CONFERENCE
FREE-RADICAL MECHANISMS OF BRAIN PATHOLOGIES: THEORETICAL AND PRACTICAL ASPECTS
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ABSTRACTS

Protective Effect of Gangliosides Against Oxidative Stress in Cerebral, Cardiac, and Other Tissues

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Together with antioxidants (compounds that directly react with free radicals) and antioxidant enzymes, naturally occurring agents that can inhibit free-radical processes via other mechanisms are of considerable interest.

Our studies have shown that preincubation of brain synaptosomes with nanomolar concentrations of individual gangliosides (GM1, GM1a, etc.) decreases the accumulation of malonic dialdehyde (MDA) and reduces the destruction of polyenic fatty acids for induction of lipid peroxidation (LPO). It was found that individual gangliosides protect the plasma membrane proteins and normalize the activity of enzymes and the specific binding of ligands to β-adrenergic receptors of synaptosomes, which had been reduced by LPO. The inhibitory effect of gangliosides on LPO was confirmed by experiments on the isolated heart, demonstrating that ganglioside infusion into the bloodstream normalizes cardiac function. *In vivo* experiments confirmed the ability of gangliosides to prevent disturbances of lipid distribution in the plasma membrane and lysophospholipid accumulation, and to partially normalize the activity of Na^+,K^+-ATPase. A modulatory effect of very low concentrations of gangliosides (10^{-13}-10^{-12} M) on free-radical processes was also revealed in experiments with human neutrophils and murine macrophages. The results obtained indicate that the effect of gangliosides on LPO and other free-radical reactions in the brain, heart, and other organs is mediated by the signal-transducing systems.

Effect of Adrenalectomy, Administration of Adrenal Hormones, and Stress on Free-Radical Processes in Rat Brain and Blood

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Adrenalectomy (AE) was performed in male outbred albino rats weighing 300-350 g. During 5 days these animals received physiological doses of adrenalin (AD), corticosterone (CS), and aldosterone (ALD) alone or in combination. In each group some the rats were subjected to a 2-h immobilization stress. Free-radical processes (FRP) were studied in the brain and blood serum. In was found that 1) AE induced no statisti-

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cally significant changes in the brain and serum, judging from H_2O_2-induced peroxide-dependent chemiluminescence (CL), the content of TBA-active products (TBAap), and superoxide dismutase activity (SOD), and had no effect on the cholesterol (Chl) and phospholipid (Phl) contents in brain membranes; 2) under the chosen experimental conditions immobilization stress did not change FRP (an intermediate phase between the initial "antioxidant" phase and FRP activation); 3) AD resulted in a decrease in serum Chl concentration, Phl accumulation, and a lowering of the Chl:Phl ratio in the brain (particularly in the right hemisphere); 4) AD administered against the background of AE led to Chl accumulation in the left hemisphere and a decrease in the Chl:Phl ratio and an increase in the Chl content of the right hemisphere; 5) AD administered against the background of immobilization stress or AE stimulated FRP in the brain and reduced the accumulation of Fe ascorbate-induced TBAap in the serum; 6) AD and AS lowered the serum contents of Chl and TBAap, the effect being more pronounced in adrenectomized rats, while in stress-exposed rats AD and ALD increased the serum Chl concentration; 8) CS and ALD stimulated SOD and lowered the TBAap content in the right hemisphere, whereas in the left hemisphere ALD increased the Chl content, and CS administered against the background of AE lowered the TBAap content; 9) ALD and CS induced Chl accumulation and increased the Chl:Phl ratio in the left hemisphere while eliciting opposite effects in the right hemisphere. The differential effects of adrenal hormones on FRP in the brain hemispheres and in the blood and their relation to stress and AE are considered.

Comparative Efficiency of Antioxidants in the Therapy of Experimental Choreiform Hyperkinesis

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The study was performed on a rat rst model of choreiform hyperkinesis described previously. The model implies local disturbances of the inhibitory GABA-ergic mechanisms in the central zone of rostral part of the neostriatum induced by intrastrial microinjections of the GABA-receptor blocker picrotoxin (3-5 μl of a 0.01% solution). The efficiency of several antioxidants vis-a-vis the duration, amplitude, and frequency of choreiform hyperkinesis was studied under conditions of nonrestricted behavior. The following preparations were compared: 1) SOP-1 (mexidol, oxypin), 100 mg/kg intraperitoneally at peak hyperkinesis (n=30); 2) α-tocopherol, 100 mg/kg intramuscularly and intraperitoneally, 24 h prior to picrotoxin injection (n=23); 3) glutathione, 100 mg/kg intraperitoneally at peak hyperkinesis, (n=6).

The administration of SOP-1 alone had no effect on the syndrome, which was mitigated during 1 h by the administration of SOP-1 in combination with the GABA-positive preparation sodium valproate (100 mg/kg intraperitoneally).

Pretreatment of the animals with μ-tocopherol prevented the development of hyperkinesis in 40% of the animals; the frequency and amplitude of involuntary movements were significantly (p<0.01) decreased in 42.5% of the animals, and only 17% developed hyperkinesis.

The administration of oxidized glutathione at peak hyperkinesis alleviated the syndrome for 1 h.

The different antioxidants were of different therapeutic efficacy in the case of experimental choreiform hyperkinesis. This proves the role of LPO in the pathogenesis of this syndrome and calls for further studies aimed at developing clinical treatments for some forms of hyperkinesis.

The Use of Antioxidants for the Correction of Motor Side Effects Caused by Psychopharmacotherapy

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The aim of this study was to test the effectiveness of the antioxidant α-tocopherol in the treatment of neuroleptic-induced extrapyramidal motor complications resistant to routine correction therapy.

α-Tocopherol was administered in a daily dose of 600 mg for 21-28 days. Its effect was studied by the open method. The standard AIMS and TDRS scales were employed together with a clinical assessment of the patient's condition on days 0, 2, 7, 14, 21, and 28 of therapy. Psychotropic therapy was not changed throughout the study.

Forty-seven patients were included in the study, 16 of them (11 females and 5 males) were of young or middle age (17-50 years, mean 33 years) and 33 (24 females and 9 males) elderly (53-85 years, mean 67.4 years). All the young and middle-aged patients had a history of schizophrenia. Twelve elderly patients had a history of schizophrenia; 9 patients, schizoaffective psychosis; and 12, affective psychosis. Neurological complications were diagnosed on the basis of the clinical classification [J. Delay and P. Deniker] and international criteria [N. R. Schooler and J. M. Kane].

α-Tocopherol proved to be quite effective in the therapy of extrapyramidal motor complications caused by psychopharmacotherapy in both groups. A marked improvement of the condition and a minimum 25% reduction in the severity of dyskinesia on the AIMS scale were recorded in 68.8% of middle-aged patients and in 72.2% of elderly patients. A 50% or greater decrease on the AIMS scale parameters was observed in 43.8 and 48.5% of middle-aged and elderly patients, respectively. The antioxidant had no effect in 31.2% and 27.3% of the patients, respectively.

Our results indicate that α-tocopherol is a promising preparation in the treatment of chronic extrapyramidal motor complications caused by psychopharmacotherapy which are resistant to routine correction.

Effect of Carnosine and Its Derivatives on the Brain's Resistance to Hypoxia

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Analysis of the antioxidant function of carnosine, a naturally occurring peptide, which is metabolized in the human and animal organism (hydrolyzed, methylated, decarboxylated, acetylated, etc.), and its derivatives has shown that they may serve not only as pH buffers but also as oxygen free-radical buffers. Carnosine is most active in interaction with hydroxyl radical, however its methylation, which leads to anserine formation, increases the ability of carnosine to interact with superoxide anion and to neutralize hypochlorite anion, a toxic product of the myeloperoxidase reaction. Homocarnosine elicits a pronounced stabilizing effect on cell structures and macromolecules, including DNA. Moreover, carnosine and its derivatives exert a marked antiaggregatory effect on human platelets.

Systemic administration of carnosine to animals exposed to stress lowers the concentration of peroxidation products in the blood and tissues, including the brain. It increases the organism's tolerance for stress and its consequences and improves the organism's ability to adapt to stress. Taken together, these observations led us to assume that carnosine can serve as an effective therapeutic agent capable of moderating oxidative stress and increasing the brain's resistance to it.

In fact, the administration of carnosine to rats (200 mg/kg) prior to hyperbaric hypoxia simulating an altitude of 10,000 m in a pressure chamber) increased both the survival rate and provided better recovery of the animals upont heir return to normal conditions. The effects of carnosine on physiological and biochemical parameters of rats exposed to hyperbaric oxygenation are reported, and the possibility of using this agent in the therapy of cerebral circulation disorders is considered. The possibility of regulating the carnosine level by endogenous metabolic enzymes and converting carnosine *in situ* into metabolites that are potentially more effective regulators of metabolism makes carnosine a promising candidate as a natural adaptogenic agent.

Antioxidant Activity of Preparations Used in the Therapy of Parkinson's Disease

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The effects of L-DOPA and Midantan (both agents are widely used in antiparkinsonism therapy) on Fe ascorbate-dependent lipid peroxidation (LPO) have been examined in two model systems: rat brain homogenate

and liposomes prepared from egg lecithin. The preparations were studied in the concentration range of 0.1 μM-1 mM. It was found that at 0.1-1 mM L-DOPA markedly inhibits LPO in liposomes. At lower concentrations the preparation had no effect on LPO in liposomes. At 0.1 μM-1 mM, L-DOPA almost completely inhibited LPO in brain homogenates, the effect being weaker at lower concentrations. Kinetic curves illustrating the effect of L-DOPA on the accumulation of LPO products in liposomes and brain homogenate were constructed. Midantan exhibited no antioxidant activity in these model systems. The data are considered in terms of the hypothesis that disturbances in LPO regulation are involved in the pathogenesis of Parkinson's disease.

Comparative Analysis of Lipid Peroxidation in the Rat and Musk-rat Brain in Asphyxia

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Asphyxia was achieved by: 1) a 4-5-min arrest of artificial ventilation of an animal immobilized with a myorelaxant or 2) immersion of the animal's head in water for 1-2 min. After immersion, the content of diene and triene conjugates, Schiff bases, and malonic dialdehyde in the rat brain remained unchanged. The superoxide dismutase (SOD) activity was about 2-fold higher, and the catalase activity was more than 2-fold lower than in intact rats (for both cases $p<0.05$). It should be mentioned that lipid peroxidation (LPO) in the liver, heart, and blood serum was increased, while there were no changes in SOD activity, and catalase activity either was decreased or remained unchanged. There were no statistically significant changes in LPO intensity and catalase activity after the cessation of hypoxia caused by the arrest of artificial ventilation, and no correlation was established between these parameters and electrical brain activity. However, an inverse correlation ($p<0.05$) was established between catalase activity in the brain and the heart rate. It can be assumed that these peculiarities in the functioning of the antioxidant system are part of the protective mechanisms responsible for the relative stability of LPO processes in the brain during asphyxia. In the musk-rat, immersion of the head into water caused no statistically significant changes in the brain content of diene and triene conjugates, Schiff bases, and SOD activity; the malonic dialdehyde content decreased ($p<0.05$), whereas catalase activity increased more than 2-fold ($p<0.01$). A similar dynamics of LPO and activity of antioxidant enzymes was recorded in other tissues of the musk-rat.

There were no specific features in the mechanisms of antioxidant protection of the brain as compared with other organs of the musk-rat. It is obvious that in this mammal, an animal adapted to prolonged breath holding, the system of LPO regulation functions in a different way than in the rat. In the musk-rat, this system, ope- rating together with known physiological mechanisms, not only limits LPO processes but also weakens them.

