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TRANSMEMBRANE POTENTIAL OF RAT LIVER MITOCHONDRIA IN HYPOTHYROIDISM

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Changes in oxidative phosphorylation of mitochondria caused by a deficiency of thyroid hormones in the body are now well known although the molecular mechanism of these changes still remains unexplained. The rate of oxidation of substrates in the presence of ADP has been shown to be depressed compared with normally [6]. It is also known that mitochondria, isolated from animals with hypothyroidism swell more slowly in media containing injury factors (oleate, phosphate anions, thyroxine) [10]. The reasons for these differences have not hitherto been analyzed. It was reported previously that the transmembrane potential (TMP) of liver mitochondria of hyperthyroid rats is higher than normal, whereas the system responsible for maintaining TMP in these mitochondria is less resistant to Ca^{++} ions than in the organelles of normal animals [2]. In the present investigation, by recording the value of TMP of mitochondria from the liver of hypothyroid animals, changes opposite to those observed when the thyroid hormone level was raised were observed: a decrease in TMP and an increase in resistance of the potential-maintaining system to Ca^{++} ions.

EXPERIMENTAL METHOD

Wistar rats weighing 180-200 g were used in the experiments. Mitochondria were isolated in 0.3 M sucrose with 10 mM Tris-HCl, pH 7.4, by the method in [5]. Experimental hypothyroidism was produced by thyroidectomy. The animals were used in the experiments 3-4 weeks after thyroidectomy. Animals undergoing a mock operation served as the control. The TMP level was judged from the intensity of quenching of fluorescence of a dis-C_3-(5) probe in a suspension of energized mitochondria (the $\Delta F/F$ parameter) [7]. The quantity of endogenous Ca^{++} in the mitochondria was determined by flame photometry on a Hitachi-207 atomic absorption spectrophotometer. The protein concentration was determined by the method of Lowry et al. [8].

EXPERIMENTAL RESULTS

TMP of energized mitochondria from the liver of hypothyroid rats was maintained longer than in preparations from control animals. This can be seen on curves of fluorescence of the probe in a suspension of mitochondria loaded with Ca^{++} ions (Fig. 1a, b). Additionally, as will be clear from this figure, more Ca^{++} had to be added to the preparations for development of a rapid decline of TMP than in the case of mitochondria of normal animals (Fig. 1c, d). Measurement of the parameter $\Delta F/F$ of the probe, the value of which is proportional to TMP [7], showed that this ratio is 83 ± 4% of normal (results of preparative isolations from eight normal and eight hypothyroid rats with four or five repetitions for each mitochondrial preparation). Consequently, if thyroid hormones are deficient in the body, the picture observed in the mitochondria is opposite to that revealed for organs of rats with hyperthyroidism [2]. In the latter case an increase in TMP was combined with a decrease of resistance of the mitochondria to the damaging action of Ca^{++} ions.

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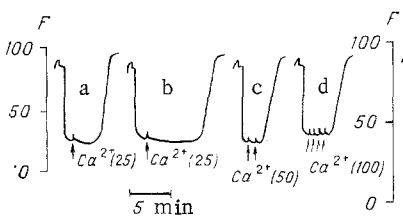

Fig. 1

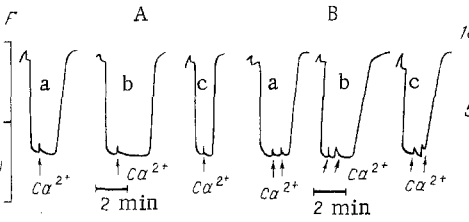

Fig. 2

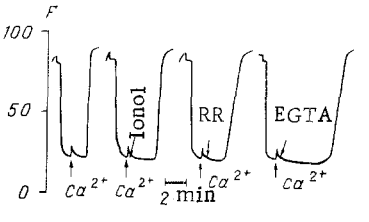

Fig. 3

Fig. 1. Effect of different Ca^{++} loads on TMP of mitochondria of normal (a and c) and hypothyroid (b and d) rats. Here and later, mitochondria (1 mg protein in 1 ml) were incubated in medium containing 100 mM KCl, 100 mM sucrose, 10 mM Tris-HCl, 1 mM KH_2PO_4, and 1 mM succinate; pH 7.4. Concentration of probe dis-C_3-(5) was 10 μM. Time of addition of Ca^{++} indicated on figure. Numbers in parentheses show total quantity of cation in nanomoles per milligram mitochondrial protein. F) Intensity of fluorescence of probe (in relative units). Temperature 20-22°C.

Fig. 2. Effect of single dose of L-thyroxine (300 μg/100 g body weight 46 h before isolation of mitochondria) on Ca^{++} retention time (A) and calcium capacity (B) of mitochondria. a) Normal animals; b) hypothyroid; c) hypothyroid + thyroxine. Total Ca^{++} concentration in experiments A was 25 nmoles/mg protein and in B 50 nmoles/mg protein.

