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INFLUENCE OF TRACHEA STENOSIS ON FUNCTIONING OF POLY-ENZY MEMBRANE MITOCHONDRIA LIVER SYSTEMS

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ABSTRACT

The aim. A comparative study of activity of multienzyme systems of respiratory chain of mitochondria of liver, which have different sensitivity to hypoxia in conditions of moderate tracheal stenosis.

Material and methods. 50 outbred rabbits weighing 2.3-2.5kg were used. Stenosis was created by applying a wide silk ribbon to trachea and narrowing the lumen from 1/3 to 2/3 [8]. Degree of narrowing was determined directly during slaughter of animals, as well as by the nature of the spirogram, clinical signs and by measuring the lumen of stenotic section of trachea relative to original using tracheoscopy. Rabbits were killed by decapitation after 3rd, 7th and 15th day of the experiment and physiological, biochemical and biophysical parameters of blood and liver were determined.

Results. Pre incubation at 37°C, NADH-oxidase activity of mitochondria isolated from liver of normal rabbits increases markedly and this high level of activation persists for 6 hours. At the same time, with a 7-day tracheal stenosis, activity of NADH-oxidase undergoes degradation. However, with an increase in duration of tracheal stenosis, activity of NADH-oxidase is restored almost completely to normal level. Thus, disturbances in energy metabolism with moderate tracheal stenosis actually begin in the NAD-dependent section of the respiratory chain and, with an increase in the duration of exposure, extend to the succinate oxidase section.

Conclusion. An increase in the activity of poly-enzymatic respiratory chain systems with moderate tracheal stenosis is associated not with an increase in the synthesis of these enzymes, but with an improvement in the access of substrates (NADH, succinate, cytochromes) to the active center of the enzyme system as a result of minor damage to the inner mitochondrial membrane. This is manifested in a violation of the activity of poly-enzymatic systems of the...
respiratory chain and access of exogenous cytochromes to phospholipids and proteins of mitochondrial membranes, as well as a decrease in their stability when exposed to endogenous and exogenous phospholipases and proteases, leading to disruption of phospholipid-protein and phospholipid-phospholipid interactions of the inner membranes.

**Keywords:** liver, mitochondria, NADH-oxidase, succinate oxidase, cytochrome oxidase, cytochrome.

**INTRODUCTION**

Problem of tracheal stenosis is one of the most relevant modern biomedical sciences, both because of large absolute number of diseases and because of the variety of causes that cause this pathology. The cause of stenosis can be malignant and benign tumors, mechanical injuries, gunshot wounds, severe combined injuries, goiter gland hypertrophy, struma formation, infectious diseases, etc. [1], however, in our opinion, due attention is not paid to analysis of state of homeostasis of body. Narrowing lumen of the respiratory tract leads to a violation of function of external respiration and, as a result, to the development of hypoxia. This, in turn, leads to development of respiratory failure and bioenergetic disorders at various levels of body [2,3]. The end result of the development of hypoxia in tracheal stenosis is a violation of activity of enzymes of respiratory chain of mitochondria, therefore, study of the mechanism of action of oxygen starvation should primarily be aimed at studying activity of enzymes in mitochondria.

**MATERIALS AND METHODS**

50 outbred rabbits weighing 2.3-2.5kg were used. All animals are divided into two groups (each group of 10 animals): first control; second - with stenosis of trachea. To create tracheal stenosis and false tracheal stenosis 60 minutes before start of the experiment, rabbits were intramuscularly injected with 1% morphine solution at a dose of 3.5-4 ml / kg body weight. Then animal was fixed on an operating table and was administered internally 1% solution of hexonal at a dose of 30 mg/kg of body weight. Next, a skin incision was made along the midline of neck from the jugular notch of the sternum upward, 4-5 cm long. The cervical trachea was exposed in layers.

