CHANGES IN RAT LIVER MITOCHONDRIA AFTER BILATERAL SUBDIAPHRAGMATIC VAGOTOMY

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Changes in the liver mitochondria of rats after bilateral subdiaphragmatic vagotomy were studied . Two stages were distinguished in the dynamics of the response of the mitochondrial system to denervation. During the first stage (0.5-3 days after vagotomy) reversible functional disturbances due to postoperative stress took place in the mitochondria. The second stage (7-60 days after denervation) is characterized by more marked structural and functional changes with some common features with those observed in hypoxia and resulting from vagotomy itself.

KEY WORDS: *mitochondrion; liver; vagotomy.*

Different types of vagotomy combined with drainage operations are used at the present time in clinical surgery for the operative treatment of gastric and duodenal ulcer [5]. The discovery of the mechanism of action of vagotomy on the cell is thus of practical as well as theoretical interest.

The object of this investigation was to study the state of the mitochondrial system of the rat liver after bilateral subdiaphragmatic vagotomy.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 150-180 g were used. Bilateral subdiaphragmatic vagotomy (experiment) and laparotomy (control) were performed as described by Kirshenblat and Chigrina [2]. The rats were decapitated 0.5, 1, 3, 5, 7, 14, 28, and 60 days after the operation, always in the morning. Mitochondria were isolated from the liver by Schneider's method [9]. The state of oxidative phosphorylation was assessed on the LP-7 polarograph, using an open steady-state platinum electrode. Solubilization of cytochrome c from the mitochondria was determined by the TMPD* oxidase test [3]. The investigation of the Ca^{2+}-accumulating power of the mitochondria was carried out with tetracycline as a fluorescent probe, by the method described earlier [7]. Slowing of the mitochondria was recorded on the SF-16 spectrophotometer at 520 nm. Protein was determined by Lowry's method [8]. The significance of the results was determined by the Fisher–Student criterion ($P \leqslant 0.05$).

EXPERIMENTAL RESULTS

In the experimental and control groups similar changes were observed in the mitochondria during the 3 days after the operation, namely a decrease in the respiratory control (RC) [6] (Fig. 1a). Uncoupling of oxidative phosphorylation at these times was mainly connected with an increase in the rate of oxidation of substrates in state 4. No serious structural reorganizations were observed in the mitochondria during the first day after the operation: The TMPD oxidase test showed absence of solubilization of cytochrome c into the cytoplasm

*TMPD: Abbreviation for tetramethylphenylenediamine.

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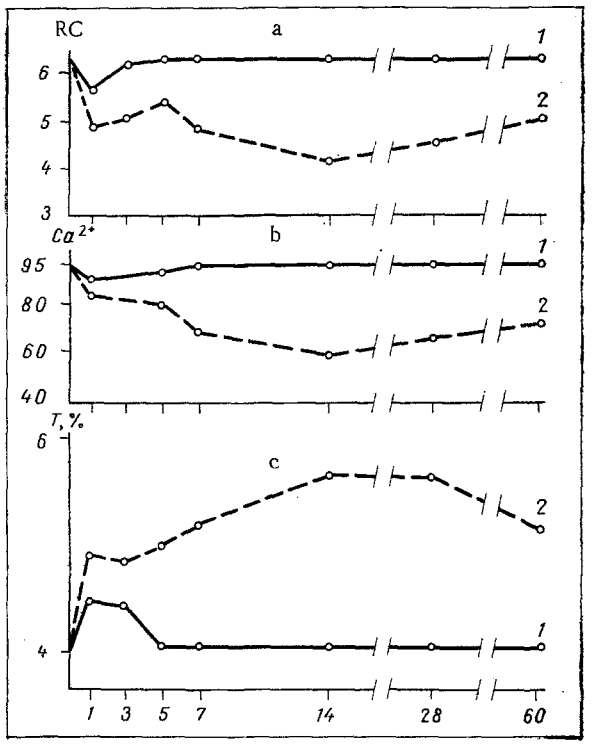

Fig. 1. State of mitochondrial system of rat liver after laparotomy (1) and vagotomy (2). a) RC of rat liver mitochondria at different times after operation. Incubation medium: 0.15 M sucrose, 0.075 M KCl, 5 mM phosphate buffer (pH 7.4), 2.5 mM $MgCl_2$. Protein concentration of sample 3-4 mg/ml. Oxidation substrate: mixture of glutamate (5 mM) and malate (5 mM). Final ADP concentration 200 μM. b) Ca^{2+}-accumulating power of rat liver mitochondria (in nmoles/mg protein) at different times after operation. Incubation medium 0.25 M sucrose, 10 mM Tris buffer (pH 7.40. Protein concentration in sample 5-6 mg/ml. Oxidation substrate succinate (10 mM). c) Light transmittance (T) of suspension of rat liver mitochondria at different times after operation. Abscissa, time (in days).

and the Ca^{2+}-accumulating power of the mitochondria in the experimental and control animals showed no significant change from normal (Fig. 1b). There was only very slight swelling of the mitochondria, as revealed by a change in the scattering of light by the suspension (Fig. 1c).

The similarity between the dynamics and character of the functional disturbances of the mitochondrial system in the experimental and control groups indicates that changes in the experimental animals compared with the control were nonspecific. The changes taking place during the 3 days after the operation were evidently the result of a general stress reaction.

The state of the rat liver mitochondria 3-5 days after laparotomy was completely restored to normal, but after vagotomy it was only partially restored (Fig. 1).

