CYTOCHROME C OXIDASE OF GUINEA PIG LIVER MITOCHONDRIA DURING ANAPHYLACTIC SHOCK

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One of the characteristic features of energy metabolism in the animal organism is its high lability. The presence of a fine adaptive regulation of the oxidation-reduction processes has been demonstrated, both under conditions of normal vital activity [8] and in pathological states [7]. Definite changes in the energy metabolism appear during allergic diseases, including rather distinct changes in experimental anaphylactic shock, which is considered as an immediate allergic reaction [12]. In particular, in acute anaphylactic shock, the redox potential of the blood is lowered [2], tissue respiration is inhibited [6, 9], and oxidative phosphorylation is changed [8, 13].

Although the state of many units of the oxidation-reduction reactions during allergy has been more or less studied, this cannot be said of cytochrome C oxidase, although it is known that this enzyme plays an important role in the tissue oxidative processes. An 80-90% inhibition of cytochrome C oxidase inhibits the tissue respiration of most cells and tissues [2].

In this work we studied the activity of cytochrome C oxidase in the guinea pig liver during anaphylactic shock.

EXPERIMENTAL METHOD

We examined 50 guinea pigs (21 males and 29 females) weighing 290-350 g, kept on a mixed diet (beets, cabbage, oats). The work was conducted on "winter" animals. The experimental pigs were divided into four groups: the first, control (11 healthy animals), the second, animals at the height of sensitization (10), the third, animals that died of acute anaphylactic shock or were killed at the 29th to 31st minute in the case of delayed anaphylactic shock (20), and fourth, animals that died of mechanical suffocation (9).

All the guinea pigs were starved for 12-14 h before administration of the resolving dose or before killing.

Sensitization was performed by intraperitoneal injection of 0.1 ml of normal horse serum. Anaphylactic shock was induced in the animals 21-30 days after the sensitizing injection by injecting 1 ml of serum into the subcutaneous vein of the shin (10 guinea pigs) or by subcutaneous injection of 10 ml of serum (10 guinea pigs). Autopsy revealed acute distension of the lungs, overfilling of the right heart (with the presence of contractions), and active peristalsis the intestines. In two pigs, there was no pronounced pulmonary emphysema; however, they exhibited hemorrhages in the intestinal wall.

The animals at the height of sensitization (21-30 days after the sensitizing injection) and the controls were killed by passing an alternating current with a voltage of 220 V through the head. The guinea pigs with delayed anaphylactic shock were killed by the same method. Mechanical suffocation was induced by placing a rubber sleeve over the head, tightly covering the respiratory pathways. The animals died of electrocution after $\frac{1}{2}$-1 min, of acute shock after 4-6 min, and of suffocation after 3-15 min.

The liver, rapidly extracted after death, was immersed in a 0.25 M ice water solution of sucrose. After chilling, the organ, dried with filter paper, was pressed through a special syringe with a large number of openings 0.8 mm in diameter. The parenchymal pulp of the organ obtained was homogenized in 0.25 M sucrose solution (1 : 9)

Activity of Cytochrome C Oxidase from the Guinea Pig Liver Mitochondria

Group of animals	No. of animals	M ± m
First	11	0,131±0,007
Second	10	0,108±0,004
Third	20	0,077±0,004
Fourth	9	0,112±0,002

with the aid of an axial homogenizer with chilling at a rate of rotation of 5000 rpm twice for 1.5 min periods at 3 min intervals.

To obtain the mitochondria, the homogenate was subjected to differential centrifuging according to the method of Sneider and Hoegebum in Allfrey's modification [9]. The entire treatment was performed at 0-2°. The mitochondrial preparations obtained were standardized according to total nitrogen on the FÉKN-57 nephelometer with the aid of preconstructed calibration curves with an accuracy within 1 μg of total nitrogen per ml.

The enzyme activity was determined in samples containing 25-30 μg of total mitochondrial nitrogen. The colorimetric method of Strauss [14] was used with certain modifications. In this method, when the mitochondria come in contact with dimethylparaphenylenediamine hydrochloride (DPPD), a red pigment is formed from the latter in an amount proportional to the activity of cytochrome C oxidase and duration of incubation from 1 to 3 min. The direct proportionality is preserved when mitochondria containing from 1 to 30 μg of total nitrogen are added. The specific substrate is the added cytochrome C, the electron donor DPPD. The intensity of the color obtained is determined with a photoelectrocolorimeter. The specific activity of the enzyme is calculated by dividing the average extinction of two parallel determinations, multiplied by 100, by the number of micrograms of total mitochondrial nitrogen in the sample and the time of incubation in minutes. We described this method in detail earlier [5].

RESULTS

The statistically treated results of the determinations are presented in the table.

An analysis of the data cited shows that the enzyme activity decreases in comparison with the control by an average of 18% at the height of sensitization ($P < 0.001$), by 41% in anaphylactic shock ($P < 0.001$), and by 15% in the case of mechanical suffocation ($P < 0.05$). A significant decrease in the activity was detected during shock in comparison with this index during sensitization and suffocation (P in both cases less than 0.001). The differences between the decrease in the activity during acute and delayed shock are statistically insignificant (that is why they are summarized in one group), as are those in the case of sensitization and suffocation.

Thus, the cytochrome C oxidase activity of the liver is appreciably reduced at the height of sensitization. Anaphylactic shock is accompanied by a profound inhibition of the enzyme activity.

The decrease in the cytochrome C oxidase activity during sensitization is comparable with the data of [15], according to which there is a decrease in the respiration during incubation of the liver tissue with serum in vitro. The decrease in the enzyme activity in the case of mechanical suffocation agrees with information on the decrease in the activity of this enzyme in the liver, kidneys, and brain when rats are "raised" to an altitude of 10,000 meters in a pressure chamber [3], as well as with numerous studies of the inhibition of tissue respiration during hypoxia. The decrease in the enzyme activity in the liver is approximately the same when anaphylactic shock lasts from 4 to 31 min. At the same time, the decrease in the activity in the case of mechanically induced anoxia of approximately the same duration was almost three times lower. This apparently is evidence that the decrease in the conduction of the bronchi during their spasm does not play a deciding role in the pathogenesis of the disturbance of oxidation-reduction processes during anaphylactic shock. Noteworthy is the fact that the decrease in the activity of the enzyme studied is quantitatively approximately the same at the height of sensitization and in the case of suffocation.

The data cited on the substantial activity of cytochrome C oxidase in the guinea pig liver mitochondria are evidence of profound changes in the enzyme activity during anaphylactic shock, occurring at the cellular level [1].

SUMMARY

Experiments on fifty guinea pigs were used to determine the activity of cytochrome C oxidase after Strauss' method (with modifications) in the mitochondria isolated from the saccharose homogenates of the liver by means of differential centrifugation by Sneider and Hoegebum's method in Allfrey modification. In twenty animals the determination was carried out during anaphylactic shock, in 10 at the height of sensitization, in 9 during mechanical strangulation, and 11 animals were used as control. A sharp reduction in the activity of the enzyme in anaphylactic shock was discovered. A less significant but statistically authentic decrease in activity was noted at the

height of sensitization and in anoxia. The data obtained are evidence of serious disturbances in the respiratory chain during anaphylaxis.

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