Nitrogen Oxide in the Central Nervous System

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The significance of nitrogen oxide (NO), a recently discovered transmitter of intercellular interactions, in brain functions is considered. The constitutive form of nitrogen oxide synthetase (NOS) is present in some neurons of the central nervous system (CNS) and in vascular endothelium. Another form of NOS can be induced by cytokines in glial cells. Calcium entry, particularly upon activation of the glutamate receptors, increases the production of NO. Long-term potentiation and coupling of local blood flow to neuronal activity may be one of the functions of NO. Together with superoxide formation, overproduction of NO leads to the accumulation of peroxynitrite, a compound generated by the superoxide-NO interaction. Peroxynitrite diffuses from the site of formation and produces hydroxyl radical, which may lead to the development of pathogenic free-radical processes in the brain in ischemia, epilepsy, and other cerebral disorders. Under normal conditions superoxide may act as a modulator of NO-dependent functions of the CNS. This and other known aspects of NO formation in the CNS broaden considerably the concepts of brain functioning and its disorders.

Monoamine Oxidases and Their Endogenous Regulation in the Mechanisms of CNS Damage in Epilepsy and Other Pathologies

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During intoxication with bipyridilic derivatives and/or stimulation of lipid peroxidation (LPO) in epilepsy or cerebral trauma (CT) free-radical processes modify the catalytic properties of monoamine oxidase (MAO): in addition to a decrease in its activity toward physiological substrates (serotonin, phenylethylamine, dopamine, etc.), compounds that normally are not MAO substrates are deaminated. The latter also include the neurotransmitter amino acids β-alanine, γ-aminobutyric acid (GABA), and taurine; their deamination upon free-radical and/or LPO-dependent brain damage is catalyzed by MAO A. Although the affinity of MAO A for the physiological substrate serotonin is more than 5-fold higher, the rate of deamination of saturating concentrations of GABA, taurine, and glucosamine is not less than 50-80% of the rate of serotonin deamination. In addition to LPO-dependent modification of catalytic properties, epileptic fits and CT increase MAO sensitivity to trypsinolysis, which, according to our data, reflects changes of the enzyme's conformation but not of its topography in the membrane. Stimulation of LPO also affects the content of endogenous MAO modulators in the brain. The study carried out together with the Laboratory of M. Sandler has shown an increase in the tribuline content of rat brain in an epileptic fit. It should be noted that the MAO A content in the inhibitory component increased to a greater degree than the MAO B content. Stimulation of LPO *in vitro* increased the MAO A content of the inhibitory component without changing the level of isatine, a tribuline component that inhibits MAO B.

Pyrazidol, a selective MAO A inhibitor, antioxidants (ditludine, ascorbate), and nucleophilic reagents prevented MAO modification, reduced the intensity of epileptic fits, and increased the survival rate in paraquat intoxication. Taken together, these findings indicate that MAO is related to the mechanisms of CNS damage in the studied pathologies.

Autoregulation of Free-Radical Processes in Stress Is a Mechanism Underlying the Adaptive Capabilities of the Brain

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Changes in free-radical processes (FRP) are a crucial general mechanism governing both pathological and adaptive processes. On the basis of the data obtained during the last decade, we have developed a concept that allows for the identification of FRP changes which are fundamental to or accompany the adaptive-compensatory brain response to stress (decrease in LPO intensity, increase in the superoxide dismutase activity, and decrease in the cholesterol-phospholipid ratio in brain membranes). We have demonstrated at least two types of such reactions differing in time and appearance: immediate, occurring in the initial period of stress, hyperoxia, reanimation, administration of antioxidants or neuropeptides, and long-term, occurring in chronic stress, repeated hyperoxia, severe brain damage, neurotransplantation, etc. We have also shown long-term adaptive FRP changes against the background of disadaptive shifts in the lipid composition of brain membranes. Comparison of the FRP dynamics in the brain and in the serum (FRP in the serum was taken as the FRP index for the whole organism) for various types of acute and chronic influences has shown that: 1) the initial "antioxidant" rapid adaptive response in many cases occurs only in the brain, and in other cases is more pronounced (in terms of amplitude and duration) in the brain; 2) for the majority of acute influences and always for chronic ones, adaptational failure of the organism (activation of FRP in the blood) occurs against the background of a normal or decreased level of FRP and, consequently, a normal or increased activity of antiradical protection system in the brain. These facts allowed us to formulate a concept regarding the autoregulation of FRP and systems of the antiradical protection of the brain and to assume that this autoregulation is an important functional-biochemical mechanism that underlies the adaptive capabilities of the brain.

Investigation of the Role of Free-Radical Processes in Epilepsy and Epileptogenesis

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Numerous disturbances of cerebral function, including Parkinson's disease, Alzheimer's disease, insults, and epilepsy, are linked to the intensification of free-radical processes. However, it is not clear whether this is a nonspecific epiphenomenon or a factor in the development of specific symptoms. In order to answer this question, it is appropriate to study the effects of specific inhibitors of the corresponding processes on the development of disease symptoms.

We studied the effect of intravenous injections of superoxide dismutase (SOD) and Olifen, a novel antihypoxant with an antioxidant activity (both preparations produced at the Institute of General and Specific Biochemistry, St. Petersburg), on seizures after kindling of the amygdala and the effect of the Fe chelator Desferal on the development of post-trauma epilepsy induced by subdural injection of autologous blood in the rat. It was found that SOD alleviates seizure activity in the 5th stage of epilepsy, inhibits spontaneous and electrostimulated electrographic manifestations of seizures, and raises the threshold values of the stimulating current. Olifen elicited similar effect with specific time- and dose-dependence. Neither SOD nor Olifen was effective in the corazole or electric shock model of epilepsy. Intramuscular injections of Desferal resulted in a decrease in Fe content at the site of blood injection and inhibited the development of an epileptic focus.

Since in this model Fe catalyzes lipid peroxidation, the results obtained confirm the ability of free-radicals to cause damage to nervous tissue, which leads to the development of epilepsy. Concerning the effects of antioxidants after the formation of an epileptic focus, the results obtained in experiments with different antioxidants and different models are controversial, indicating the importance of a specific target for each preparation in the generation of seizure activity. Superoxide radical is the target for SOD. This radical is not only involved in LPO activation but also inactivates nitrogen oxide. The significance of the latter in the development of seizure activity is unknown and is the subject of our current studies.

The Glutamate Receptor System in the Mechanism of L-Arginine-Dependent Generation of Nitrogen Oxide

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It is known that in cells of the central nervous system (CNS) nitrogen oxide (NO) is generated from L-arginine due to the activation of Ca-calmodulin-dependent nitrogen oxide synthetase (NOS), increased Ca permeability of the plasma membrane, or mobilization of intracellular Ca. These mechanisms may be triggered by stimulation of the glutamate receptors (GR), which play an important role in synaptic transduction in cerebellar neurons.

The role of NMDA- and AMPA-cainate ionotropic and metabotropic GR in the mechanism of NOS activation was studied in the subcellular synaptosome fraction (SpF) isolated from the rat cerebellum. It was demonstrated that the baseline NOS activity in SpF was dependent on the presence of exogenous NADPH and L-arginine. Stimulation of the glutamate receptors by NMDA increased NO production. The increase was dependent on the presence of extracellular Ca and was abolished by the antagonist AP-5, while the stimulation of GR by AMPA did not increase NOS activity. The agonist of the metabotropic GR ACPD and glutamate increased the NO content in SpF, which did not depend on the presence of extracellular Ca. The effect of ACPD and glutamate in the absence of extracellular Ca was abolished by the antagonist AP-4, the inhibitor of the inositol triphosphate receptors of calcitosomes dantrolene, or the calmodulin inhibitor W7. These data demonstrate the significance of metabotropic GR in the regulation of L-arginine-dependent NO synthesis in the cerebellar neurons.

Free-Radical Oxidation in the Serum of Patients with a Primary Diagnosis of Schizophrenia

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The aim of this study was to evaluate free-radical oxidation and the activity of the antioxidant enzyme catalase in the blood serum of patients with a primary diagnosis of schizophrenia who had bot been treated with psychotropic preparations. The study included 21 patients with various forms of schizophrenia and 10 healthy donors. The negative and positive symptoms of schizophrenia were estimated using special scales.

Free-radical oxidation proved to be more intense in the patients than in the donors, the intensity being higher for an acute onset of the disease. Serum catalase activity in the patients did not differ significantly from that in the donors. The results obtained may provide better insight into the pathogenesis of schizophrenia and allow us to recommend the use of antioxidants in the complex therapy of schizophrenia.

The Role of Lipid Peroxidation in the Pathogenesis of Craniocerebral Injury

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The content of lipid peroxidation products (LPOpr) - diene conjugates (DC), malonic dialdehyde (MDA), and Schiff bases (SB) - and the total antioxidant activity (TAOA) in rabbit brain were determined after a moderately severe craniocerebral injury (CCI). The content of all the LPOpr was increased and TAOA was decreased, indicating activation of pathological free-radical processes in the brain. Further, we examined the effects of preparations stimulating (phenamine and piracetam) and inhibiting (luminal) CNS function on LPO in the brain of rabbits after CCI. When the stimulators (chemically different compounds) were injected 5 min after CCI, the levels of all the LPOpr normalized and TAOA rose to baseline values (prior to CCI). After the administration of luminal, LPO and TAOA were the same as those in rabbits after CCI.

Thus, controlled changes in the functional activity of the CNS in animals with CCI may regulate LPO in the brain and evidently play a significant role in the therapy of CCI.

We then attempted to evaluate to what extent the regularities revealed in the experiment are manifested in CCI in humans. After analyzing the data obtained (123 patients), we found that:

1. The MDA content in the blood and cerebrospinal fluid (CSF) statistically higher in patients with severe CCI. There is a relationship among this parameter and the type of CCI, the permeability of the blood-brain barrier, and the dynamics of recovery processes.

2. The increase in LPO intensity and decrease of TAOA in the CSF correlates with CCI severity in children with acute focal CCI.

3. There is a correlation between the lactate and the MDA content in the CSF of children with CCI, which indicates a relationship between LPO in the brain and disturbances in its energy metabolism.

This study offers additional possibilities for the investigation of traumatic cerebral injuries and approaches to their treatment.

Peculiarities of the Psycho-Organic Syndrome in Individuals Abusing Chemically Treated Cannabis

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Indications that prolonged abuse of cannabis promotes the development of a psycho-organic syndrome can be found in the publications of V. V. Borinevich (1969), A. I. Durandin (1969), I. N. Pyatnitskaya (1975), P. V. Mikhalev and S. P. Genailo (1992). In recent years the clinical picture of drug and toxin addiction has been distorted

due to a variety of reasons (drug addiction at a young age, abuse of highly toxic compounds), including the treatment of cannabis with chemicals, usually a solvent.

The aim of this work was to study the peculiarities of the psycho-organic syndrome noted in individuals who used the solvent FK-1 for the preparation of cannabis. Thirty male addicts admitted to the psychoneurological clinic of Blagoveshchensk in 1992-1993 were studied. The second stage of the disease (according to the classification proposed by I. N. Pyatnitskaya) was diagnosed in all patients. The patients had to fill out special questionnaires dealing with clinical characteristics, primarily in the mnemo-intellectual sphere, their character, and personality.

Disorders in the mnemo-intellectual sphere were observed in most of the patients, manifested in poor comprehension and reasoning. Rapidly escalating these syndromes led to social dysadaptation. These individuals could not be taught in public schools, could not utilize previously acquired knowledge and skills, and could not perform simple tasks. Marked attention deficit and inability to concentrate were characteristic of these patients. Memory disorders were manifested in the inability to fix new information and difficulties of retrieval. The patients were emotionally unbalanced and labile, indifferent to their family members, fractious, and apathetic.

