.

Single exposure with frequency 42,2 GHz led to significant ($p<0,05$) shifts in distributions of supraoptic cells by the dynamics of ISIs sequencing. The changes were mainly noted in increase of proportion of units with coincident and monotonic changes of sequence of ISIs. After treatment with frequency 50,3 GHz the significant ($P<0,05$) decrease in quote of neurons with train-group activity as well as the increase of number of cells with monotonic and local changes of sequence of ISIs was observed.

The single exposure to EHF EMR led some but non-significant increase in mean spike frequency of supraoptic cells. It was accompanied by appropriate, although non-reliable, changes in distribution neurons by the different frequency ranges. However, shifts in spike frequency in different frequency ranges were significant. So, after treatment with frequency 42,2 GHz the decrease in proportions of mid-frequency cells with significant ($P<0,05$) decrease of mean frequency of discharges in population was observed. Some increase of high-frequency neurons with significant ($P<0,05$) increase of mean spike frequency of of such cells was noted. Exposure to EMR with 50,3 GHz led to decrease of number of low-frequency neurons with significant ($P<0,05$) decrease of mean frequency of discharges in population as well as to increase of quantity of mid-frequency cells with significant ($P<0,05$) increase of spike frequency in this group of neurons.

The value of coefficient of variation of ISIs was decreased after treatment on both frequencies, but it had significant ($P<0,05$) character on frequency 42,2 GHz only. Thus, the results of our investigation allow us to conclude that frequency 42,2 GHz in comparison with 50,3 GHz caused more pronounced shifts in BIA.

RESEARCH OF DEVIANT BEHAVIOUR VEGETATIVE REGULATIO MECHANISMS AT TEENAGERS

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Research of a mediators' role in behaviour regulation is the one of actual problems of modern neurobiology. Neurochemical basis of deviant behaviour is the imbalance of some hormones, which are determining domination of sympathetic or parasympathetic nervous system. Particularly, change of monoaminoxidaze enzyme activity, which is responsible for oxidation of adrenaline, noradrenalin and serotonin, modulates aggressive behavioural tendencies. Influence of adult's hormonal state on their behaviour has been shown. The low percentage of monoaminoxidaze enzyme and cortizol in blood at "aggressive" teenagers has been shown. This allows suggesting, that human biochemical features can increase probability of aggressive reactions. However, they can be corrected even at the worse heredity.

The purpose of the current research was a comparative estimation of vegetative parameters at teenagers before and after psychocorrection, aiming to decrease aggression level. Results of this research can be used as criteria for choosing the correction techniques depending on a gender of examinees.

24 «aggressive» male and female teenagers were examined in 4 psychocorrection sessions. To carrying out psychocorrection the combination of "aggressive" computer gameplay and the number standard techniques has been used. For revealing of heart rhythm vegetative regulation changes at examinees the electrocardiogram (ECG) registration before and after experiment has been used. For ECG processing variational pulsomerty by R.M. Baevski has been used. On the basis of this processing, statistical parameters of heart rhythm variability (HRV) were calculated and psychocorrection efficiency was supervised.

At «aggressive» examinees statistically significant ($p>0,01$) decrease of stress index (SI) and other HVR statistical parameters (IVR, VPR, PARP, RMSSD, etc.) regardless of gender was shown. However, intensity of these changes was closely dependent on sex: at male examinees HVR statistics are decreasing from sympathotony up to parasympathotony (SI decreases from 253 to 80) while at female examinees those parameters are decreasing from sympathotony to normotony (SI decreases from 248 to 138).

Obtained data shows vegetative balance displacement towards sympathetic tonus decrease; and also activation of heart rhythm self-control processes, which are preventing the over effects of sympathetic nervous system influence.

Results of the current research can be interpreted from the biochemical point of view that hormonal balance regulation influences on specificity of behavioural reactions.

PROTECTIVE EFFECTS OF THE PROLINE-RICH PEPTIDE (PRP-1) ON DEITERS' NEURONS AFTER UNILATERAL LABYRINTHECTOMY

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Still remains quite actual the problem and uncovered mechanisms of the vestibular compensation (VC), following unilateral labyrinthectomy (UL). On the other hand the hypothalamus, better studied as a higher link of the autonomic system regulation, needs to be explored from the view of its role in the motors displacement of the organism. Influence of the high frequency stimulation (HFS) of the hypothalamic supraoptic (SON) and paraventricualar (PVN) nuclei on Deaiters' lateral vestibular nucleus (LVN) neurons activity was studied during action of proline reach peptide (PRP-1) upon dynamics of rehabilitation after UL. Earlier we have shown the protective effect of PRP-1 in the case of various specific and non specific neurodegenerative diseases. Experiments were carried out on *encephale isole* male rats (n=7) on intact, as well as those with UL during PRP-1 injection (once at next day after UL0.1 mg/kg i/m). In electrophysiological experiments by mean of extracellular recording technique the spike activity flow of the single LVN neurons was monitored. Early and late responses of Deiters' neurons evoked by bilateral HFS (50 и 100 Hz, 1 sec) stimulation of SON and PVN were displayed in the form of inhibitory and excitatory tetanic (TD and TP) and post tetanic (PTD and PTP) potentiation.

The activity of 133 LVN neurons was recorded. Recordings were carried out by on-line selection and software package of spike analysis. The complex averaged and peri-event time histogram (PETH) and frequency histograms with computing of spike middle frequency were constructed.
In general electrophysiological data pointed out the tendency of LVN neurons activity changes during HFS of PVN and SON after the injection of PRP-1 composing of increase of the inhibitory and excitatory post stimulus reactions in the early stage of VC. In the late stage of VC changes are reached the norm. It must be also mentioned that post stimulus activities were relatively more pronounced at HFS 100Hz.

Hystochemical study was carried out by method of Ca^{2+}- dependent acidic phosphate (AP) revealing. At 3-d day after UL and PRP-1 administration Deiters' neurons were still exist and remind those of intact rats. At 9-th day after UL take place sharp intensification of AP. By the way at ipsi- and contralateral sides the activity of AP is the same. In other words there is alignment (balancing) of the ferment activity. Shape of changes at 17-19th day is characterized by weakening of ferment activity in the intact side. At the injured side there are found neurons with normal morphology as well as those with hyper chromatic nuclei, which show on hyper phosphorylation. In comparison with the intact side decreasing of neurons quantity is registered but morphological-hystochemical characteristic of the latter's allow to assume their survival through successful protective effect of PRP-1.

On the basis of our data involving of the GABA-ergic inhibition in the period of TD can't be excluded, which according to our intracellular recording results have been revealed during stimulation of a grate number of different brain structures. This show's on permanent deficit of inhibitory input to LVN and according to the literature data act as a tropic factor, influencing on proliferation, differentiation, maturation of the synapses and etc.Thus, our findings on direct connections of LVN with PVN and SON indicate that Deiters' nucleus acts not only as an integrative structure involved in regulation of posture and orientation in space, but also in the central regulation of autonomic functions.

STUDY OF THE NEURO-MODULATING ACTIVITY OF SOME DERIVATIVES OF CYCLOPROPANE- AND CYCLOBUTANECARBONIC ACIDS BY COMPUTER MODELING

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Cyclopropane and cyclobutane structures were found as a basic structural element in a wide range of naturally occurring compounds and in a great number of physiologically active substances. In series of possible studies of drug-receptor/channel interactions, the study of structure-activity relationship (SAR) is among perspectives. Molecular modeling of the structure of newly synthesized cyclic structures and their comparison with known analogous structures for activity and preliminary docking studies of said ligands with models of ion channels gave us possibility to definite physiological activity of mentioned compounds [1, 2].

It is well known, that amino acids with pyrethroid-like structure modulate mammalian and insect sodium channels. Some of them have neuroprotective activity and block sodium channels and high-voltage-activated calcium channels, thereby preventing excess stimulation of N-methyl-D-aspartate (NMDA) receptors and massive influx of calcium, thereby retarding spread of infarction in the brain. Neuronal nicotinic acetylcholine (nACh) receptors have received much attention recently. Inhalational general anesthetics augment the activity of γ-aminobutyric acid ($GABA_A$) receptors and inhibit the activity of α4β2-type acetylcholine receptors, causing a variety of clinical syndromes [3]. In the last few years, a variety of interesting biological properties of cyclopropane and cyclobutane based α-amino acids have been described. Some of them are known to be partial agonists at the strychnine insensitive glycine binding site of the NMDA receptor complex. Recently, it was shown that 1-aminocyclopropanecarboxylic acts concurrently as a glycine site partial agonist and as a glutamate site antagonist, thus protecting against neural cell death and exhibiting antipsychotic-like effects in animal models [4].

The results of docking analysis (Autodock 4.0) of following representatives of newly synthesized compounds (CBAA – 1-amino-2,2-dimethyl-3-chloro-4-trichloromethylcyclobutanecarboxylic acid; CBDAA – 1-amino-3-chloro-4,4-dimethylcyclobutane-1,2-dicarboxylic acid; HxCAA – 1-amino-2-hexyl-cyclopropanecarboxylic acid; PhCAA – 1-amino-2-phenylcyclopropanecarboxylic acid; CIP* – cypermethrin; ClCIP – 1-chlorocypermethrin; Perm* – permethrin; ClPerm – 1-chloropermethrin) are presented in table:

Compound	Receptor					
	NMDA		$GABA_A$		nACh	
	ΔG, kcal/mol	KI	ΔG, kcal/mol	KI	ΔG, kcal/mol	KI
CBAA	-26.97	16.9 zM	+6.32	–	-5.56	81.5 μM
CBDAA	-1.05e+7	< 0.1 zM	+4.137	–	+4.96	–
HxCAA	-26.96	17.4 zM	+16.51	–	-5.57	82.9 μM
PhCAA	-27.42	8.0 zM	+4.32	–	-5.63	74.5 μM
CIP	+2.20	–	-4.46	540.8 μM	-4.0 e+06	< 0.1 zM
ClCIP	-4.20e+6	< 0.1 zM	+2.20	–	+1e+18	–
Perm	-12.88	361.4 pM	-4.72	348.0 μM	-6.18	29.7 μM
ClPerm	+1.92	–	-3.90	1.4mM	-6.17	29.9 μM

* – are known pyrethroid compounds.

