OXIDATIVE PHOSPHORYLATION IN LIVER MITOCHONDRIA IN THE TOXIC FORM OF EXPERIMENTAL INFLUENZA

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Disturbance of mitochondrial respiration as a result of accumulation of lipid peroxidation (LPO) products, nitric oxide and methemoglobin, formed in the tissues as a result of free-radical redox reactions, has been described in the literature [2, 6, 12]. The writers showed previously that in the toxic form of influenza [3] these physiologically active compounds accumulate in the animals' blood [8]. The aim of this investigation was to assess the state of the oxidative phosphorylation system of the liver mitochondria in this form of infection, a matter of great importance for our understanding of the molecular mechanism of development of toxicosis in the pathogenesis of the disease.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice. Influenza viruses A/Victoria/35/72, with antigenic structure NZ No. 2, adapted (pathogenicity 4.2 log LD_{50}) and unadapted(spathogenic) to mice were used for infection The toxic form of influenza was induced by a pathogenic strain of influenza virus. The peak of virus reproduction in the lungs for the pathogenic strain (dose 4.0 log MID_{50}) was observed after 48 h, and amounted to 8.3 log EID_{50}, which was 100 times greater than the corresponding value for animals of the control group, infected with the apathogenic virus. The level of redox processes in the oxidative phosphorylation system in the liver tissue was monitored by EPR-spectroscopy. EPR spectra were recorded at 77K on small EPR spectrometer made by the "Svetlana" Leningrad Optical-Electrical Factory. The level of substrate-dependent reduction of iron-sulfur proteins of the electron-transport chain of the mitochondria was estimated from the intensity of the g = 1.94 line, characteristic of the reduced (low-spin) state of the paramagnetic centers [1, 9]. The level of free-radical reactions and of accumulation of nitric oxide ($^{\cdot}N = 0$) was judged from the intensity of lines in the g = 2.00 and g = 2.041 regions respectively. The signal with allowed triplet structure at g = 2.041 is characteristic of the mononitrosyl ($^{\cdot}N = O$ complex with endogenous Fe^{2+} in the presence of diethyl thiocarbamate (DETC) [5]. Accumulation of LPO products was monitored by fluorescence analysis of lipid extracts from liver tissue, just as in [4]. Mitochondria were isolated from the animals' liver by the usual method. The respiration rate of the mitochondria in a closed Clark's platinum electrode in medium of the following composition: 100 mM KCl, 20 mM Tris-HCl, 3 mM $MgCl_2$, 3 mM K^+/Na^+ phosphate, pH 7.4. As the substrates, 5 mM succinate and 5 mM glutamate were used, together with 1 mM malate. The concentration of adenine nucleotides in the mitochondria was determined by enzymic methods [10, 11]. Altogether six series of experiments were carried out, in each of which 30-40 animals were used. The graphic data are presented with twice the standard error.

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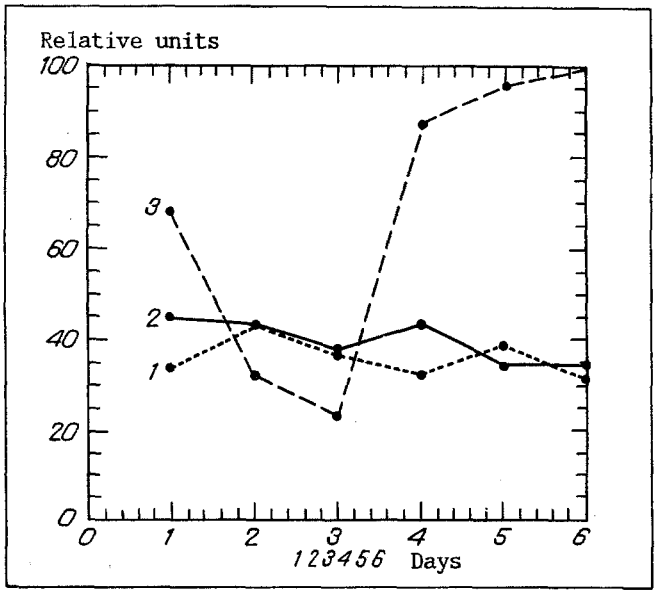

Fig. 1. Level of fluorescent products of lipid peroxidation in extract of mouse liver homogenate: 1) control, 2) apathogenic infection, 3) pathogenic infection.