These observations indicate that individuals addicted to chemically treated cannabis rapidly develop a psycho-organic syndrome with the predominance of mnemo-intellectual and neurological disorders.

Lipid Peroxidation in the Compressive Ischemia Focus in Rat Brain

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The study was performed to assess lipid peroxidation in a focus of ischemia of the cerebral cortex in rats at different periods of its formation, development, and involution.

Local ischemia was induced by a short-term (10 min) and controlled (40 mm Hg) compression of a certain area on the parietal cortex of the rat brain [I. V. Barskov and I. V. Viktorov, 1992]. This model represents retractile ischemia in neurosurgery. For pathohistological studies sections were cut from paraffin-embedded material and stained with hematoxylin-eosin and acid vanadium fuchsin. The intensity of lipid peroxidation was assessed by measuring the concentration of TBA-reactive products in standard specimens of the cortex. The content of lipids and adenyl nucleotides was measured routinely. The symmetrical area of the cortex in the opposite hemisphere was used as the control. The material for morphological and biochemical studies was obtained after 2, 5, 8, 16, and 24 h and on days 4 and 7 after ischemia had been produced.

Pathohistologically, the compressed area was characterized by local ischemia with nondiffuse hemorrhages and contained necrotic neurons. By the 7th day a gliomesodermal scar had formed in the ischemic zone.

The concentration of TBA products in the ischemic zone was twice that in the control zone during the first few hours after the procedure and was rather high on day 4, whereas by the 7th day it decreased to the control level. During the first 24 h the contents of lipids and adenyl nucleotides in the ischemic zone decreased to 50-60% of the control levels, after which they gradual- ly increased and reached the normal level by the 7th day.

The study was supported by the Russian Foundation for Basic Research.

Effect of Intracerebral Allotransplantation of Embryonic Nervous Tissue on Lipid Peroxidation in Rats with a Low Threshold of Epileptic Activity

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Lipid peroxidation (LPO) is a damaging factor in epilepsy. In previous studies performed on various models of epilepsy we found that intracerebral allotransplantation of some structures of embryonic brain into certain structures of the recipient brain reduces epileptic activity and improves some parameters of higher nervous activity. However, the molecular mechanisms responsible for the effect of neurotransplantation (NT) have not been studied in sufficient detail.

Here we report the results of a study of the effect produced by NT on LPO in the brain hemispheres of rats with different responses to the epileptogenic sound

stimuli (86 dB). The study was performed on 50 pubescent outbred and linear albino rats. Transplantation of the tissues derived from the nuclei raphe, locus ceruleus, and substantia nigra of the embryonic brain was performed by methods developed at the Institute of the Brain (Russian Academy of Medical Sciences). Brain tissues were obtained 2-3 months after NT and 1 min after a 2-min exposure to the sound stimuli by the method of L. V. Krushinskii (1960) and were fixed in liquid nitrogen. Lipid extract was prepared after Folch *et al.* (1957), and the contents of diene conjugates (DC) [Z. Plazer, 1968] and lipofuchsin pigment (LP) [B. J. Fletcher, 1973[were measured. The results were statistically processed using Student's *t* test and nonparametric methods.

There no significant changes in the DC and LP contents in rats without motor reaction to the acoustic stimuli. A tendency toward an increase in these parameters was recorded in animals with a motor reaction, while in rats with epileptic fits the LP content increased considerably and the DC content was somewhat elevated, which is indicative of more intense free-radical reactions.

After NT, the LP content was higher in rats responding and indifferent to the acoustic stimuli compared with intact rats. Although NT did not abolish the audiogenic epileptic fits, the LP content was much lower in these animals than in their counterparts without NT.

Protective Effect of the Antioxidants α-Tocopherol and U18 for Damage of Cultured Cerebellum Granular Neurons with Kainic Acid

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It is known that the neurodestructive activity of excitatory amino acids, including kainate, is associated with a prolonged increase in the cytoplasmic Ca^{2+}, which can activate Ca^{2+}-dependent enzymes (xanthine oxidase, phospholipase C) indirectly involved in lipid peroxidation in the neuron membranes. Proceeding from this, we have tested the possibility of preventing kainate-induced neuronal death by treating cultured cerebellum granular cells with the antioxidants α-tocopherol and U18, a sterically hindered phenol. Dissociated 7- or 8-day cultures of granular neurons derived from the cerebellum of Wistar rats were used. Treatment of these cultures with 100 μM kainate for 30 min induced death of 53.6±1.0% of neurons during 4 h. Preincubation of the cultures with α-tocopherol (5×10^{-4} M) or U18 (5×10^{-5} M) for 2 h reduced the number of dead cells to 30.9±0.9 and 35.6± ±4.8%, respectively.

In control cultures lipid peroxidation in the neuronal membranes was induced by 1.5-h treatment of the cultures with Fe^{2+} ascorbate (3 μM/30 μM), which induced the death of 90±1.3% of cells. Preincubation with α-tocopherol under the same conditions as in the experiment lowered mortality to 37.8±1.75%.

Our results indicate that the antioxidants α-tocopherol and U18 elicit a pronounced protective effect against kainate-induced damage of cultured granular neurons. Bearing in mind that vitamin E is a membrane stabilizer, we assumed that activation of lipid peroxidation in neuronal membranes, leading to structural disturbances, secondary Ca^{2+} entry, and calcium-induced death of neurons, is one of the possible mechanisms responsible for the damaging effect of kainate.

This study was supported by the Russian Foundation for Basic Research.

Induced Lipid Peroxidation in Hereditary Degeneration of the Retina in the Rat: Possible Reasons for Its Change in the Cortex and Retina in Early Postnatal Ontogenesis

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Hereditary degeneration of the retina (HDR) is a human disease leading to blindness. The disease is caused by a breakdown in the contact between the outer segments of retinal rods and the pigmented epithelium of the eye (PE), which causes the destruction of photoreceptors. The disease is modeled in pure strains of animals, notably on rats.

Lipid peroxidation (LPO) induced by Fe+ ascorbate in a number of tissues of Campbell rats with HDR was assessed in comparison with healthy Wistar rats.

In was found that at the early stages of postnatal ontogenesis (5th-20th day after birth) LPO in the retina and cerebral cortex of HDR rats is increased 2- to 3-fold as compared with the liver and lungs. In an attempt to explain this phenomenon it was demonstrated that the content of nonheme Fe in the microsomal fraction of the cerebral cortex is considerably lower (35-40%), and that in HDR rats the ratio between its oxidized and unoxidized forms is dramatically (10-fold) shifted in favor of the latter as compared with healthy rats. The content of hemic components (cytochrome P-450) in the cortex microsomal fraction changed much later (45th-90th day of life), being 2- to 5-fold higher than in healthy rats. At an early stage of ontogenesis (20th day of life) the content of ferritin and the degree of its saturation with Fe in the cortex of HDR rats was only 50% of that in healthy rats. The changes in the examined Fe-containing proteins occurring in the cerebral cortex of rats with HDR may account for the difference in the course of induced LPO processes in the damaged tissues.

It is demonstrated for the first time that in HDH the cerebral cortex is damaged in addition to the retina and PE.

Peculiarities of Oxidative Stress Development in Nervous System Pathologies

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There is considerable evidence indicating the development of oxidative stress (a shift in the antioxidant-prooxidant balance in favor of a predominance of the latter) in psychoneurological disorders. The heightened risk of oxidative stress generation in the cells and tissues of the nervous system is determined by the intensity of oxidative metabolism (the human brain consumes 30% of inhaled oxygen), high contents of lipids (50% of the dry brain weight), which are the substrate of peroxidation (every third fatty acid of the lipids is unsaturated), and the participation of free-radicals in neuroregulation, namely the neurotransmitter NO· and O_2·. Protection of brain tissue against oxidative damage is provided by the high activity of enzyme and fat- and water-soluble antioxidants (the ascorbic acid content of the brain is 100 times as high as in the serum), which makes the nervous tissue sensitive to a deficiency of essential antioxidants.

The specific features of oxidative metabolism determine the increased sensitivity of nerve cells to respiratory toxins which act on the electron-transport chains in the mitochondria; microcirculation disorders (insults, microscleroses, trauma) leading to ischemia and increased AKM production; increased NO-synthetase activity resulting from psychoemotional stress, the product of whose functioning (NO·) generates toxic nitrogen oxides upon interaction with O_2·.

The development of free-radical oxidative reactions in the nervous tissue is local. Although neuronal damage caused by free-radicals has been demonstrated in numerous pathologies, there is no information regarding the specific changes reflecting these processes in other tissues, specifically, serum and blood cells, which are the most convenient objects for clinical investigations. We developed an array of functional loads utilizing antioxidants for clinical diagnostics and for the choice of appropriate therapy of oxidative stress in nervous system pathologies, as well as a nondrug therapy - ultraviolet or laser radiation of the blood.

Peculiarities of Lipid Peroxidation in Some Genetically Inherited Degenerative Brain Diseases

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Huntington's disease (HD), Parkinsons's disease (PD), and Wilson-Konovalov disease (WKD) are characterized by damage to extrapyramidal structures. However, the mechanisms of neuronal degeneration in these diseases have been little investigated.

Lipid peroxidation (LPO) plays an important role in the structural and functional disintegration of membranes, contributing to progressive brain damage.

The LPO processes were studied in 81 patients; 25 of them had a history of PD, 33 HD, and 23 WKD. The control group consisted of 22 healthy individuals. Lipid peroxidation was assessed by Fe-induced chemiluminescence (CL) and serum concentration of apoB-containing lipoproteins.

Similar changes in LPO were revealed in HD and PD. These changes manifested themselves in a reduced resistance of lipoproteins to peroxidation and an increased amplitude of the slow CL burst that characterizes lipid susceptibility to oxidation. A decrease in lipoprotein susceptibility coinciding with a statistically significant increase in the amplitude of the rapid CL burst, which was proportional to the initial hydroperoxide content, were recorded in WKD patients. The blood content of the secondary LPO products reacting with thiobarbituric acid was also increased in these patients.

Biochemical analysis showed LPO disturbances in all patients. These disturbances were characterized by a shortened CL latency, reflecting the lowered resistance of lipoproteins to peroxidation. This is associated with changes in the pro- and antioxidant ratio, and its decrease may indicate an imbalance in the LPO system.

The results obtained suggest that LPO is involved in the complex biochemical mechanisms responsible for the pathogenesis of the indicated diseases and may be regarded as a basis for the development of new effective methods of LPO correction in patients with HD, PD, and WKD.

Superoxide Dismutase Activity in the Striatum of Rats with Modeled Parkinsons's Syndrome

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The activity of superoxide dismutase (SOD) was measured in the cytosol fraction of homogenates of striatum of 3-5- or 6-7-month-old Wistar rats with Parkinson's syndrome (PS), which was induced by intraperitoneal injection of the neurotoxin 1 methyl-4-phenyl-1,2,3,6-tetrahydropyridine. In 3-5-month-old rats SOD activity did not change after PS had developed. In control and experimental animals the enzyme activity was 326±31 and 364±48 arbitrary units/mg protein, respectively. In 6-7-month-old rats SOD activity decreased significantly compared with the control group: 223±35 vs. 304±12 arb. units/mg protein in the control (p=0.05). It was assumed that the resistance of SOD, a major component of the antioxidant system, plays a considerable role in the higher resistance of young (3-5-month-old) rats to the neurotoxin that induces the development of Parkinson's syndrome.