Fig. 3. Action of ruthenium red (RR), ionol, and EGTA on decay time of mitochondrial TMP of normal rats. Concentration of RR in medium 1 μM, of ionol $2 \cdot 10^{-5}$ M, of EGTA 0.5 mM. Ca^{++} concentration 50 nmoles/mg protein.

A single injection of L-thyroxine into the hypothyroid rats (300 μg/100 g body weight, intraperitoneally) after 46 h restored TMP to its normal level (the value of $\Delta F/F$ in mitochondria of hypothyroid rats was 96 ± 4% of the control; results of preparative isolations from four hypothyroid rats receiving this dose of hormone, and four normal rats with three repetitions for each preparation of mitochondria). At the same time (Fig. 2) the hormone accelerated the decline of TMP compared with mitochondria of rats "not treated" with thyroxine. The observed effects of thyroxine injected into the animals are most probably not the result of its direct action on mitochondria. This conclusion was drawn after experiments which showed that neither the direct addition of thyroxine to the incubation medium of the mitochondria (within the range of final concentrations 10^{-6}-10^{-7} M) nor preliminary incubation of homogenate from which mitochondria were later isolated, with the above-mentioned quantities of thyroxine for 30 min had any effect on the parameters of these organelles which were studied. What are the causes of the fall in the value of the mitochondrial TMP in hypothyroidism? Evidently the TMP level is determined by the intensity of work of the respiratory chain and the magnitude of "leakage" of ions, which depends on permeability of the inner membrane of the mitochondria. To judge from the data in [10], permeability of the mitochondrial membrane is no higher in hypothyroidism than in the control. It must therefore be assumed that the decrease in mitochondrial TMP observed in hypothyroidism is the result of delayed electron transport along the respiratory chain. The immediate cause of the latter is not clear. It may perhaps be connected with a decrease in the content of cytochromes in the respiratory chain of the mitochondria of animals with hypothyroidism [10], although such a fall was not mentioned by other workers [4].

Let us now consider the possible causes of the increased resistance of the mitochondria of the hypothyroid rats to Ca^{++} ions. Existing data suggest that the fall of TMP developing in the presence of Ca^{++} may be largely due to the activity of endogenous mitochondrial phospholipase, which induces hydrolysis of the lipids in the inner mitochondrial membrane [3]. In fact, as the authors cited showed [3], the decline of TMP and accompanying outflow of Ca^{++} from mitochondria into the medium are blocked by phospholipase inhibitors. Other workers observed that outflow of Ca^{++} from mitochondria loaded beforehand with this cation is stimulated by the addition of organic peroxides to the medium [9]. At the same time, it is not clear what contribution processes of peroxidation of endogenous mitochondrial lipids may make to the decline of TMP. In the present experiments the role of hydrolysis of phospholipids and peroxidation of unsaturated fatty acids of mitochondrial lipids was verified only in media with the antioxidant ionol, or with ruthenium red, an inhibitor of Ca^{++} transport into mitochondria, or with EGTA, which binds Ca^{++} ions. The results of some typical experiments

are illustrated in Fig. 3. Ionol, in quantities preventing lipid peroxidation (LPO) considerably delayed the beginning of the decline of TMP. However, even with complete inhibition of LPO reactions the decline of TMP nevertheless took place. Ruthenium red and EGTA, added to the mitochondria immediately after loading with Ca^{++} ions, also prolonged the preservation time of TMP, which would be expected in the light of data on the effect of phosphorylase on the TMP-maintaining system [3]. Incidentally, in the present experiments these substances, when added to medium with mitochondria from hypothyroid animals, had a similar action on the TMP system (not shown in this figure).

Factors activating LPO or mitochondrial phospholipase will thus bring about a corresponding decrease both in the preservation time of TMP and in resistance to the action of Ca^{++} ions. In connection with data given above on the role of lipid degradation factors in the functional state of mitochondria it is interesting to note that LPO reactions in mitochondria from hypothyroid animals followed a slower course than normally [1], and mitochondrial phospholipase activity in hypothyroid animals, as separate experiments showed, was lower than in preparations from normal animals. These findings will be discussed in greater detail in a separate communication.

The data given above suggest that the observed increase in resistance of the TMP system of mitochondria from hypothyroid animals to Ca^{++} ions is attributable at least partially to the slowing of lipid degradation in mitochondrial membranes in hypothyroidism. Another possible cause of increased resistance of mitochondria to Ca^{++} ions in the presence of thyroid hormone deficiency could be a decrease in the endogenous Ca^{++} content in the organelles and, consequently, an increase in capacity of the mitochondria for Ca^{++} ions. Such a situation could arise on account of a deficiency of thyrocalcitonin in the body, due to thyroidectomy. However, since the parathyroid glands — the source of the physiological thyrocalcitonin antagonist, parathormone, were removed in the course of this operation also, the overall disturbance of the calcium balance in the tissues may not be so very considerable. In any event, determination of the Ca^{++} content in the mitochondria of normal and hypothyroid animals revealed virtually no difference between them: 21.9 ± 4 and 20.8 ± 3.1 nmoles/mg protein respectively (data for mitochondria of four normal and four hypothyroid rats).

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