EXPERIMENTAL RESULTS

As the data in Fig. 1 show, a marked increase in the content of fluorescent LPO products was observed in liver homogenate from animals infected with the pathogenic virus on the 4th-6th day of the experiment. Meanwhile the amplitude of the g = 1.94 signal in the EPR spectrum decreased (Fig. 2), indicating structural and functional changes in the oxidative phosphorylation system in the toxic form of influenza. This could be the result either of concentration changes of the substrates involved in redox processes or modification of the properties of the protein components of the mitochondrial membranes as a result of activation of peroxidation. It must be pointed out that no increase in the intensity of the free radical signal (g = 2.00) could be recorded in pathology (Fig. 2).

Comparative analysis of the respiration rate of the mitochondria in states 3 and 4 (in the presence of succinate and glutamate together with malate) showed a significant decrease (by 20-30%) of this parameter in pathology, whereas the respiration rate of the liver mitochondria of animals infected with the apathogenic strain of the virus was at the control level (Fig. 3). The respiration rate in state 4 is known to be determined mainly by both proton conductivity of the inner mitochondrial membrane and activity of components of the respiratory chain transporting electrons to oxygen. The respiration rate in state 3, besides the above-mentioned factors, is also limited by activity of H^+-ATP-synthetase and adenine nucleotide translocase, i.e., the enzyme system responsible for phosphorylation.

The state of the phosphorylating system in mitochondria can be estimated from their content of adenine nucleotides. The results indicate that the content of individual adenine nucleotides and their total pool in the mitochondria of animals infected with both pathogenic and apathogenic strains of viruses were at the level of the corresponding parameters for the control group of animals, namely: 1) ATP = 14.9 ± 1.9 nmoles/mg protein immediately after isolation of the mitochondria (when the total nucleotide content was 26.7 ± 5.9 nmoles/mg protein), 2) in highly energized state 4: ATP = 21.1 ± 2.9 nmoles/mg protein, ADP and AMP 3.9 ± 1.7 and 1.2 nmoles/mg protein respectively (when the total nucleotide content was 26.2 ± 2.7 nmoles/mg protein); 3) in active state 3, the content of ATP, ADP, and AMP was 19.0 ± 1.8, 4.6 ± 0.7, and 1.4 ± 0.4 nmoles/mg protein respectively (when the total nucleotide content was 24.9 ± 2.8 nmoles/mg protein). It can accordingly be concluded that no disturbances were present in the phosphorylating system of the mitochondria in the toxic form of influenza.

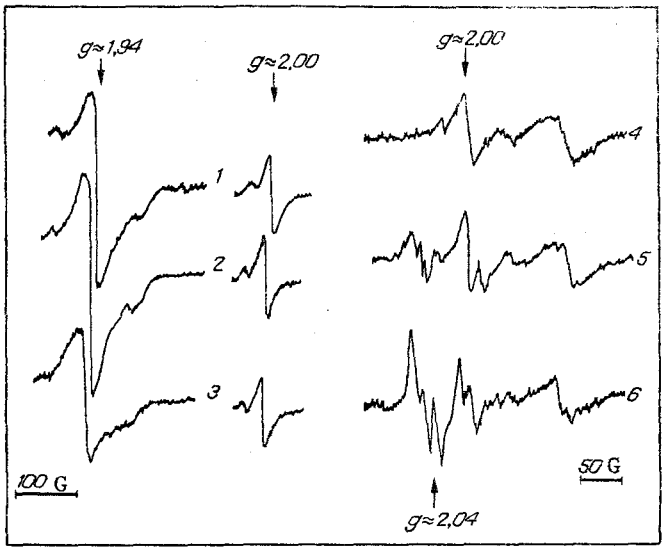

Fig. 2. EPR signals of iron-sulfur centers of mitochondrial electron-transport chain (g = 1.94), and of free radicals and mononitrosoyl complexes of iron with DETC (g = 2.03) in mouse liver. 1 and 4) Control animals; 2 and 5) animals infected with apathogenic virus; 3 and 6) animals infected with pathogenic virus.