It's evident from presented calculations, that amino cyclopropane(butane)carboxylic acids and pyrethroids shows high affinity against NMDA and nACh receptors, whereas only pyrethroids shows affinity against $GABA_A$ receptors. It should be noted, that **Perm** is active against all three studied receptors.

The investigation of the bioactivity of the individual isomers, or pairs of isomers yielded valuable knowledge about the site(s) of action and emphasis the three-dimensional structure-activity relationship. Particularly, how changes in the molecular structure of

drugs and receptors/channels result in kinetic changes in the functions of receptors/channels. These results afforded predictive capability for further synthetic endeavors.

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PECULIARITIES OF PHOSPHOLIPIDS AND THEIR FATTY ACIDS COMPOSITION ABNORMALITIES IN RAT BRAIN CHROMATIN STRUCTURE AT UNILATERAL GANGLIOSYMPATHECTOMY

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Nowadays the revealing of the biomolecular mechanisms of wide scope of the sympathetic nervous system functional activity becomes an issue of crucial importance. In this respect the role of the upper cervical sympathetic ganglion as a modulator of organism's adaptational-trophical reactions; particularly in its compensatory reactivity concerning to the hard-metabolizing compounds in the brain cells is incomparably high. Its role is especially as a peripheral neuroendocrine center in regulation of metabolic processes of phospholipids (PL) and their fatty acids (FA) in the intranuclear structures of the brain cells that underlie many pathological states etiology and pathogenesis. The purpose of the present investigation is to study the role of the right upper cervical sympathetic ganglion in processes of neuroendocrine regulation and in regulation of the brain immunobiological activity by means of qualitative and quantitative determining of changes in PL and their FA composition, as well as their physical and chemical properties. The changes in contents and in ratio of the individual PL and their FA have been studied at rats' unilateral gangliosympathectomy (removal of upper cervical right sympathetic ganglion).

The decrease of total PL contents testifies the depression of chromatin matrix activity as a result of changes of hydrophobic interaction between DNA and histones as well as the conformational changes of proteins with a new type of protein-protein interrelations inducing the decrease of RNA-polymerase accessibility to the promotor region of gene. In the realization of those changes the decrease of sphyngomyelins (SM) and increase of lysophosphatidylcholines (LPC) are of an important significance.

The decrease of SM also induces the decrease of RNA-polymerase I and II activity and changes in quantitative correlation between bound and free form of enzyme. It is very important the variable changes in the contents of Mono-, Di- and Triphosphoinositides. Those changes carry out expressed decreasing character in PL of ganglioectomated site chromatin and also testify the destroying in the phosphatidylcholinic and phosphoinositidic cycles functioning, which are well known in signal transduction processes. In this case the increase of the level of saturated FA and the decrease of the level of unsaturated, especially polyenic FA of chromatin PL in ganglioectomated hemisphere are of a significant importance and are also the statement of RNA-polymerase activity inhibition. In chromatin of the intact hemisphere the increase of the relative quantity of polyenic FA, particularly of arachidonic acid takes place. An obvious increase of DPI, TPI and especially arachidonic acid, a slight increase of LPC and relatively PS testify the possible compensator perturbations.

So, the results obtained indicate the significant role of sympathetic nervous system, especially its peripheral part - UCSG, in regulation of minor components of chromatin - PL metabolism and their fatty acids, testifying the adaptational-trophical action of sympathetic nervous system.

THE SEARCH OF RELATIONS BETWEEN AGGREGATIVE STATE OF BLOOD AND INTERLEUKIN IL-1ß AT PATIENTS WITH ISCHEMIC STROKE

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Interdependence between containing of interleukin, aggregative state of blood cell's disorder and neurological state was investigated at 114 patients with ischemic stroke (IS) during acute and sub acute periods of the disease. 32 practically healthy persons were examined as a control group.

Carrying out examination allowed to determine that during acute period of IS, there are marked disorders of aggregative peculiarities of blood cells which are characterized both by the raise of aggregative peculiarities of erythrocytes and blood platelets, and by lowering of erythrocytes deformability. At the same time the indices IL-1ß raise the level of norm almost fourfold. During sub acute period of the disease indicating indices in comparison with period of diseases also expose to marked improved changes, however, they, nevertheless, don't reach the level of norm, and the indices IL-1ß are considerably lowed and practically don't distinguish from the norm. During both periods of IS between indices IL-1ß positive correlative relations was revealed which is the evidence of accompaniment of aggregated platelet lyses with expression of interleukin IL-1ßsynthesis.

It is noteworthy that during both periods of diseases between indices of blood platelet aggravation, interleukin IL-1ß and neurological status positive, correlative relations is revealed which is more marked during acute period at the same time the correlative relation was absent at died patients (42 men).

The results of our investigation let us suppose that previously supposed interrelation between two processes – activation, aggravation and degranulation of platelets and expression of interleukin synthesis are proved facts. However, it only testifies that the lysis of aggravated platelets is accompanied by the raise of interleukin IL-1ß but it can't be the evidence of worsening of clinical state of health of the patients with IS, owing to revealed relation. So, the present investigation ought to be considered as an attempt of search of interrelations between considered factors. Conducting of wide-ranging, randomized investigations would contribute for revealing of cause-effect relationship between the state of microcirculation and the system of cytokine at different pathological states.

THE ROLE OF BIOLOGICAL ACTIVE SUBSTANCES IN THE ACTIVITY OF SUCCINATDEHYDROGENASE IN SOME BRAIN STRUCTURES UNDER VIBRATION

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In present ecological conditions the influence of negative environmental factors on human organism is in great interest. Vibration, due to reflector impact on CNS, causes disturbances in biological redox processes of tissues and organs. It can lead to changes of the level of enzymatic activity of brain structures. The activity of anaerobic metabolism enzyme such as succinatdehydrogenase is rather sensitive characteristic of cell respiration under changed physiological state of organism in the conditions of vibration influence. The natural preparations, mainly photogenes with adaptive activity, are used to increase immunity, resistance of organism, prevention and complex treatment of many diseases. Biological active substances of plants are close to natural metabolites of organism and well compatible with them, many of them are necessary for vital functions. Licorice roots (*Glicyrrhiza glabra L.*) are widely used in medicine for prevention and treatment of vide range of diseases. At present, number of effective preparations with immunopotentiating, adaptive, antioxidant, antistress activities is produced from them. It is experimentally proved that such properties of Licorice roots are caused by variety of biological and pharmacological active substances in them. In the present work we investigated changes in the activity of succinatdehydrogenase in thalamus and hypothalamus of rabbits under combined influence of vibration and Licorice roots.

Experiments were carrying out on 16 mature rabbits of breed the Chinchilla. Animals were provided by Licorice roots (150 mg per 100 g weights of body). Animals were exposed to vibration influence on vibroplatform EV-1 with frequency of 60 Hz, amplitude 0,4 mm for 30 days, 2 hours session daily. The criterion of the enzyme activity was bleaching time of methylene blue in homogenate *in vitro* in anaerobic conditions. The decolouration time, the above activity of enzyme is shorter. Three groups of animals were studied. The analysis of the received data has shown that 30-day-long influence of vibration (I group) led to fall of succinatdehydrogenase activity in studied brain areas. In the II group under combined influence of vibration and Licorice roots there was marked fall of the enzyme activity, i.e. decolouration time of methylene blue was extended in thalamus by 12,0 % ($p<0,001$), in hypothalamus - by 203,6 % ($p<0,001$) in comparison with I group. In the III group exposed to combined influence of vibration and Licorice roots (30 days) after preliminary 30 day feedings in homogenate and solutions the hyperactivity of enzyme in studied brain structures was observed, i.e. decolouration time methylene blue was shortened in thalamus by 35,7 % ($p<0,001$), and in hypothalamus - by 32,7 % ($p <0,001$) in comparison with the vibrated animals.

In anaerobic metabolism conditions the requirement of cells in glucose is more and its natural intake usually cannot satisfy to available requirement. It is supposed that mono- and disaccharides (to 20%) as well as water-soluble polysaccharides that Glicyrrhiza glabra L. roots' contains have compensatory value for maintenance of requirement of tissues in these substances, so they stimulated anaerobic metabolism, and therefore, compensatory power supply of tissues. The above-mentioned fact allows concluding that preliminary 30-day feeding of rabbits with *Glicyrrhiza glabra L.*, further 30-day combined influence *Glicyrrhiza glabra L.* and vibration substantially raises the activity of succinatdehydrogenase in thalamus and hypothalamus. This testifies activation of compensatory possibilities of anaerobic metabolism under vibration as a stress factor directed for maintenance of energetic homeostasis of the brain in the conditions of long influence of vibration.

ULTRASTRUCTURAL PECULIARITIES OF WHITE RAT BRAIN MITOCHONDRIUMS IN EXPERIMENTAL EPILEPTIC ATTACK BY KORAZOL AND IN CYTOPROTECTIVE ACTIONS OF ANTIOXIDANTS

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Now among various ways to cause experimental convulsive condition most adequate to epileptic human attacks is the psychomotor form of epilepsy induced by corazol. Considering that mitohondria of eukaryotic cells is the most sensitive indicator, defining functional activity and energetic power supply of the cells, essential interest represents the analysis of ultrastructural changes of mitochondria in conditions of real processes proceeding *in vivo*. The purpose of the present research consisted in carrying out special observation in directions of ultrastructural changes of rats brain mitochondria after corazol influence , and on a background of application by antioxidants actions a-tocopherol (TF) and thiosulfat sodium (TSN), having in view available information on volatile reorganizations of mitochondria morphology.