Glutamate Lowers the Membrane Potential in Mitochondria of Cultured Granular Neurons from the Cerebellum

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Excessive stimulation of cortical neurons and granular cells of the cerebellum with glutamate (GLU) induces a sustained increase in the intracellular Ca concentration ($[Ca^{2+}]_i$), stimulates free-radical formation [D. Choi, 1988], and lowerd the ATP concentration in these cells [A. Bogachev, 1992]. We have suggested that the increase in $[Ca^{2+}]_i$, accompanied by the generation of free-radicals and peroxidation compounds, disrupts the mitochondrial function in the glutamate-receptive neurons.

This study was performed on dissociated cultures of granular neurons isolated from the cerebellum of 7-8-day-old rats. The fluorescent dye rhodamine 123 (R123) was used as a membrane potential marker. After a 10-min incubation of control cultures with R123, an intense fluorescence of the mitochondria was observed during 30-40 min. Experimental cultures were preincubated for 15 min in salt solution containing 100 μM GLU. There was no fluorescence in GLU-treated cells, indicating the absence of a potential on the mitochondrial membranes. A similar effect was observed after treatment of the cells with the calcium ionophore A23187 (5 min, 20-30 μM), which lowered the membrane potential of the mitochondria. The accumulation

of R123 in GLU-treated mitochondria was preserved upon blockage of NMDA and calcium potential-depdnent channels by cobalt chloride (2 mM). Presumably, the drop of the membrane potential in neuronal mitochondria after GLU treatment is associated with an increase in $[Ca^{2+}]_i$, which may also lead to excessive production of free-radicals that increase the nonspecific permeability of mitochondrial membranes.

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Combined Application of Antioxidants in Neuroses

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Bearing in mind the role of lipid peroxidation in the genesis and maintenance of chronic emotional stress [Yu. A. Aleksandrovskii et al., 1988; Yu. A, Aleksandrovskii and M. V. Pokrovskii, 1990; etc.] and the concept interpreting anxiety as a marker of these disturbances, we have attempted to compare the efficiencies of various antioxidant therapies of marginal psycho-neurological states. Sixty-four patients (45 females and 19 males, mean age 37±7 years) were enrolled in the study. All of them had the anxiety affect against the background of neurotic (54 patients with three classical forms of neuroses) or neurotic-like (10 cerebro-asthenic patients). The patients were assigned into 4 groups with comparable ratios of astheno-subdepressive, astheno-hypochondriac, and astheno-phobiac syndromes. Fifteen patients received routine psychopharmacotherapy (PPT) and antioxidants (AO), and 8 patients received group therapy (GT). In the control, 15 patients were on PPT and 26 patients on GT combined with PPT. The following vitamins with antioxidant activity were administered perorally: vitamin E (α-tocopherol acetate, daily dose 200 mg), vitamin C (ascorbic acid, daily dose 400 mg), and vitamin F (linetol, daily dose 30 ml). The effectiveness of therapy was estimated by the method of M. Ya. Sereiskii with modifications of S. M. Plotnikov, which allowed us to assess the dynamics of psychic deviations and social and work adaptation, and with the aid of the Spielberger-Khanin and Luscher tests. The results are summarized in the table.

Dynamics	Therapy			
	clinical	Luscher index of anxiety	reactive anxiety	personal anxiety
GT+PPT+AO	1.63±0.71*	−1.31	0	−0.25
PPT+AO	1.60±0.51*	−0.07	+3	−0.14
GT+AO	1.42±0.64	−0.15	+2.34	−3.16
PPT	1.33±0.72*	−0.34	−0.8	+9.53

Note. Asterisk indicates $p<0.05$.

The application of vitamins E, C, and F against the background on GT and PPT improved the psychological condition and social-work adaptability of the patients. The absence of statistically significant changes in the quantitative parameters of anxiety implies that antioxidants act via some other psychotropic mechanisms together with the known anxiolytic mechanisms.

Blood Levels of Lipid Peroxidation Products in Different-Aged Patients with Parkinson's Disease

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The blood level of lipid peroxidation products (LPOP) was studied in 62 patients with Parkinson's disease and 51 healthy donors. The patients and donors were divided into 2 subgroups: subgroup I, middle-aged (45-59 years) and subgroup II, elderly (60-74 years) individuals. In the healthy subjects the blood LPOP level was virtually the same in both subgroups. In the patients the LPO level was higher in subgroup II. Comparison between the subgroups showed that healthy donors and patients of subgroup I did not differ in the blood LPOP level, whereas the level was higher in subgroup II patients than in healthy donors. The patients of both subgroups were additionally divided into two further subgroups: patients who received a comparatively effective drug therapy and those who did not receive it. The LPOP blood level was considerably lower in subgroup I patients who received the therapy compared with the patients of the same age who did not receive it. In nontreated patients the blood

LPOP level was higher than in healthy donors. The blood LPOP level in the subgroups of treated and nontreated elderly patients was virtually the same, but was higher than that in healthy donors of the same age. We concluded that the regulation of LPOP is impaired in middle-aged patients with Parkinson's disease who not received effective therapy and in elderly patients irrespective of therapy. The results are considered in the light of the importance of LPOP regulation in the pathogenesis of Parkinson's disease.

Lipid Peroxidation (LPO) in the CNS and Blood Rats of Various Age with Modeled Parkinson's Syndrome.

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The experiments were performed on outbred albino rats aged 3-4, 8-10, and 26-28 months. It was found that systemic administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as well as intracaudal and intranigral administration of the product of MPTP oxidation 1-methyl-4-phenylpyridinium (MPP+) induced the development of the three major symptoms of Parkinson's syndrome (PS): oligokinesia, rigidity, and tremor. The severity of the symptoms depended on the type of neurotoxin, dose, route of administration, and age of the animal. The most pronounced changes were induced by intranigral injection of MPP+. The degree of manifestation of symptoms was markedly increased with age. The development of PS was accompanied by alterations in LPO regulation in the striatum. The intensity and direction of these alterations were age-dependent. In 3-4-month-old rats the intensity of LPO was lowered in both the striatum and the blood. By contrast, in 8-10- or 26-28-month-old rats the LPO intensity in the striatum was higher, the increase being greater in the older rats. In these rats the LPO intensity was also higher in the blood. It was concluded that impaired regulation of LPO in the striatum is an important factor in the pathogenesis of Parkinson's syndrome.

Dynamics of Free-Radical Processes in the Rat Brain During the Postreanimation Period

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We studied the effect of a 10-min circulation arrest on free-radical processes (FRP) in the brain hemispheres and blood serum and on the brain content of major membrane lipids (cholesterol and phospholipids)1 h, 4, 7, and 14 days, and 1 month after reanimation. The experiments were performed on male outbred albino rats. The circulation arrest was produced by intrathoracic clamping of the major vessels ascending and descending from the heart. Free-radical processes in the serum were analyzed in order to assess the response of the whole organism. Intensification of FRP in the brain was observed 1 week and 1 month after reanimation. In the brain, judging from H_2O_2-induced chemiluminescence and the content of TBA-reactive products, an increase of FRP was noted. A considerable decrease in the brain cholesterol content was recorded 1 h after reanimation, which reflects the "antioxidative" initial phase of stress characteristic of the brain. Cholesterol accumulation and increase in the cholesterol-phospholipid ratio were observed 1-2 weeks after reanimation, being indicative of a reduction in membrane plasticity due to excessive activation of FRP. Generally, the changes occurred in both hemispheres. In the serum, FRP activation had a phasic character: FRP were enhanced 1 h and 1 week after reanimation and significantly diminished after 2-4 weeks. The decrease in the intensity of FRP may testify to the activation of compensatory processes in the form of a hypercompensatory effect or the organism's response to the presence of a "pathological" focus. The different dynamics of FRP in the brain and serum confirm the concept of FRP autoregulation in antiradical protection of the brain.

Effects of Haloperidol on Free-Radical Processes in Rat Brain Hemispheres: Correlation with Thermoencephaloscopic Data

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Intraperitoneal administration of haloperidol (4 mg/kg) to Wistar rats induced inhibition after 5 min and cataleptic stupor after 10-12 min. At that time the animals were decapitated, and the intensity of free-radical processes (FRP) in brain hemisphere homogenate was assessed. There were no differences in superoxide dismutase activity in the brain and serum of control and haloperidol-treated rats. In the left hemisphere the content of TBA-active products (TBAAP) induced by Fe ascorbate was decreased 2.3-fold ($p<0.03$) and in the right hemisphere, 1.9-fold ($p<0.02$). The content of noninduced TBApr did not change significantly in the left hemisphere, but increased 1.9-fold ($p<0.03$) in the right hemisphere. Haloperidol had no effect on the blood content of TBAAP. A study of the brain heat fields using thermoencephaloscopy, an approach based on the recording of infrared radiation from the surface, showed that administration of 4 mg/kg haloperidol induces heat asymmetry of the brain, the right hemisphere being warmer than the left. The maximum reaction was observed 10-15 min after haloperidol administration, the difference being 0.5°C. A similar asymmetric heat reaction is characteristic of rats with genetic catalepsy and of Krushinsky-Molodkina rats after a sound-induced epileptic fit. Thus, haloperidol elicits a specific effect on free-radical oxidation in the brain (reduced induction of TBAAP) and an asymmetric effect (activation of perioxidation reactions in the right hemisphere), which may be directly associated with the functional asymmetry of the brain in catalepsy.

Changes in the Activity of NO Synthetase in Different Structures of Rat Brain During the Postreanimation Period

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The dymanics of NO synthetase (NOS) activity was studied in a rat post-reanimation model: outbred albino male rats were reanimated after a 15-min circulatory arrest. The cortex, cerebellum, and subcortical structures were homogenized and assayed for NOS activity by the formation of the paramagnetic mononitrosyl complex NO· with diethylcarbamide and divalent Fe (EPR recording) and the total intensity of free-radical generation (H_2O_2-induced luminol-dependent chemiluminescence, CL) was measured. In control animals, NOS activity in the cerebellum, right and left cortex, and in subcortical structures was 2.40±02, 1.96±0.24, 1.80±0.29, and 2.35±0.33 units (pmol NO/min/mg protein), respectively. One hour after reanimation it was 36, 25, 29, and 35% lower ($p<0.006$, 0.09, 0.05, and 0.05), respectively. In about half of the animals the activity reached the control level 2-3 weeks after reanimation, whereas in the other animals it was decreased, accounting for 49, 41, 43, and 47% of the control, respectively. There was a correlation between NOS activities in the left and right cortex ($r=0.84$, $p<0.02$) and in the subcortex and left/right cortex ($r=0.88$, $p<0.01$, $r=0.83$, $p<0.03$). These correlations disappeared 1 h after reanimation. Correlations were established after 2-3 weeks between NOS activities in the subcortex and right cortex ($r=0.79$, $p<0.02$) and in the cerebellum and right cortex/subcortex ($r=0.86$; $r=0.84$, $p<0.005$).

There was a correlation between NOS activity in the cerebellum and the degree of neurological deficiency (assessed in points) on days 1 and 2 after reanimation ($r=0.78$, $r=0.63$, $p<0.05$). In all the brain structures, with the exception of the right cortex, NOS activity tended to increase 10-15% and in 2-3 weeks it was 36% ($p<0.01$) higher in the cerebellum and did not differ from the control in the cortex and subcortical structures.

Lipid Peroxidation in Human Brain Tumors

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Lipid peroxidation (LPO) was assessed in human brain tissue, gliomas, and meningiomas by measuring the malonic dialdehyde (MDA) content, total antioxidant activity (TAOA), superoxide dismutase (SOD) activity, and Fe content.

