MECHANISM OF DISTURBANCES OF ENERGY FORMATION IN ACUTE ISCHEMIA OF THE LIVER

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Respiration of rat liver mitochondria after the addition of ischemic toxin to the incubation medium was compared with respiration of mitochondria isolated from ischemic rat liver. The changes in respiration in both cases could be prevented by preliminary addition of dithiothreitol and reduced by subsequent addition of cytochrome c or dithiothreitol to the incubation medium. The similarity between the mechanisms of disturbance of energy formation is postulated.

KEY WORDS: *mitochondria; ischemic toxin; ischemia of the liver; cytochrome c; dithiothreitol.*

Ischemic toxin (IT) of protein nature, isolated from ischemic organs and tissues, has marked biological activity [1, 4, 6]. It affects the structure and function of the liver mitochondria by interacting with the thiol groups of the mitochondrial membrane [2, 3]. It was important to compare changes in the rate of O_2 uptake by the mitochondria after addition of IT in vitro with disturbances arising in acute ischemia of the liver in vivo.

EXPERIMENTAL METHOD

Liver mitochondria were isolated from Wistar rats by differential centrifugation in a mixture of: 0.25 M sucrose, 5 mM Tris-HCl, pH 7.4, 1 mM EDTA. The rate of O_2 uptake was recorded polarographically. Acute ischemia of the liver was produced by ligation of the hepatic artery and portal vein for 1 h. The content of ATP, ADP, and AMP [7], protein [11], and cytochrome P-450 (by means of the Hitachi-356 spectrophotometer [9]) in the tissue was determined and benzopyrene metabolism was studied [10].

EXPERIMENTAL RESULTS

Treatment of the mitochondria with IT considerably accelerated respiration (Fig. 1a, b). Addition of cytochrome c under these conditions restored the original velocity of electron transport in state 3. Dithiothreitol (DTT) prevented the acceleration of respiration by IT. Addition of chlorocarbonylcyanide phenylhydrazone (CCP), just as in control experiments, accelerated respiration considerably (Fig. 1c). Addition of DTT after IT reduced the oxidation of substrate in state 4 to near-control values (Fig. 1d).

The content of ATP and ADP in the ischemic liver was reduced whereas the AMP content was increased; the total content of adenine nucleotides was reduced (Table 1).

The ADP-dependent respiration of mitochondria isolated from the ischemic organ was lower than in the control (Fig. 2a, b). Incubation of the mitochondria of the ischemic tissue with carnitine (Fig. 2c) did not increase the rate of respiration in response to addition of ADP. Addition of CCP to the incubation medium caused equal acceleration of respiration in mitochondrial preparations from the intact and ischemic liver (Fig. 2c). Incubation of the mitochondria of the ischemic liver with DTT on the addition of ADP at once con-

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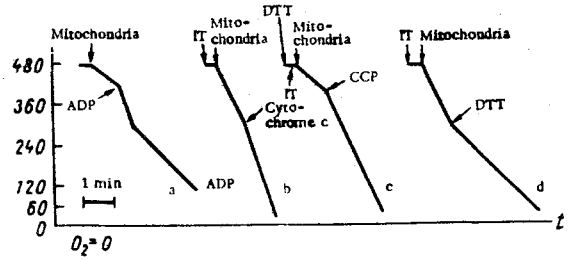

Fig. 1

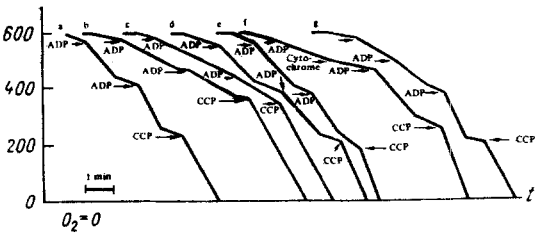

Fig. 2

Fig. 1. Effect of IT on O_2 uptake by liver mitochondria in vitro. Medium: 125 mmoles sucrose, 50 mmoles KCl, 10 mmoles Tris-HCl, pH 7.4, 100 μmoles ADP, 5 mmoles succinate, 3 mg mitochondria, 1 μg IT (as protein), 50 μliters DTT, $3 \cdot 10^{-7}$ mole CCP, 10 μmoles cytochrome c. a) O_2 uptake by intact mitochondria; b) effect of IT on respiration of mitochondria; c, d) interaction between IT and DTT.

Fig. 2. O_2 uptake by mitochondria isolated from ischemic liver. Medium: 100 mmoles sucrose, 50 mmoles KCl, 10 mmoles Tris-HCl, pH 7.4, 10 mmoles succinate, 3 mg mitochondria, 150 μmoles ADP, $3 \cdot 10^{-7}$ mole CCP, 50 μliters DTT, 10 μmoles cytochrome c, 0.5 mmole carnitine, 5 μliters of 0.1% solution of bovine serum albumin. a) Mitochondria of intact liver; b) mitochondria of ischemic liver; c) incubation of ischemic mitochondria with carnitine; d) incubation of ischemic mitochondria with DTT; e) incubation of ischemic mitochondria with bovine serum albumin and DTT; f) addition of cytochrome c to incubation medium; g) addition of DTT during experiment.

TABLE 1. Content of Adenine Nucleotides (in nmoles/g) in Rat Liver (M ± m)

Tissue	ATP	ADP	AMP	Total adenine nucleotides
Intact	1846±68,72	1162±35,17	457,7±44,37	3467±59,52
Ischemic	296,2±28,68	245,8±40,15	1348,2±59,52	1885±98,38

*P < 0.05.

verted the respiration from state 4 into the state of active respiration (Fig. 2d); meanwhile their incubation with bovine serum albumin and DTT changed respiration of the mitochondria only slightly (Fig. 2d, e). A marked effect was observed after the addition of cytochrome c and, in particular, of DTT to the incubation medium during the experiment: The velocity of succinate oxidation rose sharply until the state of active respiration in the presence of ADP (Fig. 2f, g).

A decrease in 3,4-benzopyrene metabolism (from 0.078 to 0.045 nmoles/mg P/min; P < 0.05) was observed in the supernatant obtained after isolation of the mitochondria of the ischemic liver, although the cytochrome P-450 content was virtually unchanged.

Addition of IT thus changes the structure and function of the mitochondrial system by disturbing ATP formation; this also arises in vivo during acute ischemia of the liver. In both cases this effect is evidently mainly due to an increase in permeability of the mitochondrial membranes and a decrease in the rate of oxidative phosphorylation; addition of cytochrome c in fact restored the velocity of electron transport before state 3.

Agents capable of binding thiol groups of the mitochondrial membranes probably play an important role in the mechanisms of the changes in energy formation in ischemic tissues. One such factor may be IT, for its effect is reduced or prevented by DTT, which restores the functional activity of thiol groups. The fact that carnitine and serum albumin had no effect on ADP-stimulated respiration is evidence that its inhibition is not due to inhibition of adenine-nucleotide translocase by acyl derivatives of fatty acids [5, 8]. Metabolism of the typical substrate of the hydroxylating system was reduced in the supernatant fraction containing microsomes and soluble structures in acute ischemia of the liver, although the cytochrome P-450 content was not reduced under these circumstances.

Acute ischemia of the liver thus leads to sharp disturbances of intracellular energy formation, similar in character to those arising under the influence of IT on mitochondria in vitro. This suggests that the changes observed have the same mechanism.

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