ROLE OF PEROXIDES IN CHANGES IN ENERGY METABOLISM IN BRAIN MITOCHONDRIA EXPOSED TO BLOOD SERUM FROM PATIENTS WITH SCHIZOPHRENIA

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Clinical and experimental investigations have shown that the body fluids of patients with schizophrenia exhibit increased biological activity and give rise to changes in various forms of metabolism in test objects, which as a rule are present in schizophrenic patients also [3, 7, 9, 11]. Accordingly the body fluids and, in particular, blood serum and plasma and active components isolated from them, can be used to simulate *invitro* some of the pathochemical disturbances arising in mental diseases and to investigate some aspects of their pathogenesis.

The writer showed previously that blood serum from patients with schizophrenia and its ultrafiltrate cause depression of energy metabolism in unpurified cat brain mitochondria (MC) [5-9]. The degree and character of changes in oxidation and phosphorylation under the influence of blood serum depend on the type of treatment and on the form of the mental disease, and they increase as it progresses [6-8].

Functioning of the respiratory chain of MC is known to be closely linked with the formation and decomposition of peroxides, capable of inhibiting various mitochondrial and cytoplasmic enzymes [4]. According to the present writer's hypothesis, subsequently confirmed, one cause of the change in MCH function is the toxic action of peroxidation products which accumulate in MC if exposed to the action of blood serum from schizophrenic patients [6-9].

This problem was tackled by a method of inhibition analysis using substances decomposing peroxides or preventing their formation (antioxidants): propyl gallate (0.25 mM), 1,4-dithioerythritol (0.25 mM), reduced glutathione (2 mM), 1,4-dithioerythritol (9.25 mM), reduced glutathione (2 mM), catalase (1.5×10^{-4} mM), and diphenyl-p-phenylenediamine (0.25 mM). The antioxidants were added to the sample after the rate of respiration of MC in metabolic state 4 had been recorded by a polarographic method [14]. The test object consisted of MC from the cat cerebral cortex. The intensity of accumulation of peroxidation products also was judged from the rate of formation of malonic dialdehyde (MDA) in the test object [4]. The MC were isolated by the method of Ozawa et al. [15].

The low-molecular-weight fraction (mol. wt. under 10,000) was obtained from the serum by ultrafiltration through semipermeable filters (from LKB, Sweden, and Amicon, USA), and also by precipitation of proteins in an acid medium [8]. The experimental results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

Intact brain MC were found to have a high respiration rate in metabolic state 3: V_3 = 81.0 ± 2.3 natoms O_2/min•mg protein; the efficiency of phosphorylation ADP/O was 2.6 ± 0.02 nmoles ADP/natom O_2; the rate of phosphorylation (Vp) was 201.0 ± 4.8 nmoles ADP/min•mg protein; the respiratory control (RC = V_3/V_4) was 3.9 ± 0.1, where V_4 is the rate of respiration of the mitochondria in metabolic state 4. The substrate was a mixture of 10 mM glutamate with 5 mM malate. Under the influence of healthy human blood serum only ADP/O and Vp were reduced somewhat (by 11 and 15% respectively).

Blood serum from patients with episodic and continuous forms of schizophrenia (Fig. 1) caused various changes in the energy metabolism of MC, and depressed nearly all its indices by a greater degree than healthy human serum. The respiration rate in metabolic state 4 (V_4) was increased under these experimental conditions.

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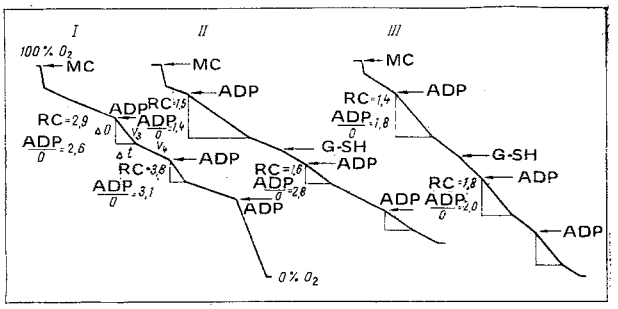

Fig. 1

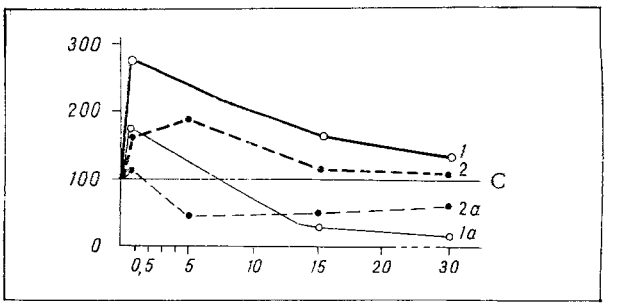

Fig. 2

Fig. 1. Effect of reduced glutathione (G-SH) on energy metabolism of mitochondria from cat cerebral cortex, when modified by exposure to blood serum from patients with different types of schizophrenia (polarographic record). Blood serum (0.037 liter/liter) added to sample before mitochondria. Concentration of ADP 150 μM, of G-SH 2 mM. RC) Respiratory control. I) Intact MC, II) action of blood serum from patients with continuous, and III) episodic forms of schizophrenia. Incubation medium contained (in mM): sucrose 170, KCl 30, Tris-buffer 20, P_{inorg} 10, EDTA 0.2, glutamate + malate 10 + 5 mM; V 0.8 ml; mitochondrial protein in sample 2-2.6 mg/ml, concentration of blood serum 0.037 liter/liter.

