ROLE OF HYDROLYSIS OF MEMBRANE LIPIDS OF MITOCHRONDRIA IN THEIR THYROXINE-INDUCED SWELLING

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Swelling of mitochondria induced by thyroxine is accompanied by the accumulation of free fatty acids in the organelles; the level of these acids returns to its initial value during contraction of the mitochrondria in the presence of ATP. EDTA also causes contraction of the mitochondria, but without any corresponding utilization of fatty acids. Thyroxine-induced swelling of mitochondria is evidently due to activation of membrane phospholipase A_2 and to an increase in permeability of the membranes to cations as a result of hydrolysis of phospholipids.
KEY WORDS: mitochondria; thyroxine; phospholipase; fatty acids.

The activators of mitochondrial phospholipase A_2 are known to include Ca^{++} ions [1]. There are several common features in the action of thyroxine and Ca^{++} on mitochondrial structure and function [6, 7, 10] and, in particular, the formation of a factor of fatty acid nature, which disturbs mitochondrial permeability. EDTA, which inhibits Ca^{++}-induced swelling [5], also blocks thyroxine swelling of mitochondria [6]. When Ca^{++} is added in a certain concentration to mitochondria it does not cause swelling if the mitochondria are able to accumulate it [1]. With the addition of thyroxine, Ca^{++} begins to flow outward [1]. Turakulov et al. [3, 4] have found a factor of polypeptide nature which is responsible for the accumulation of Ca^{++} in mitochondria; incubation of mitochondria with thyroxine leads to the loss of this factor.

In this investigation the fatty acid concentration was determined in mitochondria during thyroxine-induced swelling.

EXPERIMENTAL METHOD

Mitochondria were isolated from rat liver in 0.25 M sucrose with 0.25 mM EDTA [8]. Mitochondrial anoxia was produced by careful evacuation of the air from the Thunberg tube containing a suspension of mitochondria with a protein concentration of 1-2 mg/ml. The tube was then placed on a magnetic mixer and the mitochondria vigorously agitated to utilize any possible traces of residual O_2, after which thyroxine solution was poured from a retort into the suspension until its final concentration therein was 10 μM. The degree of swelling was estimated either from the intensity of scattering of light by the object or from the transmission of light through it. The incubation medium consisted of: KCl 0.125 M, $MgCl_2$ 0.5 mM, Tris-HCl 20 mM. Measurements were made at 25-27°C. Determination of the free fatty acid (FFA) concentration as a test of phospholipase activity was carried out by the method of Anderson and McCarty [10] with certain modifications. At the required moment of time a 1-ml sample of the suspension of the organelles was taken and added to 10 ml hexane. The resulting phases were mixed for 2 min on a magnetic mixer to ensure better extraction of the FFA. After the sample had been allowed to stand for 10 min, to enable separation of the phases, 5 ml of the hexane phase was withdrawn. The hexane was removed by a vacuum pump, and 4.5 ml of a solution of rhodamine 6G in benzene was added to the FFA remaining on the walls of the vessel. After incubation for 15 min the optical density of the solution was measured at a wavelength of 545 nm. The FFA concentration was calculated from a calibration curve plotted with the aid of known solutions of palmitic acid.

EXPERIMENTAL RESULTS

Incubation of thyroxine with mitochondria in vacuo causes them to swell, although by a lesser degree than in an aerated medium (Fig. 1). This result contradicts the data indicating that thyroxine swelling does not take

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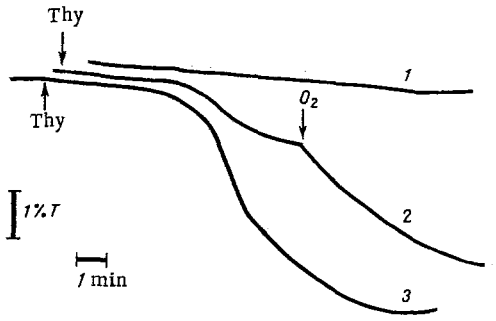

Fig. 1

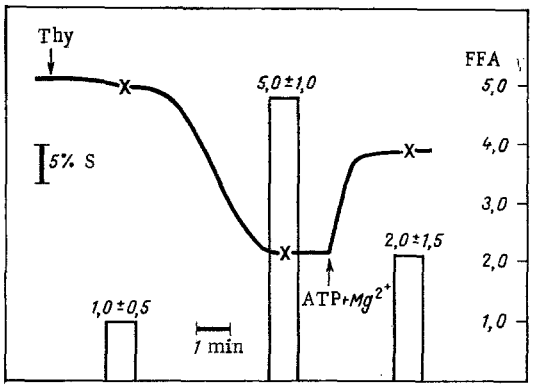

Fig. 2

Fig. 1. Effect of O_2 on development of thyroxine-induced swelling. 1) Mitochondria without thyroxine in vacuo; 2) swelling under anaerobic conditions followed by aeration of sample; 3) swelling in presence of O_2. Time of addition of hormone (Thy) and aeration (O_2) indicated by arrows. T) Light transmission of suspension (in %).