White rats (180-200 grams, not purebred) were used in these experiments. Epileptic-like concussions were induced by disposable intramuscular injection of corazol at the rate of 8 mg per 100 gram of animal weight. Control animals received 1 ml of physiological solution. With the purpose of preliminary sensibilization factors endogenous antioxidant activity, disposable intramuscular injections of 1 mg TSN and 0,4 mg of α-TF oil solution per weight of animal were performed. After euthanasia, brain slices from control and experimental animals were collected and received mitochondrial fractions for transmission electronic microscopy were fixed by 2,5% solution of glutaraldehyde in phosphate buffer with subsequent postfixing by 1% solution of osmium tetroxide in phosphate buffer. After water draining in alcohol and steep in araldits, samples were concluded in a mix of araldits. Ultrathin sections made on ultramicrotome Ultracut-Reichert, after contrasting with uranyl acetate and lead citric acid were observed under Electron microscope Tesla BS-500 at an accelerating pressure of 80 kv.

Investigation of isolated mitochondria ultrastructure in suspension and in brain tissue after corazol injection shown the role of corazol in their dynamic re-structuring and establish mitochondrial ultrastructure changes polymorphism in the form of energetically defined conditions of mitochondrial ultrastructure, i.e. by configuration changes. The structures of mitochondria well correlated with their functional condition. We found that isolated mitochondria were affected by corazol, i.e. condensation of matrix, expansion of intermembranous space, occurrence of vacuoles and separation of external membrane.

Also increased volume of intermembranous space can be noticed in such cases. Mitochondria in suspension and in fabrics of brain after α-TF and TSN influence in the greater part restore structural integrity. Revealed by us various configuration conditions of mitochondria is not the nonspecific answer of mitochondrial ultrastructure on developing pathological process. These changes of mitochondrial structure can be explained on the basis of classic research data regarding interrelations of mitochondrial ultrastructure and their functions. TF and TSN render cytoprotective effect and thus could be used for reduction of cytoprotective effect.

Thus, results of electron microscope analysis make possible an estimation of functional condition of brain at EFP according to ultrastructural changes of mitochondria, and also cytoprotective correcting effect of antioxidants (TF, SST).

OXIDATIVE MODIFICATION OF PROTEINS IN WHITE RAT BLOOD SERUM UNDER NOISE AND A2–ADRENOBLOCKER INFLUENCE

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A great amount of real facts concerning the influence of oxygen active forms on the functional state of cells and its role in the pathogenesis of many diseases has been accumulated. The recent studies showed that products of oxygen metabolism first damage protein structures.

We represent here the results of investigations on the influence of noise (acute acoustic stress) and α_2–adrenoblocker on blood serum protein oxidative modification (POM). POM was evaluated basing on dinitrophenylhydrasone (the interaction products of protein oxidized aminoacid residuals with 2,4-dinitrophenylhydrasine) content shifts, which were registered using spectrophotometer SP-26 at wave lengths 356, 370 and 430 nm.

Experimental animals (white male rats, 130-150 g) were subdivided into 6 groups. Rats of the first group (intact animals) served as control group. Rats of the 2^{-nd}, 5^{-th} and 6^{-th} groups underwent noise influence with level of 91 dBA with maximum energy in the range of medium and high frequency waves during 2 hours. The animals of the 3^{-rd} - 6^{th} groups were administered α_2-adrenoblockers intraperitoneally in the dose of 2 mg per body mass.

Beditine (for the first time synthesized in the Institute of Fine Organic Chemistry, NAS RA) and isadoxan, as a comparison standard, were used as α_2-adrenoblockers. All experimental animals were decapitated in 2 hours; serum was collected for studies.

Marked increase in POM compared to the intact group animals was revealed in the result of acoustic influence that was shown by elevated level of dinitrophenylhydrason in blood serum. It is worth mentioning that the increase degree of the latter at various wave lengths is different, what is obviously explained by POM different derivative product formation.

Thus, if POM product level registered at the wave length of 356 nm is almost twice higher under acoustic influence, whereas at the wave length of 430 nm it is higher about 20 times. Intraperitoneal administration of α_2 - adrenoblockers also increases POM intensity, though in a lesser degree compared to the acoustic exposure, especially in case of beditine administration. The study of POM products registered at wave lengths of 356 nm and 370 nm has revealed their higher growth under isadoxane influence than that of beditine, whereas POM product amount at wave length of 430 nm was almost the same under the influence of the studied α_2-adrenoblockers.

Beditine preliminary administration, unlike idasoxane, not only fails to increase POM product amount, but significantly prevents their increase. It is important to mention that dinitrophenylhydrasone level decrease under beditine and noise combined influence is registered at all 3 wave lengths.

Thus, summing up the data obtained we can come to the following conclusions.

I. Noise influence causes expressed increase of dinitrophenylhydrasone content.
II. α_2-adrenoblockers increase blood serum POM compared to the intact animals, in which beditine does it in a lesser degree.
III. Beditine preliminary administration prevents the increase of blood serum POM products.

CALCINEURIN IS A KEY ENZYME UNDERLYING THE MOLECULAR MECHANISM OF HEMORPHINS ACTION IN PATHOPHYSIOLOGY OF SARCOMA-45

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In an early *in vitro* study we showed that hemorphins modulate brain and lymphocytes Ca^{2+}/calmodulin(CaM)-dependent protein phosphatase calcineurin activity by binding with CaM, exhibiting concentration-dependent biphasic response on enzyme activity. We have identified CaM as an essential component in starting up the molecular mechanisms of hemorphins action and calcineurin as a key enzyme underlying the molecular mechanisms of hemorphins action in the brain and immune system. It is well known that calcineurin play an important role in the brain and immune system. This enzyme is involved in the signal transduction cascade leading to T-cell activation by involvement in gene expression and production of several cytokines (interleukin-2 (IL-2), IL-4, tumor necrosis factor-alpha (TNFα) and etc.) via dephosphorylation of nuclear factor of activated T-cells (NFAT) family members. In the brain calcineurin regulates neurotransmitters and neuropeptides and neurohormones release. Calcineurin is involved in pathophysiology of severe diseases, such as Alzheimer disease, diabetes, cancer and etc. In present work we study the influence of hemorphins on plasma and brain calcineurin activity in pathophysiology of sarcoma-45 (S-45). In the experiments were used 3 hemorphins: LVVYPW, LVV-hemorphin-7 (LVVYPWTQRF) and hemorphin-7 (YPWTQRF).

For the experiments were used rats (Wistar line, 100-120 g). Rats were randomly divided into 5 groups (n=10 in every group): control group injected by saline, and 4 experimental groups inoculated with sarcoma-45. 3 of experimental groups after inoculation of S-45 received treatment with hemorphins. Each group received the treatment with specific hemorphin during 8 days (single intraperitoneal injection of hemorphin (1 mg/kg) daily).

Results obtained indicated down regulation of calcineurin activity in rats bearing S-45 in both plasma (2.7 fold) and brain (2.3 fold). All 3 groups of rat received treatment with different hemorphins exhibited increase in calcineurin activity. More pronounced results in all 3 groups were received for plasma calcineurin activity, when enzyme activity was almost reaching to the level of calcineurin activity in the control group. It is necessary to emphasize that all 3 hemorphins demonstrated the inhibition of tumor growth. It is suggested that hemorphins as a members of endogenous protective system of the organism come into play mainly in response to pathophysiological conditions. In that case hemorphins, like other pleiotropic neuropeptides, serve as one of the factors that switch on the compensatory systems in the organism. This is based on several mechanisms and by implication of different signaling pathways in order to recover the homeostatic disturbance. We think that in given case the hemorphines act as homeostatic agents via modulation of calcineurin-NFAT pathway.

SOME CLINICAL LABORATORY PECULIARITIES IN PATIENTS WITH NEUROENDOCRINE SYNDROM WITH NORM- AND HYPERPROLACTINEMIA

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There were studied 107 female patients with neuroendocrine syndrome (NES) at the age of 23-35 (26.8 ±0.6). After clinical diagnostic verification the patients were divided into 2 clinical groups: the I group involved the patients with normoprolactinemia (40 patients) and the II one - with hyperprolactinemia (67 patients). According to the case histories 77 females (71.9%) had the diagnosis Hypothalamic syndrome in puberty period (HTSPP) and only half of them underwent treatment what indicates transformation of NES from HTSPP. Hereditary aggravation (on endocrine pathology) was determined in 22.5% patients of the group and 40.2%o - of the II one, and expressed premorbid state in patients with normoprolactinemia was revealed in 10.0% and reliably ($p<0.001$) more (by 55.2%) in women with hyperprolactinemia.

The leading complaint in absolute majority of the patients was infertility (in the I group secondary infertility was noticed twice often), dysfunction of menstrual cycle according to the secondary amenorrhea type (10.0%; 37.3%), opsomenorrhea (72.5%; 62.7%). The maintained menstrual rhythm was noticed only in the I clinical group (17.5%o). Galactorrhea was detected in 31 (46.3%) patients with hyperprolactinemia which didn't depend on PRL level. Headache (31.3%), weakness (4.4%) paresthesia of various types (7.4%) were nonspecific complaints observed only in the II group patients. Objective data revealed that 63 (58.9%) patients were overweight, among them 41 women of the II group. Body mass index (BMI) was on average 29.8 ± 0.4. Microadenoma not more than 2 mm in size was revealed by CT and MRI methods only in 4 patients in the II group. Hormonal monitoring exactly revealed normoprolactinemia (PRL - 17.9 ± 1.24 ng/ml, in norm - 8.39 ±20.15 ng/ml) and hyperprolactinemia 29.3 ± 2.06 ng/ml), $p<0.001$. LH level was lower than the average norm index (3.76 ± 0.07 mlU/ml; 2.81 ±0.16 mlU/ml) in both groups with reliable difference ($p< 0.002$) unlike FSH which was almost the same. Hypoestrogenia was typical for both clinical groups and that reflected both cycle dysfunction character (secondary amenorrhea, opsomenorrhea) and PRL level. All patients were euthyroid. The changes in blood lipid spectrum were revealed in 44 (41.1%) patients. The changes were characterized by general cholesterol (CS) increase and pCS - low density lipoproteins (LDL) but the difference between the I and II groups was not reliable ($p<0.05$).
Ultrasonic study method of the small pelvis determined that 2 uteral sizes became less (posterioanterior and width) and that was directly associated with the cycle dysfunction type (amenorrhea, opsomenorrhea) and E2 level in blood.