Stenosis was created by applying a wide silk ribbon to trachea and narrowing the lumen from 1/3 to 2/3 [4]. Degree of narrowing was determined directly during slaughter of animals, as well as by the nature of the spirogram, clinical signs and by measuring the lumen of stenotic section of trachea relative to original using tracheoscopy. Rabbits were killed by decapitation after 3rd, 7th and 15th day of the experiment and physiological, biochemical and biophysical parameters of blood and liver were determined.

Mitochondria were isolated from rabbit liver using generally accepted method of differential centrifugation [5] with some modifications [6].

Mitochondria were frozen at -15°C and, after thawing, NAD.H-, succinate, and cytochrome c-oxidase activities were measured by adding 1ml 3 µmol of NAD.H, 10 µmol of succinate and 20 µmol of ascorbate + 1mg of cytochrome c to the polarograph cell. If necessary, 0.4 mg of cytochromes was added during the oxidation of NAD.H or succinate [7]. All enzymatic activities were expressed as ngatom of oxygen consumed in 1 min at 25 °C per 1 mg of mitochondria protein. Measurement medium: 0.66 M sucrose containing 50 mM Tris-HSI buffer pH 7.4 and 5 mM histidine.

If necessary, mitochondria were heat-treated in 10 mMtris-HCl buffer, pH 7.4, containing 0.25 M sucrose at 37°C. Effect of phospholipase A2 and trypsin on the activity of polyenzymatic systems of mitochondrial membranes. Phospholipase A2 - 0.006 μg mg of protein - for succinate oxidase, 0.03 μg mg of protein - for cytochrome oxidase) at 20 °C and through certain time intervals aliquots were selected to measure the activity of polyenzyme systems [8]. Protein-specific [9].
RESULTS AND ITS DISCUSSION

Activity of polyenzyme systems of membranes of mitochondria of liver of rabbits with tracheal stenosis. We have shown that in case of tracheal stenosis, the disturbances begin on its substrate section and again NAD.H-dependent oxidation pathway is first to suffer.

It is seen that the magnitude of succinate oxidase and cytochrome oxidase activity does not change with a 7-day stenosis of the trachea, however, it significantly increases on the 15th day of the experiment (Fig. 3.1 a, c). In contrast to these enzymes, NAD.H-oxidase activity increases with a 7-day trachea wall, and on the 15th day of the experiment returns to normal (Fig. 1 b).

All this indicates that at the initial stage of moderate tracheal stenosis, the warehouse of the main supplier of energy substrates for the respiratory chain and liver tissue - NAD - dependent oxidation is enhanced. Thus, impaired energy metabolism and liver cell function with moderate tracheal stenosis begin with an acceleration of the electron transport function in the NAD - dependent section of the respiratory chain. These disorders are absent if the supplier of reducing equivalents for the respiratory chain is the succinate oxidase oxidation pathway and the final portion of the respiratory chain is the cytochrome oxidase oxidation pathway. With an increase in the duration of tracheal stenosis, a violation of the electron transport function extends to the remaining sections of the respiratory chain of mitochondria. Therefore, adaptation to oxygen starvation is accompanied by a reciprocal switch of NAD.H-oxidase and succinate oxidase systems of the mitochondrial respiratory chain, which depend on the duration of moderate tracheal stenosis. Apparently, switching the aforementioned oxidase systems of the respiratory chain of the liver mitochondria somehow ensures the functional integrity of the liver cells of animals under moderate tracheal stenosis.

Fig.1. The activity of polyenzyme systems of membranes of mitochondria of the liver of rabbits with moderate tracheal stenosis.