Fig. 2. Dynamics of accumulation (in % of control) of malonic dialdehyde in mitochondrial fraction of cat brain under the influence of blood serum from patients with schizophrenia and an ultrafiltrate of it. 1 and Ia) Action of ultrafiltrate and blood serum of patient with continuous schizophrenia; 2 and 2a) action of corresponding fractions of blood from patient with episodic schizophrenia. Concentration of blood fractions 0.037 liter/liter; incubation medium contained (in mM): KCl 0.175; Tris-HCl 0.025 (pH 7.4, 37°C). Control (C): incubation medium + mitochondrial brain fraction (1.4 mg protein in 1 ml medium). Abscissa, incubation time (in min); ordinate, accumulation of malonic aldehyde (in % of control, taken as 100%).

The antioxidants used in the investigation had virtually no effect on indices of energy metabolism in the fraction of intact MC during exposure to healthy human blood serum or serum from patients with episodic schizophrenia. In the latter case, the energy metabolism of MC was restored to normal only by catalase, a result which can evidently be attributed to the broad spectrum of action of this substance.

During exposure to blood serum from patients with more severe disturbances of mental activity (continuous schizophrenia) the antioxidant restored the normal efficiency of phosphorylation and increased or normalized the rate of phosphorylation. The respiratory control and respiration rate in metabolic states 3 and 4 remained unchanged in most cases.

These observations are evidence that the mechanism of the change in energy metabolism of MC when exposed to blood serum from patients with different types of schizophrenia is not always the same and that various pharmacologic agents are required to restore it to normal. In fact, as was stated previously [6, 7, 9], the main cause of depression of energy metabolism during exposure to blood serum from patients with episodic schizophrenia is an increase in activity of Mg- and Na^+,K^+-dependent ATPases, and normal metabolism is restored by inhibitors of these enzymes.

As was shown by a joint investigation with V. G. Khzardzhyan, antioxidants in the concentrations used in the present study to do not affect the activity of these enzyme systems. Only under the influence of glutathione-SH was Na^+,K^+-ATPase activity reduced to 36%.

It can be concluded from these results that the positive effect of antioxidants on the functioning of MC, modified by the action of blood serum from patients with continuous schizophrenia, was due chiefly to their normalizing influence on peroxidation.

As the mental disease progresses, the patients' serum thus induces a broad spectrum of disturbances of metabolism in MC, and peroxidation products accumulate in the test object in addition to changes in ATPase activity.

It is important to note that the combined use of antioxidants and ATPase inhibitors has the optimal action on metabolism of MC when modified through exposure to blood serum from patients with continuous schizophrenia [9].

Further evidence of the accumulation of peroxides in MC during exposure to blood serum from patients with continuous schizophrenia is given by data showing that under these experimental conditions the intensity of formation of MDA, a peroxidation product of thiobarbituric acid, is increased (Fig. 2). In the case of continuous schizophrenia, moreover, this process takes place twice as actively under the influence of an ultrafiltrate or "protein-free" fraction of serum compared with the effect of whole blood serum. Blood serum from patients with episodic schizophrenia had virtually no stimulating effect on MDA formation, but its low-molecular-weight fractions increased the intensity of its formation, but by a lesser degree than serum from patients with continuous schizophrenia. The experimental results are in agreement with those of clinical studies according to which most patients with schizophrenia are characterized by a low level of energy metabolism and an increase in the concentration of peroxides in the blood; under these circumstances the activity or concentration of endogenous antioxidants (catalase, peroxidase, glutathione, etc.) is as a rule reduced. This is a particulary pronounced feature of the disease if it follows a prolonged course without remissions [1, 10-13].

There is also evidence of the beneficial therapeutic action of certain antioxidants and metal chelating agents on patients with schizophrenia, accompanied by normalization of their blood energy metabolism indices [1, 2, 10-13]. However, the empirical use of these pharmacologic agents in some cases has not given a positive effect, possibly because of the absence of reliable biochemical criteria for their rational use in each concrete case. Accordingly information on the character of the change in metablism in the test object during exposure to active components of patients' serum, reflecting the character of the change in types of metabolism investigated in patients, may be useful as a prelude to the development of methods of pathogenetic, individually-chosen treatment of mental diseases and, in particular, for the rational choice of therapeutic agents with an appropriately directed biochemical action.

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