SPECIFIC FEATURES OF PHOSPHOLIPID MOIETY OF PROTEOLIPIDS FROM PRIMARY AND SECONDARY LYMPHOID ORGANS OF SOME MAMMALS

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In the course of investigations aimed to elicit the specific features of integral hydrophobic membrane proteins-proteolipids (PLs) and lipids bound with them in cellular membranes of brain, especially rich in these compounds, and other organs, it was important to study peculiarities of protein and lipid moieties of PL, isolated from the primary (thymus) and secondary (spleen, lymphatic node) lymphoid organs of the immune system of some mammals. Recently in definite cells of these three lymphoid organs expression of major PL protein of myelin and its isoform-protein DM-20 was revealed. These proteins parallel with basic protein of myelin are candidate antigens of autoimmune diseases including multiple sclerosis.

Investigations had shown that PLs are present in quite definite amounts, comparable with other organs, in all lymphoid tissues studied. Concentration of proteolipid proteins (PLPs) was higher in lymphatic node (cattle) – 1,73 mg/g of fresh tissue, somewhat lower in spleen (2,5- and 4- months old rats) – 1,38 mg/g and several times lower in thymus of rats (2,5- and 4- months old) and rabbits (3- and 5-months old) – 0,45 and 0,35 mg/g of fresh tissue respectively.

The main task of present work was isolation of soluble preparations of PLs from lymphoid organs (thymus, spleen – 3- and 4-months old rats; lymphatic node, armpit-cattle) and investigation of composition and quantity of phospholipids, loosely bound and more tightly bound with PLPs. Simultaneously the concentration of individual phospholipids in total lipid extracts of studied organs was determined. Isolation of PLs and further analysis of their lipid (phospholipid) components were performed according to methods described earlier [1].

Investigations had shown that total lipid extracts of all three lymphoid organs don`t significantly differ in their phospholipid composition. They all were characterized by relatively high concentration of phosphatidylserine, phosphatidylinositol and sphingomyelin. Together with similiarities quite definite differences were observed particularly in the phospholipid composition of more tightly bound with PLPs lipid fractions of lymphoid organs. Loosely bound with PLPs, lipid fractions of all three organs contained mainly neutral phospholipids, viz., phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin, which in sum constituted 81-86% of all loosely bound phospholipids. These lipid fractions of lymphoid organs were characterized by relatively high concentration of sphingomyelin (9,62-12,65% of all loosely bound phospholipids). Relatively high content of loosely bound sphingomyelin was also specific feature of PLs from brain and especially kidney. In lipid fractions more tightly bound wit PLPs the acidic phospholipids prevailed, forming 75,4-80% of total of the tightly bound phospholipids. The PLs of three lymphoid organs studied were characterized by the predominance of quite definite more tightly bound acidic phospholipids. PLs of thymus and spleen were quite similar in phospholipid composition of tightly bound with PLPs lipid fraction. It was characterized by high concentration of two acidic phospholipids: phosphatidylserine and phosphatidylinositol, which constituted 32,18 and 28,22% of all phospholipids tightly bound with PLPs in thymus and 30,53 and 29,65% in spleen respectively PLs of such type were revealed by us earlier in kidney medulla and in liver microsomes. In contrast to these two lymphoid organs, tightly bound with PLPs lipid fraction of lymphatic node PLs, was characterized by predominance of phosphatidylserine, which made 39,83% of all tightly bound phospholipids, concentration of phosphatidylinositol was two fold lower – 20,06%. Phospholipid composition of PLs of lymphatic node was

more similar with PLs of brain and its myelin, but in myelin the content of phosphatidylserine more tighyly bound with PLP was considerably higher – 60-70% and that of phosphatidylinositol-lower – 14,6%.

Comparing presented data with ones obtained by us earlier on phospholipid composition of PLs from different organs and their subcellular particles it may be concluded that in tissues of immune system, besides of myelin PLs, other cellular membranes of PLs are also present. Only further investigation of cellular elements of lymphoid organs may reveal localization of different PLs in these tissues.

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MECHANISMS OF AGGREGATION OF GLOBULAR OLIGOMERIC PROTEINS AND SUPPRESSION OF AGGREGATION BY CHAPERONES

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Protein aggregation arises as a result of protein misfolding in response to mutations, posttranslational modifications or changes of local conditions such as pH and temperature. Protein aggregation underlies pathogenesis of a considerable part of human neurodegenerative diseases (such as Alzheimer's, Parkinson's and Huntington's diseases, spinocerebellar ataxia, prion encephalopathies, amyotrophic lateral sclerosis, systemic amyloidosis). Many misfolded proteins in cells expose hydrophobic areas on their surface, which can interact leading to formation of disordered (amorphous) or ordered protein aggregates (amyloid fibrils).

The kinetics of aggregation of globular oligomeric proteins (bovine lens beta-crystallin, rabbit skeletal muscle glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and creatine kinase, pig heart mitochondrial aspartate aminotransferase) has been studied by dynamic light scattering (DLS). The DLS data show that the initial stage of thermal aggregation of proteins is formation of the start aggregates. Further sticking of the start aggregates and aggregates of higher order resulting with formation of large-sized amorphous aggregates proceeds in the regime of diffusion-limited cluster-cluster aggregation (DLCA). It is evident that the start aggregates resulting in formation of amorphous aggregates are similar to protofibrils formed in the course of assembly of fibrils.

A comparative study of the anti-aggregation effect of small heat-shock protein alpha-crystallin and chaperonin GroEL has been carried out. Both chaperones suppress thermal aggregation of GAPDH. The analysis of the initial parts of the dependences of the hydrodynamic radius of protein aggregates on time shows that in the presence of alpha-crystallin or GroEL the kinetic regime of GAPDH aggregation is changed from the DLCA regime, wherein the sticking probability for the colliding particles is equal to unity, to the reaction-limited cluster-cluster aggregation regime, wherein the sticking probability for the colliding particles becomes lower the unity. The interaction of alpha-crystallin with unfolded GAPDH blocks the formation of the start aggregates. Suppression of aggregation by alpha-crystallin is due to the diminution of the size of the start aggregates and increase in the duration of the latent period. The interaction of GroEL with unfolded GAPDH does not affect the formation of the start aggregates. However GroEL incorporated in the start aggregates reduces the sticking probability for the colliding start aggregates.

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INPUT OF QUERCETIN IN PHOTOTOXITY OF *H. PERFORATUM* EXTRACTS

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Apart from the ancient methods of use, modern techniques result in numerous innovations, where St John's wort (*Hypericum perforatum*) can be used in the field of health care. *H. perforatum* is used mainly in disturbances such as depression, nervous breakdown, stress, aggressiveness, etc. There are numerous compounds within *H. perforatum* preparations, where the major classes are naphthodianthrones, e.g. hypericin (HY) (1,3,4,6,8,13-hexahydroxy 10,11-dimethylphenanthroperylene-7, 14-dion) and flavonoids, the representative of which is quercetin (2-(3, 4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4). Nowadays these secondary metabolites are of great interest due to antioxidantive, anti-inflammatory, immunomodulatory, gastro- and neuroprotective activities (quercetin), as well as because of their potential application in photodynamic therapy of cancer, in blood sterilization and transfusion (HY) [1]. As drugs are often injected to patients through blood system, study of the influence of *H. perforatum* components on blood elements, in particular, erythrocytes, becomes an issue of current importance. In order to increase therapeutic efficacy of *H. perforatum* preparations the overall mechanism, as well as the action of separate components must be studied, the primary targets must be unrevealed. In the present work the influence of *H. perforatum* herbal preparations and its separate components on model cells, such as erythrocytes was studied.

The light-induced phototoxicity of *H. perforatum* extracts and HY were evaluated by calculating the erythrocyte resistance. The "thermal treated" extract (TE) and "cold extract" (CE) were received from flowers and leaves of *H. perforatum*. TE was received by Soxhlet extraction in methanol with further lyophilization and dilution in ethanol, CE – by storing in 96% ethanol a week. Human erythrocytes were received by centrifugation at 800g 15 min at 5^0C with further resuspension in buffer. After 10 min irradiation by visible light (100W, 30mW/cm^2 on the sample surface) photohemolysis degree was determined at 680 nm on spectrophotometer Specord M400 (Carlzeiss, Germany).

Under the influence of visible light both extracts – CE and TE, as well as HY have expressed photodynamic activity and reduce erythrocyte resistance. Irradiation of erythrocytes with CE and ascorbic acid has revealed the protective effect of the latter despite its concentration on the contrary with pure HY and TE. It was revealed that in CE extract there are some components, e.g. quercetin that suppresses photohemolysis sensitized by HY. The latter was certified by conducting erythrocyte photosensitization in the presence of quercetin. Suppression of erythrocyte photodestruction by quercetin is possibly connected with scavenging of $^1O_2$ and other radicals, generated during photosensitization reaction. The latter prevents apoptosis that is considered as one of the causes of neurodegenerative disorders [2]. It could be concluded that TE and CE are more powerful therapeutic tools and that agents existing in extracts, such as quercetin, can change outcomes of HY action and make it more favorable for therapeutic application.