We previously showed [10] that usually activation of membrane-bound enzymes is associated with improved access of substrates to the active center of the enzyme system and is observed with small degrees of membrane damage, and inactivation is associated with deep degradation of phospholipids and membrane proteins, usually under the action of endogenous lytic enzymes. Therefore, in the study of tracheal stenosis, it is very important to study the
change in the activity of the poly enzymatic mitochondrial systems in time, i.e. under the conditions of functioning of endogenous phospholipases and proteases, which are present in the composition of mitochondrial membranes. Studies have shown that in mitochondria of the liver of normal and experimental rabbits, at 37°C, succin oxidase activation phase is observed only at the beginning of incubation, and then the inactivation phase is better manifested, especially significantly with a 15-day tracheal stenosis (Fig. 1 g). If activity of succinate oxidase at 37°C in the control decreases by half within 390 minutes, then with 7 and 15-day tracheal stenosis, this requires, respectively, 350 and 170 minutes.

As a result of pre incubation at 37°C, NAD.H-oxidase activity of mitochondria isolated from liver of normal rabbits increases markedly and this high level of activation persists for 6 hours (Fig. 1e, curve 1). At the same time, with a 7-day tracheal stenosis, activity of NAD.H-oxidase undergoes degradation (Fig. 1e, curve 2). However, with an increase in duration of tracheal stenosis, activity of NAD.H-oxidase is restored almost completely to normal level (Fig. 1e, curve 3). Thus, disturbances in energy metabolism with moderate tracheal stenosis actually begin in the NAD-dependent section of the respiratory chain and, with an increase in the duration of exposure, extend to the succinate oxidase section.

Incubation of mitochondria of liver of normal rabbits at 37 °C for 6 hours does not affect activity of cytochrome oxidase (Fig. 1f, curve 1). A slight decrease in activity of mitochondrial cytochrome oxidase system at 37°C is observed only on the 15th day of tracheal stenosis (Fig. 1f, curve 3). Analyzing the results, we can conclude that with moderate tracheal stenosis, there are two types of disturbances in the structure of the membranes of the mitochondria of the liver, noted with other types of pathology and stressful effects. One of them is manifested in a change in the structural conjugation between separate parts of the respiratory chain, and the second is due to the degradation of the entire respiratory chain, including the more resistant to the action of endogenous lytic enzymes cytochrome oxidase.

**Cytochrome-dependent activity of NAD.H-oxidase and succinate oxidase mitochondria of the liver with tracheal stenosis.** Disorders in an inner membranes associated with changes in phospholipid-protein interactions significantly alter the ability of the membranes of respiratory chain mitochondria to accept exogenous cytochromes [11]. This is manifested in increase in NAD.H-oxidase and succinate oxidase activity observed upon introduction into the reaction. exogenous cytochromes medium.

![Fig. 2. Cytochromes dependent activity of NAD.H-oxidase (a, c) and succinate oxidase (b, d) of rabbit liver mitochondria with tracheal stenosis.](image)

a, b - increase in activity ($\Delta A_0$) of polyenzymatic systems of the respiratory chain of cytochromes before incubation; c, d - at 37°C ($\Delta A$).

Incubation conditions and designations, as in Fig. one.
These characteristics are very sensitive to the formation of “hidden damage” to mitochondrial membranes and change even with small degrees of damage, which can therefore serve as a reliable test indicating the state of mitochondrial membranes. In this regard, we studied the interaction of cytochrome mass mitochondrial membranes under various incubation conditions. From the results shown in Fig. 2 a, b, it follows that the values of $\Delta A$, reflecting changes in the respiration rate after adding cytochromes to the polarographic cell, are different in normal and experimental animals. On the 7th day of tracheal stenosis, an increase in the activity of NAD.H-cytochromes oxidase increases to some extent (Fig. 2a), and $\Delta A$ of succinate oxidase increases sharply (Fig. 2 b). With an increase in the term of tracheal stenosis, the opposite picture is observed: $\Delta A$ NAD.H-oxidase increases, and the inclusion of cytochromes, in relation to the succinate oxidase system, decreases compared with earlier periods of tracheal stenosis (Fig. 2 a, b). Incubation of mitochondria at 37°C leads to a decrease in the increase in the activity of NAD. H-oxidase cytochromes both in the norm and in the experiment (Fig. 2c, curves 1, 2). With an increase in the duration of tracheal stenosis (day 15), $\Delta A$ NAD.H-oxidase increases significantly (Fig. 2c, curve 3). Incubation of the mitochondria of the liver of intact animals at 37°C at the beginning of incubation, an increase in the activity of succinate oxidase cytochromes is sharply activated (this process rises by about 4 times within 1 hour, and then falls). Under these incubation conditions, the increase in the activity of succinate oxidase cytochromes of the mitochondria of the liver on the 7-day tracheal stenosis decreases at a high rate, and on the 15th day of the tracheal stenosis the activation phase is observed after the lag period, however, it does not reach the normal level (Fig. 2 g, compare curves 1-3). Thus, with moderate tracheal stenosis, the ability of the inner mitochondrial membrane of the liver to accept exogenous cytochromes is significantly impaired: at the initial stage of tracheal stenosis, $\Delta A$ succinate oxidase decreases sharply, and NAD.H oxidase does not change, with an increase in the duration of the experiment, the inclusion of cytochromes in the respiratory chain mitochondria is restored. A significant increase in $\Delta A$ NAD.H-oxidase is associated with profound disturbances in the NAD-dependent pathway of the mitochondrial respiratory chain.