It was found that the MDA content of tumor tissues was 4- to 10-fold lower than that of brain tissues. Meningovascular tumors had a lower MDA content compared with gliomas. The MDA content was higher in benign gliomas than in malignant gliomas. This parameter is consistent with TAOA, which is more than twice as high in tumor tissues as in the brain, and higher in meningiomas than in gliomas. SOD activity in the brain was significantly higher than in all the tumors studied.

While testing the possibility of inducing LPO in tumor tissues with the aid of Fe^{2+}, we found that incubation of tumor tissues at 37°C for 1 h led to a 160- to 600-fold increase in the MDA content. This increase may represent a possible means of controlling tumor growth by destroying the tumor cell membranes.

Effects of Various Regimes of Hyperbaric Oxygenation on Free-Radical Processes in Rat Brain

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Acute hyperbaric oxygenation (HBO, 1 atm for 1 h) prevented the enhancement of free-radical processes induced by immobilization stress (a 5-point fixation on the back), as measured by the content of TBA-active products (TBAAP) and the decrease in the superoxide dismutase (SOD) activity in the brain and blood, and lowered the cholesterol-phospholipid ratio in the brain of outbred male rats. The HBO-induced changes in free-radical processes (FRP) were similar to those occurring in other forms of acute stress (a rapid adaptive FRP inhibition followed by activation). Repeated HBO of increasing intensity (10 days for 1 h, 0.01-1.0 atm, daily increase of 0.1 atm) induced phasic changes in FRP. The intensity of FRP increased in the blood (minimal SOD activity and maximum TBAAP content) after 3 HBO sessions. The most pronounced adaptive changes in FRP in the brain (max. SOD activity and min. TBAAP content, min. cholesterol:phospholipid ratio) were observed after 8 HBO sessions and 5-12 days after the end of the HBO course. Comparison of FRP dynamics in the brain and blood during and after the HBO course indicates that FRP in the brain is relatively independent of the tendencies characteristic of the whole organism, which were estimated by the intensity of FRP in the blood. Adaptation to HBO prevented lactate accumulation in the brain induced by immobilization stress.

Adaptation of Brain Membranes to Chronic Stress: Structural and Functional Changes

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Moderate or severe stress induces both pathological and adaptive-compensatory changes in biological membranes. The problem of distinguishing these changes, which can occur simultaneously or at different times, is of fundamental significance and becomes crucial when proper evaluation of the functional state of the individual is necessary in clinical practice.

Using a model of neurosis developed as a result of chronic emotional-pain stress, we studied the dynamics of adaptive and dysadaptive changes in synap-

tic membranes isolated from the brain of outbred albino rats. After 1-2 weeks of exposure to stress we observed an increase in K_d of β-adrenoreceptors (K_d was measured with the use of [^{3}H]dihydroaldoprenolol), which was accompanied by a compensatory increase in B_{max}.

Functional changes in synaptic β-adrenoreceptors paralleled a decrease in the cholesterol-phospholipid ratio and a decrease in the intensity of free-radical oxidation against the background of an increase in superoxide dismutase activity after 1-2 weeks of stress. Normalization of all the parameters tested in the synaptic membranes of rats exposed to stress for 3 weeks coincided with the activation of peroxidation processes in the serum, indicating relatively well-developed compensatory abilities of brain membranes (as compared with the whole body) and a certain autonomy of free-radical processes in the brain in this model of chronic stress.

Correction of Age-Related Functional and Pathomorphological Alterations in the Brain by the Antioxidant Mexidol

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Free-radical damage to cell membranes due to the intensification of lipid peroxidation (LPO) plays an important role in aging. In an attempt to prevent and compensate for the age-related structural and functional alterations in rat brain, animals aged 4.5-20 months were given the synthetic antioxidant mexidol (a 3-oxypyridine). This compound effectively inhibits LPO and changes the phospholipid composition of brain membranes in favor of an increase in the content of unsaturated phospholipids (phosphatidylinositol and phosphatidylserine) and a decrease in the cholesterol: phospholipid ratio. Mexidol therapy prevented the development of pathological processes in middle-aged rats and ameliorated compensatory-reconstructive processes in older rats. The rats either developed or restored impaired space and time differentiations; sympathetic, emotional, and motivational reactions in them were improved, and their vitality was increased. The number of mitochondria in neurons and dendrites was markedly increased, synapses with preserved structure predominated, and the content of pathological lipid synthesis products was extremely low.

The results obtained indicate that treatment with mexidol prolongs the active life of rats and slows the development of the age-related destructive changes in the brain.

Modification of the Activity of Membrane-Bound Acetylcholine Esterase in Rat and Mouse Brain Synaptosomes by the Lipid Peroxidation Inhibitor Ionol *in Vitro* and *in Vivo*

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4-Methyl-2,6-ditertbutylphenol (ionol, dibunol) was intraperitoneally injected to outbred mice and Wistar rats in a dose of 20 mg/kg. The animals were sacrificed 18 h after the injection, and synaptosomes were isolated from the brain. The antioxidant (AO) induced changes in the composition and structure of synaptosomal lipids: it raised the phospholipid (PhL)/protein ratio, increased the content of some PhL fractions, lowered the cholesterol content and the cholesterol/PhL ratio, and increased microviscosity and increased the degree of lipid oxidation. After being internalized into synaptosomal lipids *in vitro*, AO affected acetylcholine esterase (ACE), which was manifested in an increase of the Michaelis constant (K_m), and of the maximum velocity (V_{max}) and a decrease in the enzyme "efficiency" (V_{max}/K_m). Similar changes in the kinetic parameters occurred *in vivo* after ionol administration.

There was a direct correlation between K_m and K_{max} on the one hand with the content of individual PhL (phosphatidylcholine, phosphatidyldiethanolamine, and

sphingomyelin) in synaptosomes, on the other. Althought ionol induced similar changes in synaptosomal lipids in mice and rats, the changes in ACE activity were different. In mice, both K_m and V_{max} were decreased to an equal extent, i.e., their ratio did not change. In mice, synaptosomal ACE was inhibited at all the substrate concentrations tested, whereas in rats it was inhibited at low substrate concentrations and activated at relatively high concentrations (this was also observed after AO incorporation into synaptosomal lipids *in vitro*). There was no correlation between the changes in ACE characteristics and the changes in lipids, which may be due to the predominant effect of AO on other pathways of enzyme regulation. Bearing in mind the changes in ACE activity for modulation of the CNS serotonin content by some agents and the ionol-induced increase in the serotonin content and inhibition of monoamine oxidase in the brain, we suggested that serotonin contributes to ACE inhibition. A reversible noncompetitive inhibition of ACE by physiological concentrations of this neurotransmitter was demonstrated *in vitro*.

The data on the inhibitory effect of AO on ACE in nerve endings may be useful in devicing the application of AO in the therapy of cerebral pathologies.

Neurotoxic Properties of Blood Plasma of Recently Confined Women and Newborns Living in the Region Affected by Nuclear Weapons Tests in the Semipalatinsk Test Area

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A study was performed to determine how the plasma of recently recently confined women and newborn children inhabiting the Altai region, whihch had been polluted as a result of nuclear weapons testing in the Semipalatinsk area in 1949-1956 and 1965, affects the function of rat brin synaptosomes. The integral transmembrane potential (TMP) on the plasmalemma and mitochondrial membranes of synaptosomes (measured by the distribution of the lipophilic cation [^{3}H]tetraphenylphosphonium) and the rate of Na-pump operation (measured by the ouabain-sensitive accumulation of [$^{86}Rb^+$], a K^+ analog) were chosen as the test parameters. The women and newborns enrolled in the study were divided into 3 groups: group I, mothers and newborns from control regions; group II, mothers and newborns from regions where the inhabitants had received a total dose of not less than 35 cSv, and group III, mothers and newborns from the city of Rubtsovsk, where the total dose was 35-100 cSv. Incubation of synaptosomes with the plasma obtained from group II mothers and newborns induced a statistically significant decrease in the integral TMP (23.6% for maternal plasma and 12.3% for newborn plasma) compared with the control group. In group III, these values were 22.5 and 20.4%, respectively. The sodium pump was inhibited 13.1 and 40.5% (group II maternal and newborn plasma, respectively), and 43.4 and 64.4% (group III). It was concluded that the plasma of recently delivered women and newborns living in the nuclear testing area elicits pronounced neurotoxic activity, which may be associated with the presence of some factor generated in the inhabitants of this region in response to environmental pollution. The role of free-radical reactions in the development of hereditary pathological alterations induced by small doses of ionizing irradiation is considered.

Consequences of Noncompensated Activation of Lipid Peroxidation for Synaptic Membranes

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The effect of LPO activators on the structure and function of synaptic membranes (SM) was examined. The following properties of SM were studied: permeability to Na^+ and K^+, which are the major potential-forming cations, and microviscosity. The Na-K permeability was evaluated by measuring the transmembrane potential (TMP) using the fluorescent probe dis-C_3-(5) and microviscosity was assessed by the degree of pyrene oxymerization. It was found that steady activation of ascorbate- (ASLPO) or NADPH-dependent LPO is not

accompanied by any appreciable changes in TMP, although the content of LPO products in SM was increased 2.5-3-fold. At the same time, local LPO activation by ultraviolet light in the integral protein region, which induced only a 30% increase in the concentration of LPO products, led to complete membrane depolarization during several minutes, it markedly raised the SM permeability to Na^+ and K^+. The most likely reason for this is the formation of nonspecific ion channels at the integral proteins, but not inhibition of the latter (for example Na,K-ATPase, which rapidly loses its activity upon ASLPO). The enzyme inhibition may be due to oxidation of an essential amino acid residue in the active center after its interaction with superoxide and hydroxyl radicals. An increase in the SM viscosity, which was detected after ASLPO with the use of pyrene, may contribute to this phenomenon. The increase in the microviscosity of the SM lipid bilayer may have a compensatory significance under the influence of LPO activators, by limiting the rise in the membrane permeability to the major potential-forming cations.

Correction of Free-Radical Processes in Rat Brain During the Postreanimation Period with Sodium Succinate

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The effect of sodium succinate (SS, 20 mg/kg/day, intramuscularly) on lipid peroxidation (LPO) in the brain hemispheres and serum and on the brain contents of major membrane lipids 1 h, 4, 7, 14 days, and 1 month after reanimation, as well as on behavior was studied in a rat model (10-min arrest of blood circulation by intrathoracic clamping of the major vessels ascending and descending from the heart). Daily administration of SS prevented LPO intensification in the brain of reanimated rats 1 week after the procedure, but not 4 days after it, whereas in control rats SS stimulated LPO in the left hemisphere. The SS-induced LPO intensification in the control was preceded by a pronounced decrease in the superoide dismutase activity on day 4 (the effect that was observed in the same time period in reanimated rats). A pronounced cholesterol (Chl) accumulation and an increase in the Chl:phospholipid ratio in the brain membranes, which were observed 1-2 weeks after reanimation, did not occur in reanimated rats given SS during the first week. In control rats SS induced a decrease in Chl concentration 1 h after a single dose (an "antioxidant" effect typical of the initial phase of stress, which was also observed 1 h after reanimation) and Chl accumulation in the right (but not left) hemisphere 1 week after daily administration of SS. Sodium succinate to a considerable extent prevented activation of LPO in the serum, which was typically observed 1 h and 1 week after reanimation, while in control rats daily administration of SS stimulated LPO in the serum. In control rats, repeated administration of SS induced a transient decline of behavioral activity in open field tests and anxiety-phobic states, while in reanimated rats it prevented the drop in behavioral activity induced by acute stress in the open field. Thus, we have demonstrated 1) "antioxidant" effects of SS occurring in reanimated rats *in vivo* and 2) opposite biochemical and behavioral effects of SS in control and reanimated rats.