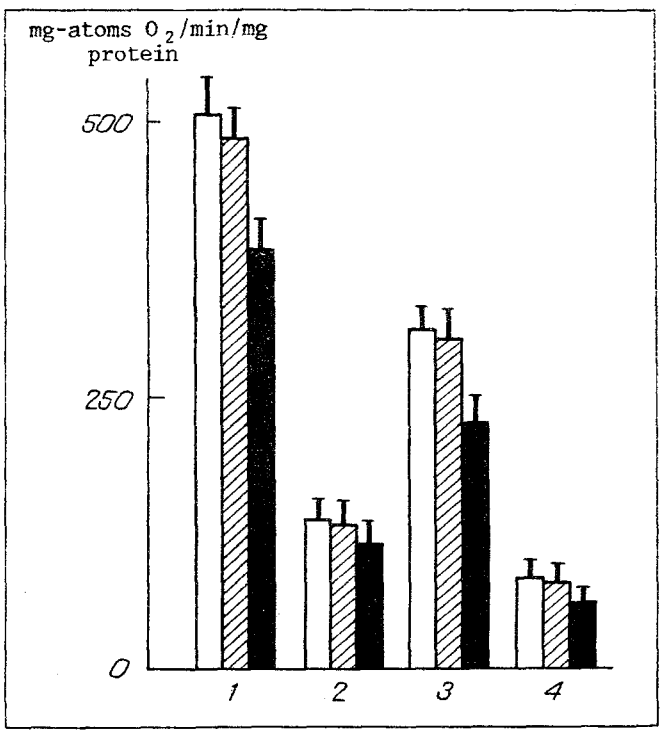

Fig. 3. Respiration rate of mitochondria in mouse liver (mg-atoms O_2/min/mg protein). 1) State 3 (substrate — succinate), 2) state 4 (substrate — succinate), 3) state 3 (substrate — glutamate + malate), 4) state 4 (substrate — glutamate + malate). Unshaded columns — control animals; obliquely shaded — animals infected with apathogenic virus; black columns — animals infected with pathogenic virus.

The results of assessment of the state of respiration in the mitochondria indicate that the proton permeability of the mitochondrial membranes remained at the control level, in the case of both apathogenic and pathogenic infection. In fact, the reduction of the respiration rate on the transition from state 4 to state 3 took place equally (whether succinate or glutamate was used together with malate) in mitochondria of the animals of the control group and of the groups of animals infected with apathogenic and pathogenic strains of the viruses (Fig. 3). Conversely, in the case of an increase in nonspecific proton permeability of the membranes during exposure to the damaging action of LPO, an increase in reversed compensatory proton transport would be expected and, as a result of that, an increase in the respiration rate in states 3 and 4.

On the basis of this analysis we are inclined to consider the cause of the decrease in the respiration rate of the liver mitochondria of mice with the acute toxic form of influenza to be changes in the protein components of the mitochondrial membrane responsible for electron transport or for transporting substrates inside the mitochondria. These changes may be caused by the accumulation of endogenous $^{\cdot}N=O$, capable of forming stable complexes with the hemic iron of the cytochromes [5], in the liver tlssue, modulating their redox potential. Evidence of accumulation of $^{\cdot}N=O$ was given by the appearance of the characteristic EPR signal of the N–O Fe^{2+} DETC complex (g = 2.041) (Fig. 2). It is suggested that Kupffer cells are the producers of $^{\cdot}N=O$ [6], when activated by virus antigen circulating in the blood stream.

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