**The inactivating effect of lytic enzymes on the electron transfer functions in isolated liver mitochondria is normal and with tracheal stenosis.** A greater susceptibility to degradation of polyenzyme systems of mitochondrial membranes under the action of endogenous lytic enzymes at 37°C is usually associated with disturbances in the structural relationships between phospholipids and mitochondrial membrane proteins. Phospholipids and mitochondrial membrane proteins from organs of intact animals are usually less affected by phospholipases and proteases, and mitochondria from animal organs with pathology or after exposure to stress factors lose their functional characteristics faster when these enzymes act [12]. A study of the effect of phospholipase A2 and trypsin on the activity of the polyenzyme systems of the liver mitochondria of control and experimental animals also confirms our assumptions. So, if the activity of succinate oxidase in the presence of phospholipase A2 in the control decreases by half within 57 minutes, then in animals with 7-day tracheal stenosis this time is reduced to 37 minutes, and with a 15-day experiment - 18 minutes. (Fig. 3 a). Accelerated inactivation is also observed for cytochrome oxidase mitochondria of the liver of experimental rabbits under the influence of phospholipase A2 (Fig. 3 c). So, if the activity of the cytochrome oxidase system of the liver mitochondria in the presence of phospholipase A2 in the control does not change for 30 min, then with 7 and 15-day tracheal stenosis during this time, the activity decreases by 32 and 20 percent. Similar data were obtained with the proteolytic enzyme trypsin. In the presence of trypsin, the succinate oxidase system of the liver mitochondria of normal animals does not change the initial activity, and activation is observed with a 7-day tracheal stenosis (Fig. 3 b). With an increase in the duration of tracheal stenosis, the stability of this system is significantly reduced and, from the very first minutes of incubation, a decrease in succinate oxidase activity...
occurs. Similar data were obtained when studying the activity of the mitochondrial cytochrome oxidase of the liver in control and experimental rabbits (Fig. 3).

**The effect of phospholipase A2 and trypsin on the activation of polyenzymatic systems of the respiratory chain of mitochondria cytochrome.** We have previously shown that lytic enzymes act primarily on the sites responsible for the incorporation of exogenous cytochromes into membranes, and this process is controlled by calcium ions [13]. In fig. Figure 4 shows that pretreatment of the control and experimental mitochondria with phospholipase A2 leads to an increase in the activity of the succinate oxidase cytochromes system to zero, both normal and with tracheal stenosis at the beginning of incubation, and then this process is restored (Fig. 4 a). If vnorm ΔA reaches the initial level, then with tracheal stenosis, complete recovery is not observed and in further incubation the inactivation process is observed both in normal and in experience.

