INCREASED ELECTRICAL STABILITY OF HEPATIC MITOCHONDRIAL LIPID MEMBRANES IN HYPERTHYROIDISM

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Thyroid hormones regulate the velocity of lipid and protein metabolism in the body by determining both the quantitative and the qualitative composition of these main components of biomembranes [9, 11]. With a fall in the thyroid hormone level the degree of unsaturation of fatty acids in the mitochondria of the heart and liver increases, and the point of phase transition in lipids isolated from these organelles is shifted considerably toward the region of lower temperatures [10, 11]. Administration of thyroxine into hypothyroid or normal animals caused the opposite changes [10, 11]. A tendency for the viscosity of the mitochondrial membrane lipids to decrease in hypothyroidism and to increase in the presence of an excess of circulating thyroid hormone has been discovered [1]. In hypothyroidism the activation energy of substrate oxidation processes by mitochondria has been shown to be increased.

So far, however, it is not yet clear whether the barrier function of the mitochondrial membranes also is affected by changes in the body level of thyroxine. There is indirect evidence that it does. For example, phospholipase is known to be activated *in vitro* in mitochondria on the addition of thyroxine [3]. Possibily a similar effect is manifested *in vivo* also in hyperthyroidism [6]. Meanwhile in model systems phospholipase reduced the electrical stability of membranes [4]. It can be tentatively suggested that in hyperthyroidism changes in the electrical stability of the lipid layer of the mitochondrial membranes may take place.

The object of this investigation was to study the effect of hyperthyroidism and thyroxine in experiments *in vitro* on the electrical stability of membranes prepared from mitochondrial lipids. Bilayer lipid membranes and phospholipid vesicles (liposomes) were used as test objects.

EXPERIMENTAL METHOD

Rabbits weighing from 2.7 to 3.6 kg were used. Hyperthyroidism was induced by daily intraperitoneal injection of L-thyroxine in 0.01 N NaOH solution in a dose of 180-200 μg/kg body weight for 6-8 days. Control animals received an injection of 0.3-0.5 ml of 0.01 N NaOH solution. Mitochondria were isolated from the rabbit liver by differential centrifugation [8] in medium containing 250 mM sucrose, 0.01 mM EDTA, and 5 mM Tris-HCl, pH 7.4. Lipids were extracted from freshly isolated mitochondria [7].

Bimolecular Lipid Membranes. Bimolecular lipid membranes (BLM) were formed on holes 1 mm^2 in area in a vertical Teflon jar immersed in an aqueous solution of 50 mM KCl with 5 mM Tris-HCl, pH 7.0, at 39°C. The electrical characteristics of the BLM were measured by means of an ED-05M electrometer and recorded on a KSP-4 potentiometer. To determine the breakdown voltage of the BLM, a voltage of 1.0 V was applied. Under these circumstances the membrane was charged up to a certain potential U* (Fig. 1a), after which its resistance fell sharply and the BLM was destroyed, with the onset of electrical breakdown. If at the beginning of breakdown of resistance the applied voltage on the BLM was reduced by the switch K to zero, the membrane was preserved and the measuring procedure could be repeated after a short interval. The potential U* in this case was the breakdown voltage. Lipsomes were prepared by dispersing lipids (0.2 mg/ml) in a 10 mM aqueous solution of sucrose, pH 7.0, fol-

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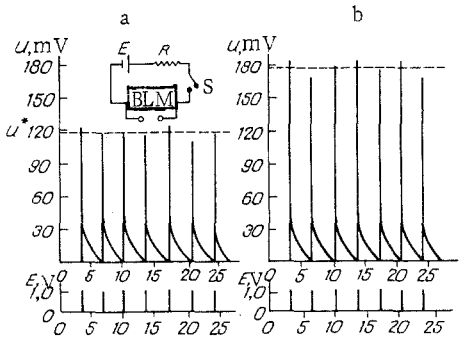

Fig. 1. Increase in breakdown voltage of BLM made from hepatic mitochondrial lipids of rabbits with hyperthyroidism. a) Healthy rabbit (control); b) rabbit with hyperthyroidism. Abscissa, time (in min), ordinate: above — potential (U) on membrane (in mV); below — voltage (E) applied to BLM from an external source (in V). U*) Breakdown voltage (in mV). Theoretical circuit for measuring electrical parameters of BLM shown in inset. R) Standard resistance; U) voltage drop on BLM; E) voltage applied to BLM from external source; S) switch.