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PRADER-WILLI SYNDROME AS HYPOTHALAMIC PATHOLOGY MANIFESTATION

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Prader-Willi syndrome (PWS) is genetic pathology which is manifested by wide spectrum of neuroendocrinal, neurosomatic, neurovegetative and others disorders, in particular by obesity and hyperphagia, growth deficiency, mental retardation, by disorders of behavior, breath, thermoregulation, reproductive function, menstrual cycle, accompanied by respective changes in hormonal profile. Frequency of diseases is 1:15 000 - 1:20 000 population. Clinical manifestations of the disease, neurological hormonal researches (decrease of prolactin, estrol level in girls, gonadotropic hormone, etc.) testify of hypothalamic character of the disorders.

It is established that PWS in caused by disorder in gene expression owing to defect of imprinting in the area of 15 chromosomes (in region of a segment q11.2-q13). Critical region involve more than 12 genes leading to dysfunction of hypothalamic area that causes neuroendocrinal disorders.

Interest to this pathology is caused by great attention to obesity problem. However, the disease is seldom diagnosed in clinical practice, in spite of introduced descriptions of phenotype and clinical symptoms in the literature, diagnostic opportunity of this pathology by modern cytogenetic and molecular genetic researching methods (FISH and mutilation test) , worked out recently introduction of the new treatment methods.

Dysfunction of hypothalamic area leads to fat metabolism disorder with over 10 times increase of fat synthesis from acetate and rather decreased process of lipolysis. Hypogonadism on hypogonadal type can be connected to hypothalamus dysfunction mainly of ventromedial and ventrolateral nucleus. Patients of both sexes are usually sterile. Inguinal and umbilical hernia, joint hypertension, posture disorders, x-shaped curvature of low extremities, etc., are apparently caused by muscular hypertension, hypertension joints, etc. Increased risk of development of leukemia and other tumors in patients with Prader-Willi syndrome are apparently caused by low reparation DNA ability, etc.

However there are no researches devoted to neuroimmunological changes in the given pathology in the literature, though wide spectrum of hypothalamic neuroendocrinal and neurovegetative disorders should assume an opportunity of neuroimmune regulation disorders in the pathogenesis of disease.

Carried out studies together with the International Association of Prader-Willi Syndrome (IPWSO) and, Lency Found (USA) of PWS problem in Armenia, in particular, diseases prevalence, as well as clinical peculiarities of syndrome, including neuromuscular disorders in PWS by using modern immunological researches, allowed revealing some immunological alteration requiring further deep investigation. Investigations allow to determine degree of neuroimmune system participation in pathogenesis of Prader-Willi syndrome and to open new page in the study of this disease.

HYPOTHALAMIC PROLIN-RICH PEPTIDE AGAINST BRAIN DEGENERATION EVOKED BY BETA-AMYLOID PEPTIDES

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By histochemical methods of revealing of Ca^{2+}-dependent acidic phosphate activity a comparative studies were carried out of morpho-functional state of hippocampal and cerebral cortex cellular structures on Alzhimer's diseases models. The model was induced by bilateral intracerebroventrical injection of β–amyloid peptides Aβ 25–35, Aβ 1–40 and Aβ 1-42, combined with systemic administration of proline-rich peptide – 1 (PRP-1). Morpho-histochemical study were carried out 18-22 weeks later after Aβ injection.

Morphological pictures of processes abundance of neurodegenerative changes in cerebral cortex under Aβ 25–35 is characterized by absence of areas continuity. The analysis of data concerning distribution of neurodegenerative processes deep into and along cerebral cortex has shown that pyramidal layer is the most frequently damaged area. The majority of neurons are subjected to central chromatolysis. However cells with normal morphology were also detected. The analogous picture was observed in hippocampal slices. In the CA3 and CA4 areas it is characterized by rounding and swelling of neuronal soma of big pyramidal cells of dentate gyrus. Although these cells were more stable to amyloid, they also undergo to tendency the cytoplasm clearing. The processes of neuronal splitting were accompanied by cancellous alveolar state of neuronal cytoplasm.

At Aβ 1–40 injection the process of chromatolysis was also detected in cerebral cortex. However, here greater in quantity and volume of "gapped" was observed compared to Aβ 25–35. As a result, in cerebral cortex occurs process of diffuse or limited evacuation of cellular layers, the spaces of which remains empty owing to absence of reaction of glial cells nuclei. In hippocampal slices at Aβ 1–40 the swelling and rounding of cellular soma with nucleus ectopy is also occurred. The central surface of cell appeared clear, and in some neglected stages it was glassy.

At Aβ 1-42 injection in CA1 area of hippocampus slices we observed total disappearance of neurons reaction in the form of semicircular pockets on equal distance from each other. As characteristic signs, we found big amorphous phaeochrous neurons of dentate gyrus of hippocampus and III – IV layers of cerebral cortex, with disorderly located neurofibrils in cytoplasm. The mentioned data testify the processes of hyperphosphoryllation that precedes late stages of Alzheimer's disease, and characterized by tau-related neurofibrillar changes.

At Aβ 25-35 and 1-40 injections and PRP-1 administration, density of cells disposition in cerebral cortex and in hippocampus increased. In cerebral cortex state of tensity of nerve cells markedly decreased; thicker apical dendrites of pyramidal neurons started to act in response. The configurations became irregular, however, further with development of regenerative processes, the dimensions decreased, the nucleus is transformed into the center of the cell; the cytoplasm is filled up by precipitate granules. Different intensity of neuronal staining was observed in slices, i.e. occured both dark-stained cells and neurons, in cytoplasm of which on the light background a Homori–positive granules were visible. The similar picture we observed in hippocampus. Everywhere the blood vessels were revealed, on external wall of which one can see a dark pericytes with processes. At Aβ 1-42 injection and PRP-1 administration, there was sharp decrease of dark cells number with neurofibrillar changes in cerebral cortex, suggecting that PRP-1 delayed tau-

phosphorylation of neurons. In CA1 area of hippocampus slices the devastated places were filled up by neural cells with weak enzymatic activity.

Thus, results of these studies on PRP-1 use after different Aβ peptides injection allow suggesting that PRP-1 causes positive changes of structural properties of neurons, increase of metabolism that determines survival of cells.

MECHANISM OF ENZYMATIC HYDROLYSIS OF DICHOLINE ESTER OF SEBACIC ACID BY HUMAN SERUM BUTYRYLCHOLINESTERASE

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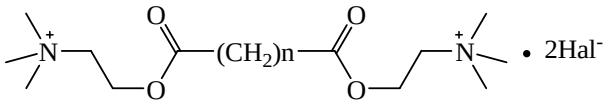
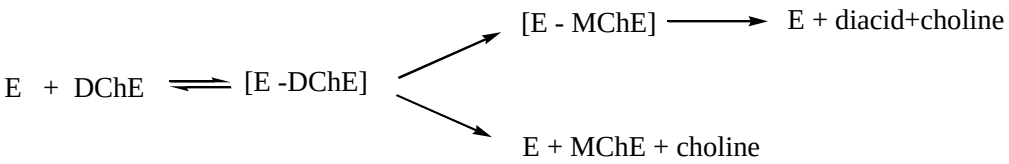
Dicholine esters of aliphatic dicarboxylic acids (DChE) with the following general formula are bioactive compounds of medical interest ascurarelike compound.



The best known compound of this family succinyldicholine ($DChE_2$, ditilin) is widely used in anesthesiology as muscle relaxant of short action [*F. Brucke, , Pharmacol. Rev.* ***1956****, 8, 265-335. D. Bovet, Neuromuscular blocking agents, in: J. Cheymol (Ed.),* ***1973****. p.244]*. The duration of their action is conditioned by their hydrolysis by plasma butyrylcholinesterase (BChE). In the works of several authors it is shown that the enzymatic hydrolysis of DChE by BuChE occurs by two sequential steps. In view of large difference between values of K_m for DChE and product of its hydrolysis monocholine ester of dicarboxylic acid (MChE), the latter is hydrolyzed forming diacid only after exhaustion of DChE in the reaction medium. However during the study of enzymatic hydrolysis of DChE with n≥6 we discovered a deviation from proposed mechanism whose essence consists in the formation of diacid in the first stage of the reaction [M*natsakanyan, M.R.; Hakobyan, L. M.; Sargsyan, J.V.; Samokish, V.A.; Halebyan, G.P. Biological Journal of Armenia 2007,* ***59****, 239-247*]. In the present work in purpose to explain the observed phenomenon the kinetics of enzymatic hydrolysis of DChE and MChE of sebacic acid ($DChE_8$ and $MChE_8$) by human serum BChE was investigated as well as the values of the parameters of Michaelis-Menten equation were determined for these substrates. It was shown that in contrast to succinyldicholine and succinylmonocholine, for which $K_{m(DChE)}/K_{m(MChE)} \approx 10^{-2}$ and $V_{m(DChE)} / V_{m(MChE)} \approx 7$, in case of $DChE_8$ ($K_{m(DChE)} - 3.0 \pm 0.38 \times 10^{-5}$ M, $k_{cat(DChE)} - 16.4 \times 10^3\ min^{-1}$) and $MChE_8$ ($K_{m(MChE)} - 2.2 \pm 0.28 \times 10^{-4}$ M , $k_{cat(MChE)} - 6.09 \times 10^3\ min^{-1}$), the values of this parameters were closer: $K_{m(DChE)}/ K_{m(MChE)} \approx 0.15$ $V_{m(DChE)} / V_{m(MChE)} \approx 2.7$. The obtained parameters for $DChE_8$ and $MChE_8$ were applied for plotting theoretical dependences of all reactants concentration on time. As attested analysis of the obtained results, the individual parameters of Michaelis-Menten equation for $DChE_8$ and $MChE_8$ do not explain observed accumulation of sebacic acid in the reaction medium (which is nearly 1.7×10^{-4} M if substrate initial concentration is 5×10^{-4} M) in the first stage of reaction if there is no deviation from pure competitive mechanism of hydrolysis. The proposed scheme of the first stage of hydrolysis explaining the observed deviation from competitiveness is based on an assumption that the part of $MChE_8$ molecules (formed as a result of $DChE_8$ hydrolysis) manages to be hydrolyzed due to $MChE_8$ slow diffusion from the region of active site of enzyme into reacting medium according to the following scheme.