Production of Antineurotransmitter Antibodies and Lipid Peroxidation Intensity in Experimental Parkinsonism

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Parkinsonism was induced in 3-5- and 8-10-month-old rats by injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or 1-methyl-4-phenylpyridine. Serum levels of antibodies to the neurotransmitters dopamine and serotonin were measured in parallel with the determination of the concentration of TBA-active prod-

ucts in striatum homogenates. It was found that, first, the younger rats are more resistant to the neurotoxins, and second, their serum contains no antidopamine and antiserotonin antibodies (serum was analyzed for the presence of the antibodies on day 5 and day 16 after neurotoxin administration). A low titer of such antibodies was detected in the control 8-10-month-old rats, and it rapidly grew after the induction of Parkinson's syndrome. Third, the content of LPO products in the striatum was lower in the younger rats compared with the control, whereas in the older rats it was higher than in the control. The results are considered in connection with the role of antineurotransmitter antibodies in the pathogenesis of Parkinson's disease and the role of LPO in the production of these antibodies.

Relationship between Free-Radical Reactions (FRR) Intensity and Lipid Content of Spinal Fluid in Epilepsy

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The elevated content of FFR products in the spinal fluid (SF) of epileptics [Kryzhanoskii *et al.*, 1984] may be associated both with FRR intensification and increased entry of lipids containing FRR products from brain tissues or blood plasma into the SF. We studied lumbar SF of 13 patients aged 17-34 years suffering from the genuine form of epilepsy and receiving routine antiseizure therapy in a clinic. Diene conjugates (DC, spectrophotometrically), Schiff bases (SB, fluorimetrically) and total lipids (TL) were measured in lipid extracts from the SF. The DC content varied from 0.13 to 0.68 (0.43±0.06) μmol/ml; the content of Schiff bases was 120-850 (477±76) arbitrary units/ml. The strong correlation not only between the DC and SB contents (r=0.91) but also between the DC and TL (r=0.83) and the SB and TL (r=0.81) contents indicates that the concentration of FRR products in the SF depends on lipid entry into SF. However, correlations were also established between total lipids and the SB/TL ratio (r=0.75) and the DC/TL ratio (r=0.50). This indicates feedback: whereas lipid entry in the SF is not determined by FRR intensity, it is dependent on the processes that govern FRR, and these stimulate either lipid leakage from brain tissues or their entry into the SF from the blood via an impaired blood-brain barrier. Gas chromatography of the fatty-acid composition of lipids of SF lipids detected a marked predominance of long-chain fatty acids, including polyunsaturated ones: $C_{20:4}$ up to 13% and $C_{22:6}$ up to 10%, which is characteristic of brain lipids, but not of plasma lipids.

Nitrogen Oxide Synthetase and NADPH-Diaphorase: Inhibition of NADPH-Diaphorase Activity by Superoxide Dismutase and Arginine in Brain Homogenates

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Nitrogen oxide (NO), a free-radical formed from arginine, has been identified as a dilatatory factor secreted by vascular endothelium. In addition, NO has proved to serve as a neurotransmitter in some peripheral nervous system terminals and is utilized as a cytotoxic factor in the immune system [Moncada *et al.*, 1991]. Generation of NO in cerebral neurons and glia raises a question about its function in the CNS. The fact that superoxide dismutase (SOD) (immunochemical visualization) is collocated with the NADPH-diaphorase activity (NADPH-DA) (histochemical visualization implying reduction of tetrazolium blue nitrate (TBN), at the expense of NADPH [Hope *et al.*, 1991] may be relevant in this regard. Biochemically, NADPH-D was characterized to an extent that is not consistent with the significance of NO in CNS function and disorders.

We measured NADPH-DA activity in homogenates prepared from rat brain cortex, brainstem, and cerebellum. Both in microsome-free homogenate and in microsomes this activity is inhibited by L-arginine but not by other amino acids. The maximum inhibition (20-40%) at 0.4 mM arginine is competitive toward

TBN, implying that arginine and TBN bind to the same active center of an enzyme, probably, NO synthetase (NOS). The absence of NADPH-DA inhibition in microsome-free liver homogenate and in the neuroglial cell fraction, which normally does not contain SOD, confirms this suggestion. Considering the arginine-inhibited (AI) NADPH-DA as a SOD parameter, we should stress its extremely high level, both total and relative to total NADPH-DA (up to 60%), in homogenates of the outer segments of bovine retina, but not in the whole retina. In Campbell rats with hereditary degeneration of the retina total AI-NADPH-DA is lowered and its contribution to total NADPH-DA is decreased.

In contrast to histochemical visualization of NADPH-D, where its localization completely coincides with that of SOD, upon biochemical determination of NADPH-D SOD constitutes only a part of total NADPH-DA. We have shown that TBN reduction is inhibited not only by arginine but also by SOD, the sum of the inhibitory effects being equal 100%. The source of superoxide in brain microsomes and cytosol is unknown. However, it should be mentioned that in a number of physiological systems superoxide, which can inactivate NO, may act as a physiological regulator of its action. The assumption that such a regulatory mechanism existsin the brain may give cause for reconsidering the significance of superoxide in the CNS.

Effect of Corazole On Lipid Peroxidation and Activity of Na,K-Catalase in Neurons and Glia

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The effect of corazole-induced (5 mg/100 g body weight) seizures on the activity of Na,K-ATPase and on the intensity of lipid peroxidation (LPO) in neuronal and neuroglial cells of rat brain was studied. The animals were sacrificed 12-15 min after corazole injection in the clonic phase of seizures.

From the enzyme activities in neuron- and glial cell-rich fractions it was concluded that 1) corazole-induced seizures lead to an almost 2-fold increase in ATPase activity for a simultaneous drop of its activity in neurons and 2) the enzyme activity calculated per gram tissue was considerably higher in glial cells than in neurons both in health and during seizures.

The function of membrane enzymes is strongly dependent on the lipid microenvironment. Changes in this microenvironment result in a shift in the enzyme activity. Lipid peroxidation is a relatively rapid means of membrane lipid modification. Our results show that under the influence of corazole-induced seizures the LPO intensity rises considerably, as evidenced by the accumulation of LPO products (diene conjugates and Schiff bases) and by the increased production of malonic dialdehyde during ascorbate- and NADPH-dependent LPO. We concluded that the membrane lipids are modified, which is consistent with the reduced activity of Na,K-ATPase. On the other hand, during corazole-induced seizures all the LPO parameters in glial cells remain within the norm or were slightly increased.

The results obtained indicate that LPO may be one of the mechanisms regulating brain Na,K-ATPase in extreme situations.

The Use of α-Tocopherol in the Therapy of Elderly Mental Patients with Manifestations of Tardive Dyskinesia (A Clinico-Biological Study)

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Eleven patients aged 58-77 years were enrolled in the study. All of them suffered from tardive dyskinesia (TD) which had developed as a consequence of long-term psychotropic therapy (mainly neuroleptic), which was resistant to routine correction. The control group was made up of healthy donors of the same age. The patients were chosen according to the criteria of N. R. Schooler and I. M. Kane. The primary mental disorders were various functional psychoses diagnosed according to ICD-10 criteria (WHO). The patients received 200 mg α-tocopherol 3 times a day during 1 month while still continuing to receive the same

doses of psychotropic drugs previously prescribed. AT the end of α-tocopherol therapy the severity of TD was decreased by 12-75% (mean 49%) according to the AIMS scale.

A 3-fold increase (compared with healthy donors) in the plasma content of malonic dialdehyde (MDA), an end product of lipid peroxidation (LPO), was recorded in patients prior to α-tocopherol administration. The blood contents of α-tocopherol and ascorbate did not differ from those in healthy donors, whereas superoxide dismutase (SOD) activity in erythrocytes was lower than normal. At the end of α-tocopherol therapy the blood MDA content was lowered and the activity of the antioxidant system was increased: the blood content of ascorbate increased by 31% ($p<0.1$) and that of α-tocopherol increased by 52% ($p<0.01$). The activity of SOD also increased, reaching the control values. A correlation was established between the decrease in MDA concentration and the clinical effect produced by α-tocopherol therapy ($r=0.87$, $p<0.01$).

Taken together, our findings indicate that there is a noncompensatory intensification of LPO in TD patients and that α-tocopherol therapy evidently normalizes LPO in these patients.

Anxiety, Atherogenesis, and Antioxidant Protection: Clinico-Pathogenetic Relationships

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In the course of studying and comparing involutionary-climacteric psychoses by a number of clinico-biological parameters, we revealed opposite tendencies in the intensity of endocrine-metabolic processes, biological oxidation, and lipid metabolism disorders, as well as differences related to sex, intellectual or physical work, residential environment (urban or rural), etc. Only one common trend was noted - the age of manifestation (45-60 years), although the incidence of atherosclerosis is higher in middle-aged individuals and declines again in old age [G. G. Avtandilov, 1972; B. A. Asetto, 1972; K. Z. Tnimova, 1972; A. M. Vikhert and V. S. Zhdanov, 1976; A. M. Lifshits *et al.*, 1988]. In contrast to atherosclerotic pathologies, climacteric psychoses are reversible, despite a sustained anxiety that models chronic stress. Emotional tension intensifies lipid peroxidation [M. V. Poyuroskii *et al.*, 1986; G. G. Neznamov *et al.*, 1986; Yu. A. Aleksandrovskii *et al.*, 1988] and stimulates the antioxidant systems of nonspecific resistance of the organism to stress factors [V. V. Sokolovskaya *et al.*, 1987; V. N. Bobyrev, V. F. Pochernyaeva, 1991]. Analyzing our clinical observations and the role of minor lipid components (naphthoquinones and tocopherols) in the regulation of the phospholipid/cholesterol ratio in biological membranes, we put forward a hypothesis that atherogenesis generates not only a damaging but also a protective potential [S. M. Plotnikov and V. A. Lider, 1992], the biological significance of which consists in antioxidant membrane stabilizing functions. We suggested that atherogenesis "kicks in" when other endogenous possibilities are exhausted or premorbidly insufficient for gentle dampening of the "metabolic impact" during the climacteric period. There are sufficient grounds for using of antioxidants to prevent and treat involutionary and vascular psychic disorders, even though these pathologies are fundamentally different.

A New Approach to the Treatment of Endogenous Depressions with Antioxidants

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In order to develop new approaches to the optimization of the therapy of endogenous depressions, we tested the possibility of using antioxidants (α-tocopherol and emoxipine) in combination with antidepressants. The study was carried out by the open method in middle-aged and elderly patients resistant to antidepressive therapy. In middle-aged patients emoxipine and α-tocopherol (600 mg/day) considerably reduced the manifestations of depressive symptoms assessed by the HAMD scale (in 53.3 and 66.7% of the patients, respectively) and abolished the resistance to tri- and tetracyclic antidepressants. In elderly patients, α-toco-

pherol used in combination with pyrazidol, ludiomil, and herphonal not only increased the efficacy of the therapy almost 3-fold (improvements were observed in 20.9 and 58.4% of the patients, respectively, compared with the control period when the antioxidants were not applied), but also reduced considerably the side effects of the antidepressants, as assessed by the SARS scale (improvements in 3.1 and 53.3% of the patients, respectively).

Comparison of this results showed α-tocopherol has the highest potentiating effect on the therapeutic efficacy of these antidepressants. The activity of the studied antioxidants is essentially independent of the chemical structure (tri- or tetracylic) and dose of the antidepressant and of the age of the patients.

Taken together, these results indicate that antioxidants are a promising means of enhancing the efficacy of psychopharmacotherapy of endogenous depressions.

