PRESERVATION OF FUNCTIONS OF LIVER MITOCHONDRIA IN RATS RESISTANT TO BLOOD LOSS UNDER LONG-TERM DEEP HYPOTENSION

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The view is held that liver mitochondrial function is rapidly disturbed in hemorrhagic shock induced by Wiggers' method, with stabilization of the blood pressure (BP) at 30-40 mm Hg. Uncoupling of oxidative phosphorylation has been found as early as 1.5-2 h after the beginning of blood loss, and it increased during the development of shock, the duration of which did not exceed 6 h [5, 10, 12].

By contrast with data in the literature, the present writers found a group of "long living" rats resistant to blood loss, and liver mitochondrial function was investigated in these animals in the course of a long period of deep arterial hypotension.

EXPERIMENTAL METHOD

The experimental situation was designed to take account of factors prolonging the course of shock. Experiments were carried out on noninbred (more resistant than inbred to blood loss) male albino rats weighing 230-330 g. The animals were given food and water ad lib., so that the liver glycogen reserves could be maintained and the duration of shock prolonged, although it did not prevent a lethal outcome [13]. According to our data the duration of shock in rats deprived of food for 18 h before the experiment was reduced by half. Under pentobarbital anesthesia (4 mg/100 g body weight) the animals were fixed in a frame and both femoral arteries were catheterized; one catheter was connected to a mercury manometer to measure BP, the other to a reservoir for the outflowing blood. About 500 U of heparin was injected intraarterially. The strict fixation was later eased, and the forelimbs were released. Hemorrhagic shock was induced by Wiggers' method in the modification of Bacalzo et al. [3], by gradual blood loss until BP reached 30-35 mm Hg, in the course of 30 min, by contrast with the technique cited, in which the same level of hypotension was reached in a shorter time (10 min). Oxidative phosphorylation in the liver mitochondria (MC) was studied in the initial period of circulatory decompensation (beginning of reinfusion of blood), during return of 70% of the blood (irreversible shock) and in the agonal state (after the last inspiration, but while the heart was still beating). Anesthetized rats, fixed and with their femoral arteries catheterized, served as the control. MC were isolated by differential centrifugation in medium containing 0.3 M sucrose and 2 mM Tris-HCl buffer (pH 7.45). Oxygen uptake was measured on the ZP-60a polarograph, with closed platinum electrode of Clark type. The respiratory control (RC), ADP/O ratio, and rate of phosphorylation [4] were calculated. Protein was determined by Lowry's method. The oxidation substrates used were α-ketoglutaric, glutamic, β-hydroxybutyric, and succinic acids. Mitochondrial ATPase activity was measured by a pH-metric method [9].

EXPERIMENTAL RESULTS

The number of "long-living" rats in the fall and winter in these experiments was about 70%, compared with 35% in the spring, and it depended on the batch of rats. Reinfusion of blood in this group of animals began on average 2.5 h, return of 70% of blood was observed 16.4 h, and the agonal state developed 20.6 h after the beginning of blood loss.

The maximal volume of blood loss amounted to 58.5 ± 1.0% of the circulating blood volume. As shock developed the rectal temperature fell gradually to 24.8 ± 0.6°C.

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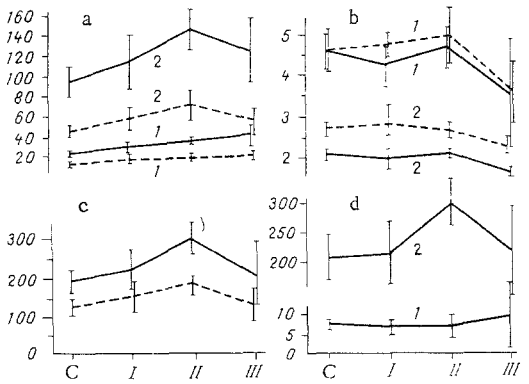

Fig. 1. Oxidative phosphorylation and ATPase activity of liver MC in course of hemorrhagic shock. Abscissa: C) control, I) beginning of reinfusion of blood, II) irreversible shock, III) agonal state. Ordinate: a) rate of oxygen uptake by MC (in μatoms/min/mg protein), 1) in state 4, 2) in state 3; b, 1) RC, 2) ATP/O; c) rate of phosphorylation (in μmoles ATP/min/mg protein) (a, b, and c, continuous lines indicate that substrate was α-ketoglutarate, broken lines – succinate); d) ATPase activity in nmoles H^+/min/mg protein, 1) latent ATPase, 2) DNP-stimulated ATPase. Incubation medium for measuring oxygen uptake: 0.1 M sucrose, 0.1 M KCl, 5 mM KH_2PO_4, 10 mM Tris-HCl buffer (pH 7.4), NAD-dependent substrates (10 mM of each), succinate 5 mM, ADP 300 μM. Incubation medium for measuring ATPase activity: 0.1 M sucrose, 0.1 M KCl, 2 mM Tris HCl buffer (pH 7.45), ATP 2 mM, 2,4-DNP 50 μM. Protein concentration in sample 2-3 mg/ml.