Fig. 2. Action of ATP and Mg^{++} on contraction of swollen mitochondria and on FFA level. Curve shows scattering of light (S) by mitochondrial suspension; columns show FFA concentration (in nmoles/mg protein). Addition of 5 mM ATP, 3 mM $MgCl_2$, and thyroxine (Thy) indicated by arrow. Times of sampling for FFA indicated by crosses on curve.

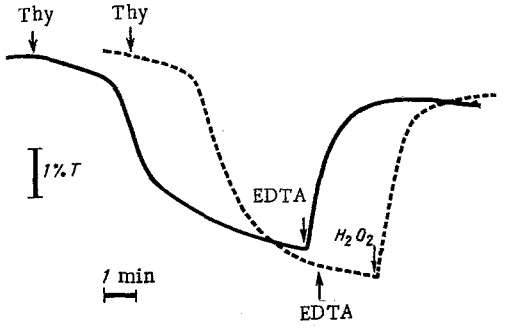

Fig. 3. Contraction of swollen mitochondria induced by EDTA. Arrows indicate times of addition of thyroxine (Thy), 1 mM EDTA, and H_2O_2. Continuous line – at time of addition of EDTA mitochondria were under aerobic conditions; broken line – on addition of EDTA mitochondria were under anaerobic conditions (state 5 after Chance). T) Transmission of light through suspension (in %).

place under anaerobic conditions [4]. In the present experiments the mitochondria were energized, possibly by residual ATP in the organelles. Supplying air to mitochondria swelling in vacuo makes a rapid and additional contribution to the swelling process.

As Fig. 2 shows, the FFA concentration in the mitochondrial suspension at the height of thyroxine-induced swelling was much greater than their concentration in the latent stage of the process, suggesting activation of the phospholipase system in the presence of thyroxine. Addition of ATP and Mg^{++} to the sample was accompanied by contraction of the organelles, in agreement with observations by other workers [7, 10], although restoration of the level of scatter of light was incomplete in these experiments. Contraction of the mitochondria under the influence of ATP was accompanied by a fall in the FFA concentration.

Mitochondria swollen under the influence of the hormone also contracted on the addition of EDTA (Fig. 3). The mechanism of this contraction is not clear and requires special analysis. All that can be said is that it is active in nature. In fact, addition of EDTA to swollen mitochondria kept in an oxygen-free medium (state 5 after Chance) did not cause them to contract, but contraction began immediately after oxygen was added (as H_2O_2) to the system. Contraction of the mitochondria under the influence of EDTA was not accompanied by a fall in the FFA concentration in the suspension.

The increase in the FFA concentration during incubation of mitochondria with thyroxine must evidently be interpreted as the result of activation of phospholipase A_2, although the mechanism of this activation is not yet clear. It can tentatively be suggested that phospholipase is activated by Ca^{++}, which flows outward under the influence of thyroxine. However, it still remains unclear whether this effect can be explained by loss by

the mitochondria of the polypeptide factor responsible for their "calcium capacity" [2, 3].

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CYCLIC AMP IN THE ORGANS AND TISSUES DURING ADAPTATION TO EXTREMAL FACTORS

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Extremal factors of different nature (hypoxic hypoxia, carbon monoxide poisoning, exposure to chemicals and to noise, hypokinesia) caused similar changes in the cyclic AMP level in the organs of albino rats (liver, cerebral hemispheres, heart). A considerable increase in the cyclic AMP concentration was found in the first stages, followed by a progressive fall during subsequent exposure, especially if the intensity of the factor was high. It is suggested that the universality of this response reflects one of the central adaptive mechanisms of the cell and of the organism as a whole.

KEY WORDS: adaptation; hypoxia; cyclic AMP; extremal factors.

The discovery of cyclic adenosine-3',5'-monophosphate (cyclic AMP) as an intracellular mediator of neurohormonal regulatory influences has provided a new approach to the study of the cellular mechanism of adaptation to extremal environmental factors. A few papers describing the study of the cyclic AMP system in hypoxic states have now been published. An increase in the cyclic AMP concentration and adenylate cyclase activity has been demonstrated in brain tissue in acute hypoxia [3, 6], and a sharp increase in the cyclic AMP concentration has been found in the early stages after acute isolated ischemia of the brain [7] and myocardium [5] in dogs.

Considering the importance of the determination of the principles governing the response of the cyclic AMP system to extremal factors in order to understand the mechanisms of goal-directed changes in the resistance of the body, changes in the cyclic AMP concentration in the organs and tissues were studied during exposure to extremal factors of varied nature.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 150-180 g were used. The effect of extremal factors was studied under dynamic conditions at two levels of intensity (acute limiting – Lim. ac.) and mean lethal (LD_{50}) exposure. Hypoxic hypoxia was produced by "raising" the animals in a pressure chamber to an "altitude" of 6000-10,000 m (the mean rate of "ascent" was 150-200 m/min). Acute poisoning with carbon monoxide (CO), styrene, and

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