CHANGES IN OPTICAL DENSITY OF MITOCHONDRIA OF THE RAT LIVER AND HEART IN NEUROGENIC DEGENERATION

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Electrical stimulation of rats causes swelling of the liver mitochondria and interferes with their contractility. Increased swelling of the mitochondria of the heart also takes place, and if stimulation is prolonged they contract more strongly. Similar changes in the mitochondria of the heart take place after injection of adrenalin.

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Application of an extreme stimulus to rats, such as injury to the duodenum or electrical stimulation of the animals, causes the development of degenerative changes in the stomach [4, 5], liver [7], and heart [2]. Similar disturbances may arise in the myocardium of rats after injection of large doses of adrenalin [1, 3].

Such changes in animal organs appear relatively early: after stimulation of immobilized rats for only 15 min, for instance, ultrastructural changes were found in the chief and parietal cells of the gastric mucosa, manifested by swelling of the mitochondria [6]. The state of mitochondrial function can also be judged from changes in the optical density of their suspension in various media (the cyto-biochemical method).

The object of this investigation was to study the optical density of mitochondria isolated from the liver and heart of rats subjected to an extreme stimulus: electrical stimulation or injection of large doses of adrenalin.

EXPERIMENTAL METHOD

Experiments were carried out on 160 male albino rats weighing 160-250 g. Square pulses (5 V, 50 Hz, 10 msec) from a type GIP-1 stimulator were applied to the rats through electrodes implanted into the forelimbs. Adrenalin was injected intraperitoneally in a dose of 0.5 ml of 1:1000 solution 10 min before the rats were sacrificed by decapitation. Mitochondria were extracted from the liver by the method of Hogeboom and Schneider [12] in 0.25 M sucrose solution, and from the heart by the same method as modified by Cleland and Slater [11] in 0.25 M mannitol solution. The optical density of the isolated mitochondria was determined by Cleland's method [10] in a medium of 0.125-0.15 M KCl solution on a type SF-4 spectrophotometer at wavelength 520 mμ. Before measurement of the optical density of the mitochondria began, 0.1 ml of phosphate buffer solution, pH 7.4, was added to their suspension. To cause the mitochondria to contract, 30 min after the start of measurement 0.005 M ATP solution and 0.03-0.05 M $MgCl_2$ solution were added in a volume of 0.05 ml, and the measurements continued for a further 30 min.

To compare the extinctions obtained in the control and experimental groups of rats, they were calculated per mg protein determined by the biuret method, making allowance for results obtained by Lehninger and Schneider [13] indicating that in the zone of change of extinction from 0.15 to 0.7 the curve of adsorption plotted against protein concentration of the mitochondria is a straight line. Swelling of the mitochondria was expressed as a coefficient of swelling, determined by the magnitude of the decrease in optical density during the first 30 min of measurements, estimated per mg protein. The contractility of the mitochondria was estimated in a similar way by means of a coefficient of contraction.

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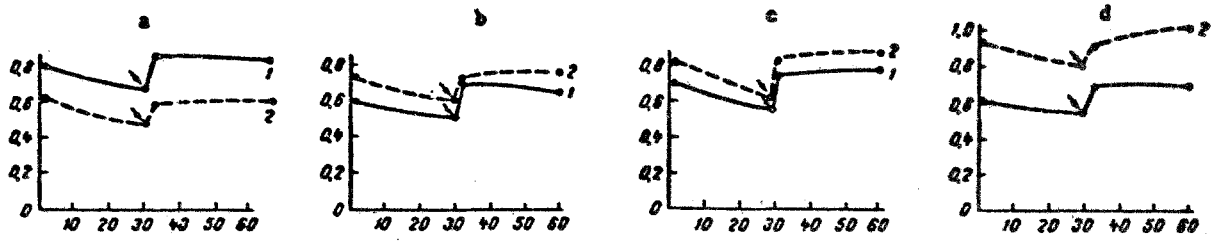

Fig. 1. Changes in optical density of mitochondria of liver (a) and heart (b, c, d) of rats undergoing electrical stimulation for 1 h (a, b) and 3 h (c), and injection of 0.5 ml 1:1000 adrenalin (d). 1) Control; 2) experiment. Arrows indicate time of addition of ATP and $MgCl_2$. Results given are mean data: a) of 10 experiments, b) of 15, c) of 12, d) of 14 experiments. Ordinate, extinction (E520 mμ); abscissa, time (in min).

EXPERIMENTAL RESULTS

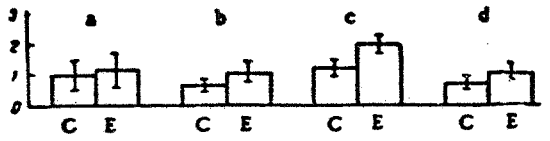

Fig. 2. Coefficient of swelling of mitochondria of liver (a) and heart (b, c, d) of rats subjected to electrical stimulation for 1 h (a, b) and 3 h (c) or receiving 0.5 ml 1:1000 adrenalin (d). C) control; E) experiment.

Optical density of mitochondria isolated from the liver of rats receiving electrical stimulation for 15 min and 1 h (Figs. 1, a and 2, a) was studied in the experiments of series I. Initial extinction in the experiments was appreciably lowered. This indicates that the mitochondria were in a swollen state. In rats subjected to electrical stimulation, the contractility of the mitochondria was also appreciably reduced by the addition of ATP and $MgCl_2$ to their suspension, the coefficient of contraction in this group being about one-third smaller than in the control. During electrical stimulation of the animals for 15 min the changes were similar but less marked.

In the next series of experiments, the optical density of mitochondria isolated from the heart of rats subjected to electrical stimulation for periods of 15 min, and 1 and 3 h was determined. After electrical stimulation for 15 min an increase in swelling of the mitochondria was found. Stimulation of the rats for 1 h led to increased swelling of the mitochondria (Fig. 1, b); the coefficient of swelling increasing by 60% over the control level (Fig. 2, b). In the heart, in contrast to the liver, as Figure 1 shows the initial extinction was higher in the experiment than in the control, because the mitochondria were more contracted before centrifugation. However, in response to addition of ATP and $MgCl_2$ the mitochondria contracted equally.

Similar results were obtained in rats receiving electrical stimulation for 3 h (Figs. 1, c and 2, c).

In view of the great importance of the sympathetic nervous system in the mechanism of development of degenerative changes in organs and the important role of adrenalin in this process [8], in the last series of experiments we studied changes in the optical density of mitochondria from the heart of rats receiving adrenalin injections.

After injection of adrenalin, swelling of the mitochondria increased and the coefficient of swelling rose to almost twice the control level (Figs. 1, d and 2, d). The initial extinction, as in the experiments with electrical stimulation of the rats, was higher than in the control. The contractile power of the mitochondria also increased. Hence, the character of the change in optical density of the myocardial mitochondria under the influence of adrenalin and electrical stimulation was similar.

A common feature to the response of the mitochondria of the heart and liver to electrical stimulation, which may lead to degeneration of these organs, is an increase in their swelling in a medium containing KCl. However, judging by the results of determination of optical density, electrical stimulation of the rats caused more intensive changes in the liver mitochondria: the initial extinction was diminished and their contractile power disturbed. This is in agreement with results obtained by Shen Wen-mei [9], who irradiated rats with a regenerating liver by means of x-rays. Changes in the optical density of the mitochondria in the early stages (15 min and 1 h) after application of an extreme stimulus may be interpreted as one of the early signs of developing degeneration.

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