EFFECT OF CORONARY CONSTRICTORY NEUROPEPTIDE ON [^{35}S] TAURINE UPTAKE IN RATS HEART AT THE BLOCKADE WITH STRYCHNINE – INHIBITOR OF TAURINE RECEPTORS

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In hypothalamus neurosecretory cells (NO and NPV) there are synthesized releasing hormones, as well as cardiotrop neurohormones, which are specific regulators of cardiac blood circulation and cardio-vascular system in a whole. In the adaptation mechanisms it was very important to find mediators, feed-back between heart and hypothalamus. In 1967 A.A.Galoyan indicated that biosynthesis of peptide hormones and peptidergic system takes place in atria ganglionary cells, which are signal systems for cardiotrop hormones "K" and "C" release into general blood flow. The neurohormones "K" and "C" are mediators like acetylcholine and catecholamines. The sulfoamino acid taurine belongs to the system of mediators, which changes the cardiac muscle reaction to adrenaline and acetylcholine influencing their receptors. A complex regulatory system exists in heart, including Ca, K, GAMP and taurine. To understand the conjunction of those mediators in the regulation of cardiac blood circulation, we have studied the effect of coronary constrictory neuropeptides (CCF) and strychnine (taurine blockader) on [^{35}S] taurine uptake rate in different parts of the heart at incubation for 10 min, 30 min, and 60 min. It is established that CCF in concentration of $3 \cdot 10^{-8}$M during 10 min increases taurine uptake in the right and left atria for 53% and 84.2%, respectively, and in left ventricle for 72%. At blockade of taurine receptors with strychnine in concentration $5 \cdot 10^{-4}$M, significant change of taurine uptake is not observed in all studied parts of heart. Combination of CCF and strychnine induces increase of the uptake only in the left atria for 129.5%. The investigations fulfilled indicate that at incubation of the slices from different parts of the heart during 30 min does not induce a noticeable change in taurine uptake. At 60 min incubation an increase of taurine uptake is observed only in the left atria for 129%, and in all other parts of heart an inhibition of the uptake is observed in the right and left atria for 81.7% and 59%, respectively, and in cardiac ventricle for 67%. Strychnine removes the CCF inhibiting effect and induces increase of uptake in the right ventricle, in the right and left atria for 101%, 125% and 120%, respectively. CCF in combination with strychnine an inhibition of taurine uptake takes place in all studied parts of heart except the right atrium, where an increase of uptake is observed for 136%.

Thus, the obtained results indicate that in the basis of taurine physiological effect on the heart is the change of cardiac muscle stimulation under CCF and taurine through the change of cell membrane permeability. The effects of CCF and strychnine can be considered on taurine receptors level.

DNA METHYLATION AND ITS POSSIBLE CHANGES IN THE BRAIN AT THE RETROGRADE AMNESIA

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The aim of present study was to investigate DNA methylation reaction in the cerebral cortex and cerebellum of animals with amnesia before and after injection of the psychotropic preparations piracetam and fenozepam. It is estimated the antiamnestic action during the formation of the conditioned passive avoidance reflex with the subsequent use of electric shock as a factor of amnesia. It is established that during the conditioned reflex formation the content of 5-methylcytosine in cerebral cortex is increased (80%) as compared with the control (47%). Piracetam activated the function of neocortex and increased methylation level of DNA in the cerebral cortex of rat brain. Fenozepam is not related to the conditioned reflex activity and does not induce the changes of DNA methylation both in two studied regions of rat brain. Thus, DNA methylation may correlate with the functional activity of brain cells in the process of the conditioned reflex formation. Piracetam modulating the functional activity of brain cells can also change methylation level of genome.

EXPERIMENTAL APPROACH TO STUDY THE SECRETED PROTEASES OF BRAIN

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The cysteine proteases caspase 3 and cathepsins are involved in both neuronal plasticity and neuropathology. Caspase-3 and cathepsin B activities in extracellular medium (balanced salt solution, BSS) of primary rat neuroglial cerebellar cultures were studied. At acidic pH, cathepsin B cleaved Ac-DEVD-AMC, a synthetic caspase 3 substrate, this activity being significantly higher than that of caspase 3 at pH 7.4. This activity is blocked by peptide inhibitors of both caspase 3 and cathepsin B. Substitution of culture medium for balanced salt solution stimulated cathepsin B secretion in both types of cultures. Ischemia (oxygen–glucose deprivation) significantly decreased secretion of cathepsin B activities into the culture medium.

This work was supported by the Russian Foundation for Basic Research (grant No.08-04-00870).

INFLUENCE OF NEW ADRENAL MEDULLARY CARDIOACTIVE PEPTIDES ON BRAIN AND SOME ORGANS GLYCOGENPHOSPHORYLASE ACTIVITY

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Development of new approaches and techniques to investigate specific CNS protein has led to quick progress in studies of the adrenal medullary protein spectra. In adition to the catecholamines and nucleotides large amounts of chromogranines and some identified and anidentified proteins and peptides: vasoactive intestinal polypeptide, substance P, neuropeptide Y, somatostatin, neurotensine, opioid peptides, dopamine-ß-hydroxylase, O-glycosylated polypeptides. Even this short list provides some perspectives onto the search for new bioactive compounds in the adrenal medulla. Cardioactive compounds we discoverd earlier [1,2] in the bovine adrenal medulla are interesting in this respect. Interest in these compounds as well as further studies on their identification are associated with the involvement of adrenals in the realization of the coronarodilating effect of hypothalamic neurohormones "K" and "C" [3].

Eight cardioactive compounds have been isolated from the bovine adrenal medulla. These compounds differ from each other in terms of their pharmacological characteristics (the duration of action, the time taken to increase and the magnitude of the peak effect). These differences appear to be associated with their structural characteristics and particularly with the amino acid composition. The most interesting are two of these compounds, lettered as $M3_1$ and $M4_2$ with Mr (4100-4200 D) and can be classified as the glycopeptides. $M3_1$ and $M4_2$ factors as a hypothalamic cardiotropic neurohormone "C" are resistent to various denaturing agents, high temperature (110^oC) and enzymatic treatment, to the effect of alkali (1N NaOH) and acids (6N HCl). These factors demonstrate analogous to hypothalamic neurohormone "C" inhibition of cAMP PDE activity and dependence of this effect from their coronary dilating potency as well.

In this study we attempted to investigate the possible participation of mentioned ($M3_1$ and $M4_2$) compounds in the regulation of glycogenphosphorylase activity in brain and some organs of white rats. Experimental data showed that $M3_1$ factor results in distinct changes of phosphorylase in the heart and skeletal muscle. On the background of a decrease of a total phosphorylase activity balance there is some prefential increase in the activity of Pa in the heart (48%) and skeletal muscle (62%), $M4_2$ does not effect on a total phosphorylase activity, but markedly increase activity Pa in the brain and in the heart. Glycogenphosphorylase is known to be controlled in several ways. In our case, probably there is covalent modification on this enzyme which manifests itself in the alteration of the Pa/Pb coefficient in the heart and skeletal muscle especially.

Results obthained provide indirect evidence about the intensification of glycogenolysis in these tissues. This assumption has been confirmed alongside whit the data by observation on studies of altered glycogen level in heart an skeletal muscle,where $M3_1$ factor decreased it (by 60%) and (by 42%) respectively. From these data we can specifically see the coronary dilatory effect of adrenal medullary factors $M3_1$ and $M4_2$.

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RECOVERY PROCESSES IN RATS AFTER UNILATERAL PYRAMIDOTOMY, DESTRUCTION OF RUBROSPINAL TRACT AND ACTION OF BACTERIAL MELANIN

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Effects of bacterial melanin on the processes of posttraumatic recovery of instrumental reflex (IR) and limb movements were studied after unilateral pyramidotomy on the bulbar level (n=12) and destruction of rubrospinal tract on the level of C3-C4 segments in adult white mongrel rats (n=12), weighing 180 - 220 g and initially trained to the IR. These operations caused unilateral limb paresis. On the next day after the operation, one part of the animals was injected intramuscularly with the solution of bacterial melanin (BM), obtained by biotechnological method, at a concentration of 6 mg/ml with an estimated optimally tolerable dose of 170 mg/kg.

It is well known that corticospinal and rubrospinal tracts have almost the same function in the organization of movements and this explains the process of functional compensation after lesion in one of these tracts. Clinical signs for the isolated damage of each tract are also very similar and in both cases unilateral paresis is manifested. Recovery of IR and balancing movements in pyramidotomized rats of control group was completed within 19 days in average, and in rats with the destruction of rubrospinal tract - within 13.7±1.3 days in average. In rats injected with BM the complete recovery was over in average within 3 days in pyramidotomized animals and within 2 days after the rubrospinal tract destruction.

Such a complete and rapid recovery was also confirmed by morpho-histochemical examination of brain tissue of operated animals, shows that in these experiments applied bacterial melanin has a role of a neuroprotector in the animal organism, accelerating the process of posttraumatic rehabilitation. Similarity of responses registered from lumbar motoneurons of "melanin" and intact animals by extracellular registration in response to tetanic stimulation of pyramidal tract above the transection area, confirms as well the fact of impulse conduction complete recovery in pyramidal tract after trauma.