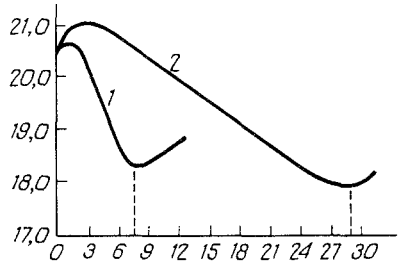

Fig. 2. Change in light transmission by lipsome suspension on continuous addition of $(NH_4)_2SO_4$. 1) Liposomes from hepatic lipids of healthy rabbit; 2) liposomes from hepatic lipids of rabbits with hyperthyroidism. Suspension contained 0.2 mg lipids/ml and the protonophore carbonyl cyanide-m-chlorophenylhydrazone in a concentration of 0.1 μM. Abscissa, concentration of ammonium sulfate $(NH_4)_2SO_4$ in cuvette (in mM); ordinate, light transmission (in %).

lowed by disintegration by freezing and thawing [5]. The breakdown voltage of the liposome membrane was determined by the method of electrical breakdown of membranes by a proton diffusion potential [2], developed previously, and recorded as the change in light transmission of the suspension. To speed up recording of the breakdown potential the method was modified: The salt of the weak base $(NH_4)_2SO_4$ was supplied continuously to the cuvette. Light transmission was measured on the FET turbidimeter, the output of which was connected to the KSP-4 potentiometer. Ammonium sulfate was supplied continuously to the cuvette (length of optical path 2 cm), with continuous mixing of the liposome suspension (volume 7 ml).

EXPERIMENTAL RESULTS

Typical traces showing changes in the breakdown voltage of BLM prepared from mitochondrial lipids of normal animals and of rabbits with hyperthyroidism are given in Fig. 1. The mean data for a series of membranes from lipids of five rabbits with hyperthyroidism and three normal animals are given in Table 1. The results show a significant increase in the break-

TABLE 1. Changes in Breakdown Voltage of Membranes in Hyperthyroidism (M ± m)

Experimental conditions	BLM		Liposomes	
	Breakdown voltage, mV	Number of membranes	Critical $(NH_4)_2SO_4$ concentration, mM	Breakdown voltage, mV
Control	110,0±3,7	18	7,5	141,3
	112,0±3,8	19	7,2	140,8
	102,0±3,8	12	7,9	142,1
Hyperthyroidism	176,9±4,7	21	21,6	154,7
	189,50±2,78	19	26,6	157,3
	210,20±3,78	28	40,1	162,5
	160.60±3,14	26	27,6	157,8
	165,0±3,58	26	29,6	158,0

down voltage in the case of hyperthyroidism. The same rule was observed when electrical breakdown phenomena were studied in suspensions of liposomes. As will be clear from Fig. 2 and Table 1, the concentration of $(NH_4)_2SO_4$ at which the course of the light scattering curve changed was shifted from 7.2-7.5 mM for mitochondrial lipids from normal animals to 22-40 mM for lipids of animals with hyperthyroidism. When the breakdown voltage was calculated by the equation suggested in [2], the values obtained were excessively high compared with those for BLM in the case of control animals and unduly low in the case of animals with hyperthyroidism (Table 1). The reasons for these divergences were probably connected with the assumptions made when the equation was deduced. Nevertheless, it is quite evident that in the case of liposomes the breakdown voltage was higher for membranes from mitochondrial lipids of animals with hyperthyroidism than from control animals.