The Use of Antioxidants in the Treatment of Tic-Accompanied Hyperkineses in Children

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The principles of complex combined therapy with antioxidants and GABA-positive preparations that we had developed earlier in the experiment were employed in the therapy of tic-accompanied hyperkineses in 49 children aged 6-14 years (of whom 30 had neurosis-like and 19 had nervous tics).

Vitamin E (5 mg/kg intramuscularly for 15-20 days) and GABA-positive preparations (piracetam, pantogam, phenybut) were used in doses 20-25% lower that those conventionally applied in pediatric neurology). In the control group 40 children with tics received routine therapy (tranquilizers and neuroleptics). The control parameters (evaluation of hyperkinesis in points, subjective feelings, neurological status, EEG and seismogram dynamics) were recorded prior to and 30 days after the therapy.

The most prominent effect was observed in children with nervous tics. The number of recoveries was statistically significant ($p<0.01$) compared with the control. In children receiving complex therapy the intensity of tics was reduced by the 13th-14th day. During a 1-year period the number of relapses was 2.5-fold higher in the control group.

In the children with nervous tics the number of recoveries was 2-fold hihger, and the hyperkinesis intensity was reduced significantly ($p<0.01$) in unrecovered children. A positive EEG dynamics was recorded. In catamnesis the number of relapses was 2-fold higher in the children had received routine therapy. Our results show that the use of an antioxidant is effective in combined therapy of tic-accompanied hyperkineses in children.

The Use of Antioxidants and Some Regulatory Peptides for the Prevention of Cerebral Hemorrhages in Experimental Hemorrhagic Insult

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The effects of the novel water-soluble antioxidant emoxipine (20 mg/kg) and three neuropeptides: thyroliberin analog (TRH, 1 mg/kg), delta-sleep-inducing peptide (DSIP, 100 μg/kg), and Semax, an ACTH (4-7), PHP (20 mg/kg) were studied in a model of hemorrhagic insult. The study was performed in Krushinsky-Molodkina rats, which are genetically predisposed to audiogenic epilepsy, i.e., acoustic stress induces in them hyperkinesia, seizures, acute arterial hypertension, and a sharp increase in the blood flow resulting in subdural, subarachnoidal, intraventricular, and cerebral hemorrhages. A study was preformed of the effects of the four preparations on the frequency and intensity of hemorrhagic manifestations in experimental disorders in the cerebral circulation. None of these preparations induced any significant changes in the intensity of seizures, although DSIP slightly increased the latency. Previously, DSIP was found to reduce penicillin- or strychnine-induced epileptic activity [G. N. Kryzhanovskii *et al.*, 1987]. Morphological studies showed that all the preparations tested significantly decreased the frequency of cerebral hemorrhages in experimental insult. Emoxipine and TRH reduced the frequency of subdural and subarachnoidal hemorrhages 2-fold, DSIP, 3.4-fold, and Semax, 4.1-fold. Thus, emoxipine, TRH, DSIP, and Semax elicit pronounced angioprotective effects. The

mechanisms responsible for the decrease in cerebral hemorrhages induced by these preparations are unknown. The major hemorrhage-reducing factors may be a decrease in the vascular wall permeability, an increase in the plasma membrane resistance to hypoxia, and improved microcirculation.

Age-Related Changes in NO Synthetase Activity and Generation of Free Oxygen Radicals in Rat Brain Structures

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The activity of NO synthetase (NOS) and the total intensity of radical formation was determined in homogenate prepared from the cerebellum, cortex, subcortical structures, and hippocampus of young (YR, 2-month-old), adult (AR, 5-month-old), and old (OR, 23-month-old) male Wistar rats. The enzyme activity was determined by measuring the paramagnetic mononitrosyl complex of NO· with diethyldithiocarbamate and divalent Fe (EPR recording) and the intensity of radical formation was assessed by H_2O_2-induced luminol-dependent chemiluminescence (CL). The highest NOS activity was recorded in the cerebellum and subcortical structures, the enzyme activity being lower in the cortex than in the cerebellum 1.1-, 1.2-, and 1.6-fold in YR, AR, and OR, respectively. With age, NOS activity in the cerebellum increased from 1.02±0.09 units (pmol NO/min/mg protein) in YR to 1.53±0.08 units in AR ($p<0.04$) and 1.61±0.12 units in OR ($p<0.01$). The activity did not vary substantially with age and was similar in the right and left cortex.

There were no significant age-related variations in NOS activity in the hippocampus (mean 1.41-1.73 units). A tendency toward an increase in NOS activity with age was observed in other subcortical structures: 1.14±0.08 in YR, 1.39±0.09 in AR, and 1.6±0.13 units in OR ($p<0.05$). The intensity of chemiluminescence was higher in the cerebellum than in the cortex; 1.2-fold in YR ($p<0.1$) and AR ($p<0.001$) and 1.3-fold in OR ($p<0.002$). In the subcortical structures of YR and AR CL intensity was 1.2-fold lower than in the cerebellum ($p<0.03$) and in AR it was 1.1-fold higher ($p<0.01$). In all the structures CL intensity decreased as follows: YR>OR>AR. The relationship between NOS and CL was different in different age groups. There was a statistically insignificant tendency toward a positive correlation between NOS and CL in YR (r varied from 0.27 to 0.69 in the brain structures studied), no correlation between NOS activity and CL in AR, and an inverse correlation in OR (r varied from 0.41 to 0.57, $p<0.08$-0.02 in different areas).

Lipid Peroxidation in Premature Infants with Perinatal Encephalopathy against the Background of Therapy with Metabolite Complexes

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The aim of this study was to assesslipid peroxidation (LPO) in the erythrocytes of premature infants with perinatal encephalopathy (PE) and to estimate the effectiveness of the therapy with metabolite complexes for the correction of LPO disturbances. Fifty-two premature infants were observed, of whom 24 with PE received therapy with metabolite complexes (experiemntal group, EG). The control group (CG) consisted of 16 premature infants with a similar severity of PE, who received routine therapy. Twelve essentially healthy premature infants were also observed (HG). We evaluated the parameters characterizing the intensity of LPO and membrane metabolism: the degree of mechanical and peroxide hemolysis (MH and PH, respectively), percentage of hemolysis augmentation, malonic aldehyde (MA) content before and after *in vitro* incubation under physiological conditions, the MA/PH ratio, and the ratio between the intensity of MA degradation and MA content (D/MA). Pronounced changes in LPO were found in infants with PE, as evidenced by an increased

MH and MA content prior to incubation ($p<0.001$), decreased D/MA ratio ($p<0.05$), and absence of hemolysis augmentation during incubation *in vitro*, as compared with healthy infants. The degree of MH in the infants receiving the therapy was 0.90±0.07% vs. 1.16± ±0.10% in essentailly healthy infants and 2.12±0.25% in the control group. *In vitro* incubation of erythrocytes under physiological conditions resulted in a 77% increase in hemolysis in EG vs. 67% in HG, while in CG this parameter was equal to 0%. The intensity of MA degradation was 29.58±0.91% in EG, which is significantly higher than in HG (25.00±1.17%). There were no statistically significant differences in the MA content, MA/PH and D/MA ratios and PH between EG and HG. It was found that therapy with metabolite complexes normalizes most of the studied LPO parameters in premature infants with PE, preventing pronounced disorders in cell homeostasis.

Effects of Bemegride and Carnosine on Behavior and Lipid Peroxidation in the Blood and Brain of Albino Rats

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Previous studies have shown that administration of bemegride (100 mg/kg) to intact rats and rats after craniocerebral trauma increases seizure activity simulating an epileptic fit and is accompanied by pronounced activation of lipid peroxidation (LPO) in the blood and brain. Ionol (120 mg/kg) reduced LPO intensity and to a considerable degree prevented the kindling effect of bemegride, suggesting that LPO plays a role in the development of clonic seizures. If so, the search for novel effective and pharmacologically safe LPO inhibitors for use in the correction of epileptic activity seems fully justified. Histidine-containing dipeptides, for example, carnosine, are promising candidates for such inhibitors, since they are constituents of numerous "readily excited" tissues and elicit a pronounced antioxidant effect.

Bemegride was administered to maze-adapted rats at a daily dose of 10 or 100 mg/kg during a 23-25-day period (intraperitoneal injection). Carnosine (20 mg/kg/day for 10 days) was also injected intraperitoneally. The rats were then decapitated.

Bemegride proved to increase considerably the plasma contents of TBA-active producst, Schiff bases, and spontaneous plasma chemiluminescence and decreased the peroxide resistance of erythrocytes. It is noteworthy that the time it took the rats to find their way out of the maze was cut by half. The LPO intensity increased markedly with 100 mg/kg bemegride. The changes were particularly pronounced in the brain, where the studied parameters accounted for 240-280% of the control value. This was accompanied by a substantial (more than 1.5-fold) inhibition of the animals' orientation abilities. Carnosine in combination with 10 or 100 mg/kg bemegride normalized free-radical reactions and had diverse effects on rat behavior in the maze. At lower doses of bemegride it sharply inhibited the orientation abilities, whereas at higher doses it stimulated the reaction, bringing it almost to the control level.

Our results indicate that by activating and stabilizing antioxidant activity, carnosine can stimulate depressed and inhibit enhanced orientation abilities of rats in a maze, i.e., it can normalize behavioral reactions.

Dynamics of Lipid Peroxidation after Craniocerebral Injury and Carnosine Administration

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Activation of lipid peroxidation (LPO) develops as a component of the nonspecific reaction of the organism in various physiological strains and pathologies. Investigation of LPO dynamics after craniocerebral injury (CCI) objectively characterizes the severity of the injury and allows for an assessment of the effictiveness of the chosen therapy, for example, antioxidant therapy. Lipid peroxidation was estimated by the contents of endogenous peroxides, TBA-active products, and Schiff bases in rat brain and blood. We also evaluated the peroxide resistance of erythrocytes and spontaneous chemiluminescence of the serum (SCS).

The intensity of LPO increased considerably in the brain and blood 7, 14, 30, and 120 days after CCI com-

pared with the control. The changes in LPO intensity were the most pronounced 7 and 14 days after CCI, and therefore the antioxidant activity of carnosine was studied at these times. On day 7 and 14 after CCI erythrocyte resistance to hydrogen peroxide was decreased 120% and the content of TBA-active products was increased 34%. Substantial (55%) increase in the brain content of endogenous peroxides and Schiff bases was recorded 14 days after CCI. A substantial increase (3-fold above the control) in the content of TBA-active products was recorded 7 days after CCI. Carnosine administered to rats at a daily dose of 20 mg/kg (intraperitoneally) throughout the entire post-traumatic period had noticeable and diverse effects on LPO. On day 7 the effect of carnosine on free-radical oxidation was the same as that observed after CCI, whereas on day 14 LPO was markedly inactivated. Specifically, peroxide resistance to erythrocytes increased by 60% compared with that recorded in CCI, while the level of TBA-active products and the intensity of blood SCL were close to the control value. The brain contents of endogenous peroxides, Schiff bases, and TBA-active products were markedly reduced, exceeding the control values only by 15-20%. Thus, when administered after CCI, carnosine partially, and in some cases completely, normalized LPO parameters, which allows us to regard the compound (providing that broader studies are carried out) as a potential candidate for use in neurosurgery to stimulate the antioxidant resistance of the organism during the post-trauma period.

Relationship Between the Kinetics of Lipid Peroxidation and Autoimmune Reactions after Craniocerebral Injury

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Activation of lipid peroxidation (LPO) is known to be accompanied by changes in the immune response. For example, it was found that the adaptive LPO reduction at high altitudes is attended by inhibition of the immune response. In contrast, after adrenalectomy and under the influence of immunostimulatory agents the intensity of LPO increases, which stimulates antibody production.