PROLINE-RICH POLYPEPTIDE REGULATED THE ACTIVITY OF MEMBRANE TRANSPORTING PROCESSES AT RADIATING DEFEAT

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The Aim of this work was the research of membrane transporting processes by radiation stress and the possibilities of regulation of the broken ways by studying of Na/K-ATPase activity before and after application of proline-rich peptide (PRP).

Materials and Methods Experiments were carried out on Wistar white male rats. Animals were exposed to rays on the apparatus RUM-17. After 10 days of the exposure of 3Grej, PRP was introduced intraperitoneally during 3 days at a rate of 5mg per 100g of animal weight.

Results According to the received data at a unitary total irradiation, the activity of Na/K - and Mg-АТФаз in lymphocytes, thymocytes and spleen tissue was decreased, whereas in erythrocytes and brain it authentically increased ($p<0.001$). After application of PRP in immunocompetent cells there was tendency to normalization of studied enzymes activity; however in erythrocytes it remains to blood raised. The problem of possible pathogenetic therapy of irradiation pathology by inclusion of PRP into the complex of therapeutic means is discussed.

EFFECT OF PROLONGED VIBRATION CONJOINED WITH ACTION OF PRP-1 ON RESPONSES OF SUPERIOR VESTIBULAR NUCLEUS NEURONS, EVOKED BY STIMULATION OF HYPOTHALAMIC PARAVENTRICULAR AND SUPRAOPTIC NUCLEI

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As known, the vibration action (VA), even by weak of their level, can be evoked the vibration disease, striking the nervous, the cardiovascular and motor systems. Under prolonged VA observes the disturbance of vestibular analyzer functions in type of subjective complaints on giddiness, violation of movement and other. In present time the important place take in the main new approach, directed to the setting of CNS functions by means use of endogenous neurohumoral factors of peptide nature. The special point in this respect take a new hypothalamic proline-rich peptide-1 (PRP-1) produced by paraventricular (PVN) and supraoptic (SON) nuclei of hypothalamus.

The studies of plasticity were made, detected by changes of synaptic transmission and survival of superior vestibular nucleus (SVN) neurons in conditions of prolonged VA and PRP-1 administration. On the Albino rats were studied the hypothalamic-vestibular connections by means bilateral recording of evoked activity of SVN single neurons at high frequency stimulation (HFS 100 Hz, 1sec) of PVN and SON. Experiments were carried out at three series: 1 – intact animals; 2 – animals subjected by 15 days VA (frequency 60 Hz, amplitude of vibrations 0,4 mm, 2 hours daily); 3 – animals, whom parallel with 15 days VA in first two days over 2 hours before exposition were injected PRP-1 (0,05 mg/kg i/m). The registration and programmed mathematical analysis of impulse activity were made in real time on-line.

In the first series analysis of pattern reaction of neurons of right-side SVN on HFS of ipsi SON and contra PVN was revealed: the most intensity of tetanic potentiation (TP) – 65,8% and 60,5% respectively, combined with posttetanic (TP+PTP) – 23,7% and 13,2% respectively. In the neurons of left side SVN at HFS of contra SON TP is composed 51,1%, ipsi PVN – 62,2%, TP+PTP – 22,2%, and 20,0%, respectively. In second series on HFS contra PVN in right side SVN has been revealed reactions of neurons with TP – in 41,2% cells, PTP – 32,5%, TP+PTP – 11,7%, but under stimulation ipsi SON – TP in 26,5%, PTP – 32,4%, TP+PTP – 23,5% cells. The activity of neurons of leftside SVN on HFS of ipsi PVN originated TP in 20,0% cells, PTP – 28,6%, TP+PTP – 11,4%, TD+PTP in 14,3%, but at HFS of contra SON were revealed TP in 31,4%, PTP – 22,9%, TP+PTP in 17,1% of neurons. In 3-th series of experiments in conditions of PRP-1 administration and prolonged VA of HFS of hypothalamic nuclei on SVN nuclei was revealed of following poststimulus manifestations of excitability: at HFS of ipsi SON in right SVN with TP in 72,7% cells, PTP – 15,1%, TP+PTD and TP+PTP per 6,1%, by absent of areactive neurons; by stimulation of contra PVN took place TP – 54,5%, PTP – 6,1%, presence areactive cells – 36,4%. Under HFS of ipsi PVN in neurons of neurons of left side SVN 74,4% were composed the cells with TP and PTP, TP+PTP per 2,8%, but by stimulation of contra SON 60% with TP and 14,3% with TP+PTP.

The disturbances of vestibular functions originating in postvibration period in useful degree has been conditioned by changes in neuromediatory processes, connected with involvement of powerful adaptive

systems. As known, under action of vibration significantly changes the reception of corticosteroids, reflecting the dependence of shifts of cholin-monoamine and GABA-ergic neuronal activity from level of glucocorticoids and functional state of hypothalamo-hypophyseal-adrenocortical system.

Thus, the mechanisms of neuromediatory adaptation in conditions of VA, covering the GABA-mediated inhibition in CNS, evidently activates under action of PRP-1. PRP-1 systemic use was prevented the pathological disbalance of excitatory and inhibitory hypothalamic-vestibular poststimule manifestations of activity and provided in conditions of prolonged VA the adequate adaptive redistribution of neuromediation.

PROLINE SPECIFIC DIPEPTIDYL PEPTIDASES

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The increasing number of biological processes appears controlled by proline-specific peptide hormones. Proline is a unique residue which forms special conformation of peptide chain protecting it against degradation by usual peptidases even with broad specificity. Proteolytic processing of proline neuropeptides and hormones from precursors and their degradation is realized by proteases specific for proline bonds. Dipeptidyl peptidases (DPPs) compose a family of serine proteases, which remove N-terminal dipeptides from polypeptides and proteins with proline or alanine on the penultimate position. DPPIV (E.C. 3.4.14.5) is a more widespread and intensive studied member of DPP family. Its substrates are: chemokines, neuropeptides, hormones, growth factors, etc. In the hematopoesis system, DPPIV is expressed on the surface of resting and activated T-cells as antigen CD26. DPPIV/CD26 plays an important role in immune response, in protection of cells from cytotoxic derivatives of adenosine through binding adenosine deaminase (ADA, E. C. 3.5.4.4). CD26/DPPIV participates in activation of T-cells, cell adhesion, modulation of proline-containing peptides, can act as a tumor suppressor or activator. At multiple sclerosis CD26/DPPIV level on T-cells was found to be 3- 4-fold higher than in control. Another member of DPP family, DPPII (E.C.3.4.14.2) is discovered in lysosomes of fibroblasts, macrophages, must cells, neutrophil granulocytes, and lymphocytes of different tissue. Its high specific activity is observed in human hemisphere, pituitary gland and hypothalamus of rats. DPPII activity in brain changed at neurodegeneration, increased in the brain parts with damaged neurons. It is suggested that DPPII may influence on the cicatrization processes after lesion of brain.

We studied the influence of acute and chronic experimental aluminum toxicosis as a neuro-degeneration model, on the activities of DPPIV, DPPII and ADA in brain and blood of rats. In brain, a significant decrease of ADA activity and a weak increase of DPPII were observed at both types of toxicosis. In blood plasma, DPPIV, DPPII and ADA activities increased at both types of toxicosis. We monitored the DPPIV/CD26 and ADA activities, as well as IgG and IgM titers, after rat's immunization with human erythrocytes. The similarities between immunoglobulins and enzymes development allowed us to suggest the participation of ADA in Th2-, and DPPIV – in both of Th2- and Th1- immunity pathways. Comparison of the kinetic parameters of ADA with those of DPPIV-ADA complex in the deamination reactions manifested more effective neutralization of toxic nucleosides in the extracellular medium due to complex formation between ADA and DPPIV. The interaction of DPPIV-bound and free ADA with several inhibitors demonstrate stronger inhibiting ability of some of them towards DPPIV-bound ADA which could be important both for selective therapeutic use of these inhibitors, and for obtaining new information concerning the interaction of ADA with DPPIV.

For the first time, we have shown the ADA-binding ability of DPPII, similar to DPPIV. The dissociation constant of DPPII-ADA complex was estimated using different techniques.

THE ELEVATION OF CYTOCHROME b_{558} ISOFORMES LEVELS IN RAT TISSUE CELL MEMBRANES, MITOCHONDRIA AND NUCLEI AFTER ELECTRICAL STIMULATION OF SUPRAOPTIC AND PARAVENTRICULAR NUCLEUS OF HYPOTHALAMUS

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The electrical stimulation of SON and PVN increases the immunoreactivity of rat's hypothalamus. The molecular-biochemical mechanisms of this effect associated with the changes of the level of cytochrome (cyt) b_{558} in cell membranes, mitochondria and nucleus (brain, liver, spleen, heart, kidney) has not been determined yet. The present study aims to determine those mechanisms.

The SON and PVN of hypothalamus undergo electrical stimulation with the rectangular electrical impulse during 1 sec (three times by 5 sec intervals). A cyt b_{558} isoform of acidic character from cell membranes, mitochondria and nucleus was isolated and purified by a licensed biotechnological method, using the ion-exchange chromatography method on the celluloses KM-52 and DE-52. The amount of cyt b_{558} was determined by optical-spectral method.

The electrical stimulation of hypothalamus causes a sharp increase of the endogenous level of cyt b_{558} of acidic character in cell membranes, mitochondria and nucleus of these tissues, particularly, in the spleen.

It is known that the isoforms of cyt b_{558} localized in the immune cells components are key components of NADPH oxidase. These cyt b_{558}s also possess NADPH depending O_2^- -producing and methemoglobin-reducing activities.