At the end of the first and beginning of the second week after denervation processes leading to a secondary decrease in RC took place in the hepatocytes (Fig. 1a) as the result of a decrease in the rate of oxygen utilization in state 3 (70% of normal during oxidation

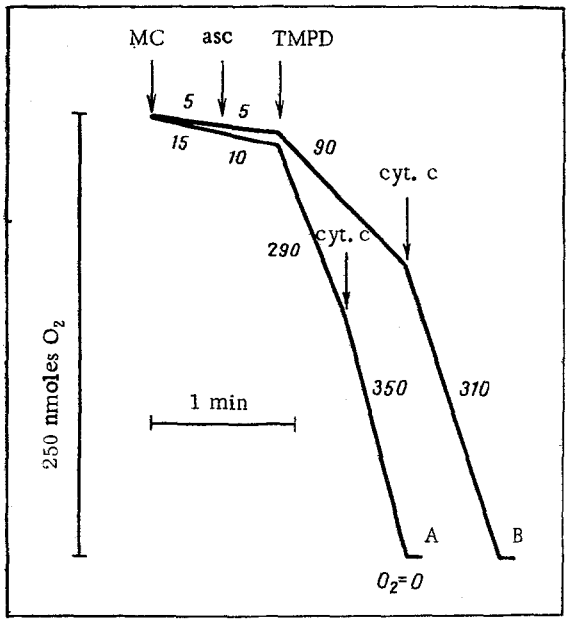

Fig. 2. Effect of cytochrome c on rate of oxidation of TMPD in mitochondria (MC) isolated from rat liver 14 days after laparotomy (A) and vagotomy (B). Incubation medium and protein concentration in sample as in Fig. 1a. Additions: asc) ascorbate (10 mM); TMPD (2 mM); cyt, c) cytochrome c (10 μM). Numbers alongside curves denote rate of oxygen utilization (in nmoles/mg protein/min).

of a glutamate + malate mixture) and an increase in the rate of respiration in state 4. The inhibition of the rate of oxygen utilization in state 3 can be explained by osmotic swelling of the mitochondria and the liberation of cytochrome c into the cytoplasm [3, 4]. This hypothesis was confirmed by the increase of 3.5 times in the rate of oxidation of TMPD when cytochrome c was added to the incubation medium (Fig. 2) and the swelling of the mitochondria observed 14 days after vagotomy (Fig. 1c). Another factor closely connected with the first mechanism of disturbance of oxidative phosphorylation under these experimental conditions was possibly activation of phospholipase A_2 [10], as judged from the significant decrease in Ca^{2+}-accumulating power [7] 14 days or more after vagotomy (Fig. 1b).

The character of the changes in the mitochondrial system remained the same 28 and 60 days after denervation, but compared with the picture after 2 weeks the degree of compensation was very small (Fig. 1).

The changes in the mitochondria discovered 14, 28, and 60 days after vagotomy were evidently the result not only of the direct action of denervation, but also of several closely interconnected nonspecific factors. It is therefore impossible to judge with certainty the mechanism of development of these changes. However, it can tentatively be suggested that one of the most important stages in the pathogenesis of the disturbances of the mitochondria after vagotomy is chronic hypoxia developing as a result of circulatory disturbances in denervated organs [1]. This hypothesis is confirmed by results obtained by other workers [3, 7].

Two stages can accordingly be distinguished in the dynamics of development of the disturbances in the mitochondrial system of the rat liver after vagotomy. The first (the first 3 days after vagotomy) is characterized by reversible functional disturbances in the mitochondria similar to those observed after laparotomy. During the second state (7-60 days after vagotomy) more marked structural and functional disturbances arise as a result of the vagotomy itself, and showing some common features with the changes observed in hypoxia.

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EFFECT OF CHRONIC CARBON TETRACHLORIDE POISONING ON THE TURNOVER OF RNA FRACTIONS IN RAT LIVER TISSUE

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Changes in the content and incorporation of 5-^{3}H-uridine after brief exposure to its labeled precursor were studied in the individual liver RNA fractions of rats during administration of carbon tetrachloride for 24 weeks. These fractions were obtained by preparative electrophoresis in 2.5% polyacrylamide gel from previously isolated nuclear and cytoplasmic RNA. Administration of CCl_4 to rats was shown to reduce the quantity of transfer and ribosomal RNA in the liver tissue. Chronic CCl_4 poisoning also disturbs the synchronization of the turnover of the individual components of fast-labeled RNA.

KEY WORDS: *carbon tetrachloride; liver; RNA metabolism.*

Administration of carbon tetrachloride (CCl_4) to rats is a widely used model of nonspecific toxic injury to the liver [6-8]. During the chronic action of this compound septal fibrosis and cirrhosis of the liver develop in animals of several species [1, 6]. However, the mechanism of action of CCl_4 is not clear and the biochemical changes taking place under these circumstances have been inadequately studied.

The object of this investigation was to study changes in the concentration and intensity of incorporation of a labeled precursor into individual RNA fractions of rat liver tissue during chronic administration of CCl_4.

EXPERIMENTAL METHOD

A subcutaneous injection of CCl_4 in a dose of 1 ml/kg body weight was given twice a week to noninbred male albino rats weighing 120-150 g. The liver of these animals was investigated between 4 and 24 weeks after the beginning of CCl_4 poisoning. At the end of

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