Oxygen uptake by the liver MC in state 4 during oxidation of α-ketoglutarate or succinate increased progressively as shock developed (Fig. 1). Oxygen uptake in state 3 increased to a maximum on reinfusion of 70% of the blood, after which it fell a little, but still remained on average higher than initially. In the irreversible phase of shock, activation of oxidative phosphorylation was found, as expressed by an increase in the rate of phosphorylation on account of intensitification of mitochondrial respiration in state 3, while the initial values of RC and ADP/O were preserved. In the agonal state, "mild uncoupling" [2] was observed, in which, despite some decrease in RC and ADP, ATP synthesis in unit time still remained within normal limits against the background of activation of respiration. Such a change in the functions of MC reflects a compensatory reaction directed toward maintaining ATP production once the disturbance of oxidative phosphorylation has begun. Changes in the above-mentioned parameters during oxidation of glutamic and β-hydroxybutyric acids were similar to those described for α-ketoglutarate. Activity of latent ATPase of liver MC did not change during the course of shock; in an agonal state only a tendency toward its increase could be observed. Activity of DNP stimulated ATPase increased in the irreversible phase of shock and returned to its initial level in an agonal state of the animals. Changes in ATPase activity and oxidative phosphorylation agreed completely in the corresponding phase of shock.

In acute hypoxic hypoxia oxidative phosphorylation in MC is undisturbed [1, 6, 7]. Acute hypoxia, even lethal, is considered not to injure MC either morphologically or functionally, so long as the blood flow in the organ is maintained [7]. However, if perfusion is disturbed the conditions are created for the appearance of several factors injuring the structure and function of MC (acidosis, activation of lysosomal enzymes, etc.) in a relatively short time [6-8]. The results provide new information on the possibility of preserving the functions of MC isolated from the liver, during the development of severe (30-35 mm Hg) and prolonged (20 h) hypotension, accompanied by a disturbance of perfusion of this organ and the development of hypoxia of circulatory type in it. As additional characteristics of the course of shock in these experiments it should be noted that the animals continued to survive on average for 2.3 h after reinfusion of all the lost blood (in the course of this time BP fell gradually from 30 to 0 mm Hg). Mechanisms maintaining the function of MC under these conditions are not yet clear. It is possible that hypothermia, which develops in the course of shock, plays a protective role.

The hypothesis that a causal connection exists between disturbance of energy formation in MC and the transition of shock into an irreversible phase has not yet been proved [11]. Activation of oxidative phosphorylation in MC observed in the irreversible phase of shock and maintenance of the initial rate of ATP synthesis until the animals were in an agonal state are evidence that damage to MC is not itself an essential factor causing the development of irreversibility in shock.

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PROTEOLYSIS OF NUCLEAR MATRIX PROTEINS OF THE RAT LIVER AND ZAJDELA'S ASCITES HEPATOMA

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Much attention is currently being paid to the study of the nuclear protein matrix. In particular, comparison of the protein profile of the nuclear matrix (NM) of normal and tumor tissue is of great interest. Studies of the protein profile of NM by electrophoresis in polyacrylamide gel containing sodium laurylsulfate has shown that strong macromolecular bands are present in the 100-200 kilodaltons region of hepatoma-27 and Zajdela's ascites hepatoma, which are absent or are weak in normal liver [1, 2, 8]. Some workers consider that differences in the electrophoretic profile of NM proteins of normal liver and hepatoma are due to unequal activity of nuclear proteinases [6, 7]. These workers state that proteinase activity in liver nuclei is higher than in tumors, and that because of this, macromolecular bands are weak on electrophoresis of liver NM. The use of proteinase inhibitors, such as phenylmethylsulfonyl fluoride (PMSF) and sodium tetrathionate, during isolation of nuclei and NM, according to their data, leads to a sharp increase in the yield of NM proteins and, what is very important, to disappearance of virtually all differences between hepatoma and normal liver [7]. However, the use of sodium tetrathionate was shown to cause the formation of disulfide bonds, as a result of which some nuclear proteins may be firmly bound with skeletal structures of the nucleus [11].

Data have been published on the action of chromatin-bound proteinases [3, 5, 9, 10, 12-14]. These proteinases act mainly on histones. Most of them belong to the class of serine proteinases, and they are very active at a high ionic strength (2 M NaCl). Proteolysis of histones was not observed at acid pH values and in the presence of EDTA, but this process was observed during prolonged incubation of chromatin (about 20 h).

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