ELECTRICAL STABILITY OF MITOCHONDRIAL MEMBRANES: ROLE OF THYROID HORMONES AND THE DIETARY FAT COMPONENT

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An important property of biomembranes is their electrical stability namely the ability of the lipid layer of membranes to withstand comparatively high membrane potentials that exist in living cells. Electrical stability of membranes can be characterized quantitatively by the value of their breakdown potential (φ^*), i.e., the minimal membrane potential at which the continuous lipid layer of the membrane is interrupted through the formation of spontaneously growing defects (pores) in it [5]. Since disturbance of the barrier functions of biomembranes in various diseases may take place by a mechanism of electrical failure of the membranes [5], measurement and study of dependence of the value of φ^* on the composition of the lipid layer and other factors under normal and pathological conditions is of definite interest.

It was shown previously that electrical stability of model and biological membranes depends on the composition of the phospholipids (PL), the degree of saturation of the fatty acids composing PL, the cholesterol concentration, the level of lipid peroxidation (LPO), etc. [5, 9, 10]. Among the physical factors affecting electrical stability of lipids, viscosity and density of the electric charge on the surface of lipid membranes may be mentioned [9]. Changes in the lipid composition of membranes connected with changes in lipid metabolism also exert a significant influence on electrical stability of membranes [2, 3].

Many investigations into electrical failure have been carried out on artificial lipid membranes. However, measurement of the value of φ^* for biological membranes and its comparison with the breakdown potential of model membranes made from lipids of the same cell membrane structures are particularly interesting.

The aim of this investigation was to assess the influence of the lipid composition of the diet and of hyperthyroidism on electrical stability of rat liver mitochondrial membranes and also on electrical stability of membranes of liposomes prepared from mitochondrial lipids. Methods of measurement of the breakdown potential of mitochondrial membranes [2] and liposomes [4] have been developed on several occasions in the writers' laboratory.

EXPERIMENTAL METHOD

Rats of two groups were kept for 3 weeks on a synthetic isocaloric diet containing 18% of protein, 26% of fats, and 56% of carbohydrates. The diet also included added minerals and vitamins. Dietary fats were butter for the animals of group 1 and sunflower oil for the rats of group 2. These two types of dietary fats differ sharply in the degree of unsaturation of their fatty acids, because of their different content of linoleic acid (under 3% in butter, about 60% in sunflower oil). Hyperthyroidism was induced by injection of L-thyroxine (100 μg/100 g body weight daily for 5 days) intraperitoneally. Mitochondria were isolated from rat liver by the method in [8]. Protein was determined by the microbiuret method. Phospholipids were extracted from mitochondria in [7] and liposomes were formed from them in a 10 mM solution of sucrose as described previously [3]. The method of measuring electrical stability of the liposomes was described previously [3]. Electrical stability of mitochondrial membranes was judged from the quantity of potassium acetate (PA) which had to be added to a suspension of energized mitochondria to cause breakdown of the membranes of these structures.

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TABLE 1. Effect of Dietary Fat Component and Hyperthyroidism on Value of φ^* (in mV) of Liposomes Formed from Mitochondrial Lipids (M ± m)

Experimental conditions	Groups of animals	φ^*
Euthroidism	1	120,0±4,2
	2	143,0±0,66
Hyperthyroidism	1	151,3±0,6
	2	150,7±0,5

Legend. Values given are averaged for three suspensions of liposomes. Each suspension was prepared from a pooled preparation of mitochondria isolated from the liver of four animals.

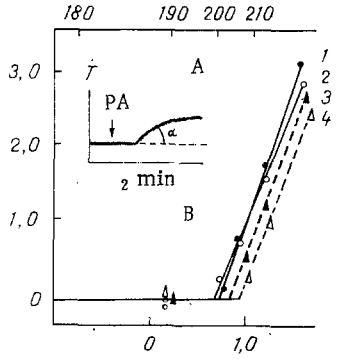

Fig. 1. Effect of hyperthyroidism and diet on electrical stability of rat liver mitochondrial membranes. A) Changes in light transmission (T) of mitochondrial suspension at 680 nm in response to addition of PA to incubation medium. Incubation medium (in mM): KCl 125, EGTA 0.1, Tris-HCl 10, succinate 1.

It was shown previously that addition of acetate leads to an additional rise of membrane potential, which is accompanied by electrical failure of the membranes and swelling of the organelles [1]. Swelling is accompanied by a decrease in scattering of light of the suspension. This means that breakdown of the membrane can be recorded as an increase in scatter of light by the mitochondrial suspension (Fig. 1A).

EXPERIMENTAL RESULTS

Curves of light transmission of mitochondrial suspensions depending on PA concentration in the medium are given in Fig. 1. The highest potential was found to be required for the development of electrical failure of the membranes in organelles of hyperthyroid animals of group 2. Conversely, mitochondrial membranes isolated from the liver of rats kept on a diet with butter required a lower potential for failure. There were no evident differences in the value of φ^* between normal and hyperthyroid rats. An intermediate position as regards the value of φ^* among the mitochondrial populations studied was occupied by organelles in animals of group 2 with a normal (euthyroid) state. Judging from the breakdown potential of the mitochondria, it was thus liver organelles of hyperthyroid animals of group 2 which had the greatest electrical stability, followed by mitochondria of euthyroid animals of group 2, and the less "stable" mitochondria of the rats of group 1, whose dietary fats were deficient in linoleic acid. Diet and hormonal status evidently affect electrical stability of mitochondrial membranes. Thyroid hormones are powerful modulators of the lipid metabolism of the body [6]. It can therefore be postulated that in this case hyperthyroidism, like the fats used in the diet, induced rearrangements in mitochondrial lipids that are responsible for the differences observed in electrical stability between the various mitochondria studied.

For this reason, in subsequent experiments correlation was studied between electrical stability of mitochondria, using the analogous parameter of mitochondrial lipids. Stability of mitochondrial lipids of rats of group 1 was found, on the basis of the value of φ^*, to be significantly lower than in rats of group 2 (Table 1).

It will be clear from the data given that hyperthyroidism caused a relatively small increase in φ^* of liposomes belonging to group 2 but, conversely, a considerable increase in this parameter in lipids of mitochondria isolated from the liver of animals of group 1. These results agree with those of a previous investigation in which electrical stability of mitochondrial lipids of hyperthyroid animals receiving a normal diet was found to be increased [2]. Hyperthyroidism in the animals of group 2 was accompanied by a very small increase in φ^* of the lipids compared with the corresponding control (about 5%), whereas in hyperthyroidism in group 1 there was an increase in φ^* by 26% respectively. It is interesting to compare this with data according to which the addition of lineolic acid to the diet neutralized a number of metabolic effects of hyperthroidism [11]. Analysis of the results reveals correlation between stability of the lipids and membranes of the corresponding mitochindria of group 2. Meanwhile, correlation of this kind was observed for group 1 only in the case of euthyroid rats, when both mitochondrial membranes and lipids were less stable than the corresponding preparations of group 2. Liposomal membranes in the group of hyperthyroid animals were equally stable in both groups. Meanwhile mitochondrial membranes from hyperthyroid rats of group 1 had much lower electrical stability than membranes of these organelles in the animals of group 2. This evidently means that stability of mitochondrial membranes does not depend only on the composition of the membrane lipids.

Further investigations are needed to explain the mechanisms regulating electrical stability of mitochondria and also connections between the values of this parameter and specific functions of the mitochondria.

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