However, the increase in the activity of succinate oxidase cytochromes of experimental animals proceeds much faster (Fig. 4a, compare curves 1-3). When trypsin is added to normal mitochondrial suspension, an increase in the increase in the succinate oxidase cytochrome system is observed (Fig. 4b, curve 1). In this case, the activation phases (ΔA / ΔA0 increase more than 4 times over 60 minutes) and inactivation (prolonged incubation reduces the effect of cytochromes) are easily distinguished. In contrast to the norm, on the 7th and 15th day of the experiment, the activation phase ΔA is also observed, however, it does not reach the norm level.

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**Fig. 3.** The effect of phospholipase A2 (a, c) and trypsin (b, d) on the activity of polyenzyme systems of mitochondrial membranes. a, b - succinate oxidase; c, d - cytochrome oxidase. Trypsin was added on a suspension of mitochondria - 120 μg mg of protein. Phospholipase A2 - 0.006 μg mg protein - for succinate oxidase, 0.03 μg mg protein - for cytochrome oxidase, A0-activity of the respiratory chain of mitochondria not exposed to lytic action, A - after treatment with phospholipase A2 and trypsin. Incubation temperature at 200C. Designations as in fig. one.

**Fig. 4.** The effect of lytic enzymes on the incorporation of exogenous cytochromes into mitochondrial membranes. a, b - an increase in the succinate-oxidase system when cytochromes is added, treated with phospholipase A2 (0.06 μg mg protein) and trypsin (120 μg mg protein). Temperature - 20°C. Designations as in fig. one.
(Fig. 4 b, curves 2 and 3). Thus, with moderate tracheal stenosis, the inclusion of exogenous cytochromes in the inner mitochondrial membrane is significantly weakened [14].

Analyzing the foregoing, we can conclude that with moderate tracheal stenosis, impaired energy metabolism begins in the NAD-dependent section of the respiratory chain and, with an increase in the duration of exposure, first spreads to the succinate oxidase section and then to the cytochrome oxidase section of the liver mitochondrial respiratory chain [15]. This is manifested in a violation of the activity of polyenzymatic systems of the respiratory chain and access of exogenous cytochromes to phospholipids and proteins of mitochondrial membranes, as well as a decrease in their stability when exposed to lytic enzymes leading to disruption of phospholipid-phospholipid and phospholipid-protein interactions of mitochondrial membranes. These facts indicate that with moderate tracheal stenosis, rather profound disturbances are observed in the electron transfer chain system in the respiratory chain of the mitochondria of the liver.

CONCLUSION

1. It was shown that with a 7-day tracheal stenosis, a violation of electron transfer in the liver mitochondria begins on the NAD-dependent section of the respiratory chain, i.e. an increase in activity and a decrease in the thermal stability of the NAD.H-oxidase system are observed. With an increase in the duration of the hypoxic effect (15th day), violations of the electron transport function extend even to the second conjugation point. At the same time, mitochondria showed restoration of the NAD.H-oxidase system of the respiratory chain. At the same time, the activity of the succinate oxidase system increases, but the thermal stability of this system drops sharply.

2. It has been established that energy compensation in case of increasing tracheal stenosis is associated with activation of the oxidizing ability of two main metabolic pathways in the respiratory chain: NAD.H-dependent and succinate-oxidase. The first is activated at the beginning of the experiment, the second, after the 15th day of tracheal stenosis.

3. An increase in the activity of polyenzymatic respiratory chain systems with moderate tracheal stenosis is associated not with an increase in the synthesis of these enzymes, but with an improvement in the access of substrates (NAD.H, succinate, cytochromes) to the active center of the enzyme system as a result of minor damage to the inner mitochondrial membrane. This is manifested in a violation of the activity of polyenzymatic systems of the respiratory chain and access of exogenous cytochromes to phospholipids and proteins of mitochondrial membranes, as well as a decrease in their stability when exposed to endogenous and exogenous phospholipases and proteases, leading to disruption of phospholipid-protein and phospholipid-phospholipid interactions of the inner membranes.

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