In a separate series of experiments the effect of thyroxine on the breakdown voltage of lipid membranes was studied. On the addition of thyroxine solution on one side of the BLM up to a final concentration of 5 μM the breakdown voltage was increased from 110.0 ± 3.7 to 162.0 ± 1.7 mV. If thyroxine was added in this same concentration on both sides, the blackening time of the BLM was considerably increased. Thyroxine itself, in relatively low concentrations, thus increased the electrical stability of the membranes. Moreover, the increase in electrical stability of membranes from rabbits with hyperthyroidism was determined most probably not by this direct effect, but by a change in the composition of the lipids of the mitochondrial membranes. It is difficult at present to estimate the biological importance of this observed increase in membrane stability in hyperthyroidism, partly because the role of the phenomenon of electrical breakdown of the membranes itself in pathology is still not absolutely clear. Two facts deserve attention: activation of phospholipase in mitochondria by thyroxine *in vitro* [3] and in animals with hyperthyroidism [6], and the decrease in electrical stability of membranes under the influence of phospholipase A_2 [4]. Can it be that the increase in electrical stability of the membranes is a special type of protective measure of the body in which the thyroxine content is increased and the mitochondrial membranes are consequently threatened with electrical breakdown? Further investigations may provide the answer to this question.

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EFFECT OF PROSTAGLANDINS ON CONDUCTIVITY OF MODEL MEMBRANES

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Prostaglandins (PG) with their broad spectrum of action on various functional states of cells, exert their influence indirectly through the cell membrane. To understand the membrane mechanism of the action of PG it is important to study their effects on model membranes.

In the present investigation the effect of PG E_1 and $F_{2\alpha}$ and of prostacyclin on conductivity of model membranes formed from gangliosides, phosphatidylcholine, and proteolipids, was studied.

EXPERIMENTAL METHOD

Bilayer membranes were formed from phosphatidylcholine by the method of Mueller et al. [4], and membranes from gangliosides and proteolipids isolated from bovine brain containing 28% protein and 72% lipids were prepared by the method suggested by the present writers previously [2, 4]. Electrical measurements were made with a high-ohmic electrometer, using a pair of Ag—AgCl electrodes [1]. The ionic selectivity of the model membranes was determined by the method suggested by Lev [3]. The measurements were made in solutions containing 0.1 M KCl, NaCl, LiCl, CsCl, and $CaCl_2$ at 26°C.

All points used to plot curves shown on the graphs represent mean values of at least six measurements on two or three different films.

The phosphatidylcholine was from the Kharkov Bacterial Preparations Factory, the PG from the Upjohn Company (USA).

EXPERIMENTAL RESULTS

Model membranes formed from components of cell membranes, namely phosphatidylcholine and gangliosides, had low conductivity. Conductivity of bilayers of phosphatidylcholine for potassium and calcium ions was $(2.3 \pm 0.3)\cdot10^{-8}$ and $(3.5 \pm 0.2)\cdot10^{-8}$ $\Omega^{-1}\cdot cm^{-2}$, respectively. The conductivity of ganglioside membranes was low for potassium ions and high for calcium ions — $(1.8 \pm 0.2)\cdot10^{-8}$ and $(36.5 \pm 0.3)\cdot10^{-6}$ $\Omega^{-1}\cdot cm^{-2}$, respectively. Membranes modified by PG E_1 and $F_{2\alpha}$ and by prostacyclin increased conductivity by several orders of magnitude depending on the PG concentration. The highest conductivity of model membranes made from phosphatidylcholine and gangliosides, modified by PG E_1 and $F_{2\alpha}$, for potassium ions (an increase of 2-3 orders of magnitude) occurred in the presence of PG in a concentration of 10^{-10}-10^{-6} M (Fig. 1a, b, curves 1 and 2), whereas the conductivity of lecithin membranes for calcium ions (increased by 3-4 orders of magnitude) was highest when modified by concentrations of 10^{-9}-10^{-5} M, and that of ganglioside membranes (an increase of one or two orders of magnitude) was highest when modified by PG in concentrations of 10^{-9}-10^{-6} M (Fig. 1c, d, curves 1 and 2). Conductivity of membranes modified by prostacyclin (Fig. 1, curves 3) differed from that of membranes modified by PG E_1 and $F_{2\alpha}$. The highest conductivity was created by prostacyclin in the case of phosphatidylcholine bilayers for all ions in a concentration of 10^{-8} M (an increase of four orders of magnitude) and in the case of ganglioside membranes for po-

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