In our studies we attempted to establish a relationship between LPO intensity and the autoimmune response after craniocerebral injury (CCI). We roceeded from the fact that a closed CCI is accompanied a pronounced LPO activation in the blood and, secondarily, in the brain of experimental animals. Destruction and necrosis of brain tissues induce a process of autoimmunization to the host brain antigens, the extent of this reaction characterizing the severity of CCI. If there is a relationship between the studied processes, the administration of ionol (120 mg/kg, three times a day) or bemegride (100 mg/kg/day for 30 days) to CCI rats (120 days after trauma), should, by normalizing LPO, produce an indirect effect on the production of autoantibodies.

Ionol partially lowered LPO intensity in intact rats. The LPO intensity was evaluated by spontaneous chemiluminescence and the blood and brain contents of TBA-active products. Bemegride and CCI (to a greater degree) activated the free-radical processes, which were markedly inhibited in the case of combined loads with antioxidant.

The autoimmune response was evaluated by the titer of antibodies against neurospecific enolase (NE), glial protein (GP), and brain antigen (BA). A nonuniform immunological reaction was noted when ionol or bemegride was injected into intact rats. The titer of anti-NE antibodies was lowered substantially, but there was no change in the response to BA. Ionol had no effect on the level of anti-GP antibodies, bemegride increased it by 21%. As a result of CCI, the titers of anti-NE and anti-BA antibodies increased by 21 and 38%, respectively, while that of anti-GP antibodies decreased. The changes in the antibody titers (particularly of anti-BA antibodies) were more pronounced in CCI rats. Ionol significantly lowered the antibody titers in CCI rats. This effect was observed after the combined administration of antioxidant and bemegride.

Taken together, our findings indicate that an increase or decrease in LPO intensity is accompanied by inhibition or stimulation of antibody production, i.e., it facilitates the autoimmune response to brain antigens. For the same reason we may surmise that directed antioxidant inhibition of LPO may be a reliable means of reducing the severity of autoimmune aggression.

Protective Effect of the Antioxidant U-18 and Superoxide Dismutase in Hypoxic Damage to Cultured Neurons of the Hippocampus

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The last decade has produced data indicating that the accumulation of Ca^{2+} in neurons is a major factor in the brain damage occurring in hypoxia/ischemia. This accumulation leads to excessive production of free-radicals (FR) that initiate lipid peroxidation (LPO) in the neuronal membranes. Application of various antioxidants in investigation of the mechanisms underlying the ischemic/hypoxic damage to neurons and the search for means of preventing neuronal destruction are of current interest.

We studied the protective effects of the lipophilic antioxidant U-18 (a sterically hindered phenol) and superoxide dismutase (SOD) on cultured neurons (SOD) from the hippocampus subjected to hypoxia (6-8 h) with subsequent reoxygenation (16-18 h).

In the presence of U-18 (25 μM) during hypoxia, the number of dead neurons and lactate dehydrogenase (LDH) activity in the nutrient medium after reoxygenation were 5.0±0.7 and 6.5±2.3%, respectively (61.2±2.6 and 26.6±2.3% in the control). The prolonged protective of U-18 may result from its ability to be incorporated into the phospholipid membranes and hamper LPO by binding FR, which are produced in excess during oxygenation. Cell mortality during reoxygenation was also decreased in the presence of SOD (300 U/ml), LDH activity in this case being 7.7±1.1% vs. 15.8±2.1% in the control.

Our results indicate that U-18 and SOD effectively prevent hypoxia-induced death of neurons and are prospective tools in the study of the mechanisms underlying neuron destruction in hypoxia/ischemia.

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Determination of NO Synthetase Activity in Brain Homogenates by EPR Spectroscopy

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NO synthetase (NOS) is a major regulator in the brain, where the enzyme catalyzes the formation of equimolar amounts of NO and citrulline from L-arginine, is involved in the regulation of cerebral circulation and couples activation of postsynaptic N-methyl-D-aspartate receptors with functional changes in neurons and glia [Moncada *et al.*, 1991]. Changes in the expression of mRNA of brain NOS have been found in central nervous system disorders [Koprowski *et al.*, 1993] and in stress [Calza *et al.*, 1993]. The evaluation of NOS activity in concentrated brain homogenates is of particular interest, since in these preparations the NOS-modulating effectors are preserved, and these preparations offer the possibility assessing the actual intensity of NO synthesis *in vivo*.

We have developed a method which is based on the known reaction between NO and diethyldithiocarbamate (DETC) and divalent Fe yielding a paramagnetic mononitrosyl complex DETC-Fe-NO (MNFK), which can be recorded using EPR spectroscopy. The characteristic three-component signal from MNFC is localized at an average g-factor of 2.03. The content of MNFC (and consequently that of NO) was determined by the intensity of the MNFC high-polarity component. The rate of accumulation of the complex upon incubation of 30-50% brain homogenates with substrates is linear for at least 30-40 min. The NOS activity measured in different brain structures of outbred and Wistar rats of different ages and sexes varied from 25 to 300 pmol NO/min/g/wet tissue weight (0.25-3.0 pmol NO/min/mg protein), which is consistent with the literature [Ohshima *et al.*, 1992]. An enzyme-catalyzed NO production was confirmed by the absence of MNFC accumulation in animals sacrificed using a microwave oven, which denatures proteins, and in the presence of 2 mM EGTA that chelates Ca^{2+}, an essential component of the NOS reaction. MNFC accumulation was inhibited by the specific NOS inhibitors N-methyl-L-arginine and nitro-L-arginine.

Activation of Lipid Peroxidation in the Brain Is an Important Link in the Pathogenesis of Influenza. The Protective Effect of Antioxidants

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Experimental and clinical observations attest to the development of neurological disorders during influenza [A. A. Smorodintsev, 1984]. This may result from postinfection alterations in the function of the hypothalamo-hypophyseal-neurosecretory system [B. A. Frolov *et al.*, 1991].

We experimentally confirmed the pathogenetic significance of lipid peroxidation (LPO) in the brain of mice infected with influenza and its poststress aggravation [B. A. Frolov *et al.*, 1986; L. K. Chetverikova *et al.*, 1987, 1991]. Our results indicate the following:

1. Lethal influenza is accompanied by a noncompensatory activation of LPO both in the infection focus (the lungs) and in the brain of the mouse.
2. The level of LPO product accumulation in the lungs and in the brain reflects the severity of the infection.
3. Poststress aggravation of influenza is attended by additional activation of LPO in the brain.
4. LPO activation develops against the background of a compensatory growth in the activity of the endogenous antioxidant protective enzymes superoxide dismutase and catalase.
5. The use of preparations with antioxidant activity limits postinfection and poststress LPO activation and elicits preventive and therapeutic effects, lowering the death rate from influenza by 20-40%.

The role of LPO activation is discussed as one of the key molecular mechanisms in postinfection disorders of central regulation, the detoxication system, and the immune response, and the prospects of the use of antioxidants as metabolic correctors in the treatment of viral infections are considered.

A New Treatment of Epilepsy Resistant to Traditional Antiseizure Pharmacotherapy

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Numerous disorders of cerebral function are linked to circulation disturbances that lead to functional hypoxia and facilitate the activation of free-radical processes, which may contribute to the development of neuropathological syndromes. Proceeding from this, we attempted to use the novel antihypoxant with antioxidant activity olifen (developed by Yu. V. Medvedev, Institute of High-Purity biopreprations, St. Petersburg) in the treatment of severe epilepsy resistant to conventional therapies.

After the antiseizure activity of olifen had been confirmed in animal studies, the drug was given to epileptic patients. (At the present time, it is approved for clinical use as an antihypoxant.) The study included 180 patients.

Good and very good results were attained in 92% of the patients with a disease duration of 14.6±1.8 years and seizure frequencies of 1 per week to 30 per day. In some patients (even in those who had been suffering from epilepsy since childhood) seizures disappeared altogether. In the group as a whole the frequencies of generalized and focal seizures decreased 5- and 2-fold, respectively. The mean duration of the seizures decreased 2-fold (in addition to EEG, we monitored blood rheology and coagulation, and pO). Treatment with olifen improved all the parameters.

It should be stressed that olifen offers the possibility of going over to monotherapy. Our results are encouraging for the treatment of severe epilepsy resistant to conventional therapies.

Lipid Peroxidation in Newborns from Various Ecological Zones

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The aim of this work was to study lipid peroxidation (LPO) in the erythrocytes of newborns from different ecological zones. LPO intensity was assessed by measuring the malonic aldehyde (MA) concentration, percentage of MA binding, MA degradation, the MA degradation/MA content (D/MA) ratio, antioxidant activ-

ity, and free-radical (FR) content. The study included 97 newborns. Group I consisted of 35 newborns from 25 maternity homes in Moscow, while group II consisted of 62 newborns from Mytishchi, an industrial city with numerous chemical plants.

In group II newborns the MA content was significantly increased compared with group I newborns (1.41±0.04 and 1.18±0.06 nmol/10^6 erythrocytes, respectively). On the other hand, in group II the level of bound MA was lower than in group I newborns ($p<0.05$).

Malonic dialdehyde binding in group II newborns was 64.36±1.27%, which is significantly lower than in group I. In newborns from Mytishchi the intensity of MA degradation and the D/MA ratio (14.06±0.25 and 10.52±0.39%,.respectively) were lower than in newborns from Moscow (18.13±0.89 and 14.41±0.83%, respectively).

There were no significant differences between the groups in the antioxidant activity.

The free-radical (FR) content was much lower in group II than in group I ($p<0.01$). The addition of 3% H_2O_2 solution to a paralled probe *in vitro* provided a significant decrease in the FR content of erythrocytes obtained from group II newborns (97.58±13.80 and 25.98±11.60 arbitrary units, respectively), while in the newborns from Moscow this parameter remained virtually unchanged after the addition of hydrogen peroxide.

It was found that unfavorable environmental factors induce considerable changes in the cell meatbolism of newborns. Timely measures aimed at the detection and correction of LPO disturbances may prevent the development of disorders in cell homeostasis in newborns and contribute to the healthy development of the growing infant.

The Role of a-Tocopherol in the Treatment of Side Effects Caused by Antidepressant Therapy in Elderly Patients

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The study included 25 elderly patients (mean age 67±5.5 years) with long-term depressions. The patients were treated for 28 days with a combination of tetracyclic antidepressants (pyrazidol or ludiomil) with vitamin E (daily dose 600 mg of 50% oil solution). The effectiveness of this treatment was compared by the intensity of the side effects induced by these antidepressants during a 28-day course of combined therapy plus the preceding 28-day therapy without antioxidants. The antidepressants were administered at the optimal individual dose (mean doses 153.8 mg pyrazidol and 112.5 pudiomil), which was not changed throughout the study. The character and intensity of somato-neurological disorders were analyzed each week using the SARS scale.

In most of teh patients the disorders were manifestations of antidopaminergic, central inhibiting, anticholinergic, and sympathicolytic activities of the drugs. α-Tocopherol effectively (statistically significant improvements in 50% and more patients) blocked peripheral anticholinergic and sympathicolytic effects of the antidepressants (orthostatic hypotension, dryness in the mouth, sweating, and tachycardia). The side effects were significantly reduced week 2 after the start of the complex therapy, the most pronounced reduction being attained in weeks 3 and 4.

In 72% of the patients at end of the therapy the side effects were only half as intense or less (the SARS scale) as at the beginning of the therapy. There was a reliable correlation between the intensity of side effects and the total effectiveness of combined therapy with antidepressants and α-tocopherol.