On the basis of these results it is supposed that the molecular-biochemical mechanisms of immune reactivity increase of the hypothalamus SON and PVN are associated with the elevation of the endogenous level of cyt b_{558} in the cell membranes, mitochondria and nucleus with the corresponding stimulation of immune system, oxygen homeostasis, regulation of genetic code, as well as the energetic balance in the respiratory burst of mitochondria.

BRAIN IMMUNITY AND ALZHEIMER'S DISEASE: LESSONS FROM ANIMAL MODELS AND NEW APPROACHES TO THERAPEUTIC INTERVENTIONS

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized clinically by a progressive cognitive decline and dementia. AD brains are marked by amyloid plaques and neurofibrillary tangles, neuronal cell loss, a prominent activation of glial cells, and innate immune responses. A growing number of studies in AD have also reported alterations in systemic immune responses including changes in lymphocyte and macrophage distribution and activation, the appearance of autoantibodies, an abnormal cytokine production. Studies in transgenic or injection animal AD models support the notion that immune cells infiltrating the brain may modulate the disease. For example, administration of aggregated fragment (25-35) of amyloid-β peptide (Aβ (25-35)) into rat hippocampus resulted in a significant cell loss. The extension of neuronal degeneration measured in the CA1 field was significantly longer in the Aβ(25-35)-treated group as compared to the contralateral vehicle-treated hemisphere. Aβ(25-35) injection caused reactive gliosis in the ipsilateral hemisphere as demonstrated by upregulation of glial fibrillar acidic protein (GFAP) expression and the presence of hypertrophic astrocytes in the hippocampus. The damaged area of the hippocampal CA1 field and the lateral band of the dentate gyrus displayed multiple darkly stained round isolectin B4-positive phagocyte-like microglial cells.

Activated astrocytes and microglia are the main internal sources of brain pro-inflammatory and anti-inflammatory cytokines. Both types of cytokines can modulate Aβ-related neurotoxicity. We studied effects of co-administration of Aβ(25-35) and proinflammatory cytokine, tumor necrosis factor-α (TNFα), on neuronal damage in rat hippocampus. TNFα augmented the Aβ(25-35)-induced damage significantly increasing the extension of degenerating area. It also increased expression of GFAP-positive astrocytes as compared to the Aβ(25-35)-treated rats. Moreover, injection of TNFα into the cerebral ventricles prevented Aβ(25-35)-induced increase in hippocampal caspase-3 activity.

Increased macrophage infiltration of the AD brain is accompanied by a decrease in the level of pro-inflammatory and an increase in the level of anti-inflammatory cytokines that represents a form of brain immune defense against AD-related pathological cascade. In our study, we used anti-inflammatory cytokine, interleukin 4 (IL-4), to modulate the effects of Aβ(25-35). In the brain, IL-4 can be synthesized by astrocytes and microglia, receptors for IL-4 being located in all types of neurons. Co-administration of IL-4 with Aβ(25-35) resulted in a substantial decrease in the size of hippocampal damage possibly due to inhibition of apoptosis-like neuronal death.

Cytokines are key participants in brain immune defense, which is activated in AD. Their roles are nuanced, highly regulated and not completely understood. Sometimes, the roles of cytokines are quite opposite to their function in other diseases, as we have demonstrated for TNF-α. Although cytokine-modulating therapies are not currently used for the treatment of AD, cytokines can ameliorate pathology in certain experimental models of AD, suggesting a potential for future therapeutic opportunities. Methods of targeted cytokine gene delivery to the brain using viral vector-based technology offer a promising opportunity to develop new therapeutics against AD.

This study was supported by RFBR grant #09-04-01157a and RFH grant #09-06-00490a.

APPLICATION POSSIBILITIES OF BILAYER LIPID MEMBRANES SUPPORTED ON METAL OR THE SALT BRIDGE FOR RECONSTRUCTION OF IONIC CHANNELS

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The selective conductance of sodium ions across the plasma membrane by the voltage-gated sodium channel underlies the propagation of action potentials in neuronal cells of both vertebrates and invertebrates. The critical role of the sodium channel in the functioning of the nervous system has made it the target of a diverse array of toxins during evolution. Now the basic tool for studying native ionic channels is patch-clamp. However this method adjusted certain restrictions on a choice of specific objects and techniques of researches. Reconstruction of a functional ion translocation system from the minimal number of components required to mimic a chemically activated membrane would certainly foster our understanding of membrane excitability in general. One of the most useful approaches, in this respect, is the reconstruction of the simple membrane system using self assembled lipid bilayers, such as planar bilayers lipid membranes (BLMs) and liposomes. Conventional BLM is ideal system for basic research. However, the membrane of thickness about 5 nm separating two aqueous solutions is too fragile to be useful for practical applications. Besides, conventional BLM is very sensitive to electrical and mechanical disturbances. In order, to overcome these disadvantages, a novel, yet very simple method was found in the late 1980s for forming planar BLMs on a solid support (s-BLM) [1], and later agar gels in the form of salt bridge support (sb-BLM) [2].

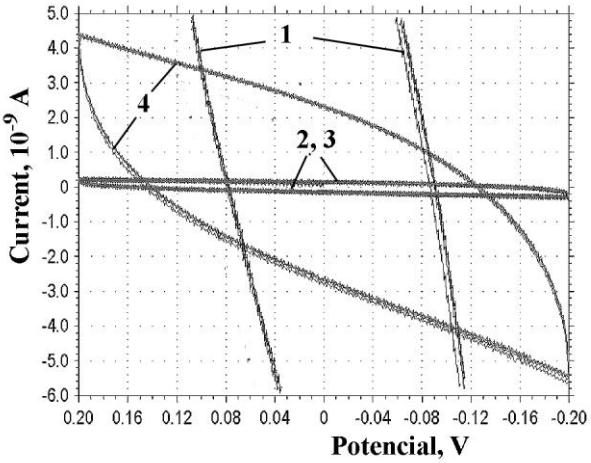

Fig. 1. Cyclic voltamperometry (CVA) curves for s-BLM. 1 - without BLM, 2 - BLM, 3 - BLM after disruption and recovery, 4 - BLM after disruption and recovery immersed in the solution of membrane proteines isolated from bovine brain.

To study the molecular mechanism of interaction of newly synthesized by as pyrethroid insecticides [3] with Na channels of insects and mammalian species we modified s-BLM and sb-BLM techniques, developed by Tien and coworkers. Typical results with application of s-BLM are presented in fig. 1.

From fig. 1 it's follows, that s-BLM received on the basis of fraction of the general lipids of bovine brain, extracted and purified by Folch's chloroform-methanol method, is completely restored after electric disruption (at ±0,8V) and shows a typical curve of Tien's cyclic voltamperometry, with - parametres - $C=4.83\times10^{-9}$ F and $R=1.36\times10^{9}$ Ohm. At immersing received s-BLM in a solution of membrane proteins the capacity increases in more than 20 times and resistance of BLM decreases almost in as much time ($C=8.38\times10^{-8}$ F and $R=7.30\times10^{7}$ the Ohm). Similar measurements on sb-BLM have shown peculiar to this system asymmetry of CVA and its sensitivity to presence in BLM of membrane proteins.

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ALTERATIONS OF THE HUMORAL IMMUNITY FACTORS UNDER THE CONDITIONS OF EARLY HYPOKINESIA

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At present there are no doubts that hypokinesia (HK) becomes a reason of deep and irreversible changes in different organs and systems of organism, including the immune system as well. The latter involves into these reactions at early stages of the hypokinetic stress, what results in expressed negative effect onto the immunological functions. That is why the study of the hypokinetic impairments' mechanisms and development of the preventive methods and means against the negative consequences become not only urgent, but of social significance as well.

A few literature data referring to the NK influence on the immune system are contradictory and controversial, what could be predetermined by different conditions and duration of HK. The abovementioned become a base for investigation of the peculiarities of the immune response formation and its course under the conditions of immunization in different terms of HK. For this objective realization the primary immune response dynamics to the sheep erythrocytes (SE) was studied under the HK conditions of 3-, 7- and 14-days duration with the following immunization.

It was implemented by means of counting the antibody-forming cells (AFC) and the rosella-forming cells (RFC) number in the spleen at the 5-th day of immunization and also by determination of the haemagglutinogens' titer in the peripheral blood at the 5-th, 10-th and 15-th days of immunization. Experiments were performed on 157 white mongrel male-rats weighing 150-170g. The animals were divided into 4 groups. The I-st was the control one, the II-nd was exposed to HK of 3-days duration, the III-rd – to HK of 7-days duration and the IV-th - to HK of 14 days duration. All the animals underwent to immunization intraperitoneally per 0.5ml of 8% SE suspension immediately after the HK term.

Counting of the AFC number in the spleen under the HK conditions of 3-, 7- and 14-days duration on the 5-th day of immunization showed the expressed inhibition of the index by 2.0; 1,7 and 2.6 times correspondingly in comparison with the control group.The similar pattern was observed in the RFC number counting (reductions was by 1.9; 1.8 and 1,5 times). All these changes definitely were reflected onto the antibodies synthesis and accumulation processes in the dynamics of immune response. A significant decrease in the antibodies titer logarithms was determined under the HK conditions of different durations, especially on the 5-th and the 7-th days of immunization. In case of 7-days HK the spike of antibodies accumulation shifted from the 7-th day onto the 10-th one, what was a result of the primary immune response development in comparably slower way. In the other terms of HK in the experimental and the control groups as well, the spike of antibodies accumulation was registered on the 7-th day of immunization.

The data obtained justify that HK as a multicomponent stress-factor evokes an inhibitory effect on the antibodies formation intensiveness and its dynamics. On the one hand, it is probably connected with the stress-realizing mechanisms, particularly with the hypothalamo-hypophysear-adrenocortical and the sympathoadrenal systems' activation, which inhibit the process of antibody formation, and on the other hand, with inhibition of the IL-1, 2, 6 TNF-α and γ-IF synthesis and secretion, what is testified by the data of recent years investigations.

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