9. F. E. Hunter, A. Scott, J. Weinstein, et al., J. Biol. Chem., 239, 622 (1964).
10. F. E. Hunter, J. M. Gebicki, B. E. Hoffstein, et al., J. Biol. Chem., 238, 828 (1963).
11. S. K. Kumar, R. Walls, and P. Hochstein, Arch. Biochem., 180, 514 (1977).
12. M. Neradilova, F. Hruba, V. Novakova, et al., Čas. Lek. Česk., 117, 264 (1978).
13. O. M. Saka and H. A. Impren, in: Advances in Thyroid Research, Oxford (1961), p. 520.

INCREASE IN BREAKDOWN POTENTIAL OF LIPOSOMES FORMED FROM LIVER MITOCHONDRIAL LIPIDS OF HYPOTHYROID RABBITS

A. I. Marzoev, O. M. Parnev, and Yu. A. Vladimirov

UDC 616.441-008.64-092. 616.36-008.939.15-073.7

KEY WORDS: liposomes; mitochondrial lipids; thyroidectomized rabbits; hypothyroidism.

It was shown previously that the breakdown potential is increased in liposomes and bilayer membranes formed from liver mitochondrial lipids of hyperthyroid rabbits [4]. The value of the breakdown potential, reflecting the electrical stability of model membranes, is determined by the spectrum of phospholipids composing them and the physicochemical characteristics of the fatty acid chains of these compounds. Thyroid hormones take part in the regulation of lipid metabolism, and under these circumstances properties of the lipids that are important for the value of the breakdown potential are changed [5, 6-9]. It was natural to suggest that if the electrical stability of the lipid membranes is increased in hyperthyroidism, a fall in the value of the breakdown potential ought to be observed in hypothyroidism, as it it is in nearly all other cases in which the biological action of thyroid hormones has been studied. The investigation described below was devoted to an experimental verification of this hypothesis.

EXPERIMENTAL METHOD

Rabbits weighing 2.0-3.0 kg were used. Hypothyroidism was induced by thyroidectomy under pentobarbital anesthesia. Mitochondria were isolated from the liver by the method previously described [1]. Lipids were extracted from the mitochondria by the method of Bligh and Dyer [6]. Liposomes were obtained by dispersion of the isolated lipids (0.2 mg/ml) in a 10 mM sucrose solution (pH 7.0). The value of the breakdown potential of the liposomes and the "aggregation parameter," reflecting the negative charge on their surface, were measured by the method in [3]. Puchkova, working in the writers' laboratory, has shown that water-soluble lipid peroxidation products formed during autooxidation or as a result of UV-irradiation can lower the breakdown potential of liposomes. Accordingly, in a series of experiments the antioxidant β-ionol was added to all the isolation media of mitochondria and liposomes in a final concentration of 0.1 μM. However, no difference could be found in the electrical parameters between lipids isolated in media with or without the antioxidant.

EXPERIMENTAL RESULTS

Typical curves showing the change in scattering of light by the liposomes on addition of potassium acetate are given in Fig. 1. The minimum of the curves corresponds to the transition from compression of the liposomes in hypertonic medium (a decrease in light transmittance) to swelling of the liposomes, taking place as a result of electrical breakdown of the membranes and entry of electrolyte inside the liposomes [3]. The concentration of potassium acetate which induced breakdown of these structures was higher in liposomes formed from liver mitochondrial lipids of hypothyroid animals than in the corresponding preparations from normal animals. This increase signifies an increase in the breakdown potential of the liposomes, calculated by the method in [3] (Table 1). It should be noted that a similar value of the breakdown potential of liposomes was observed in hyperthyroid animals also [4]. Changes in

Department of Biophysics, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 38-40, March, 1982. Original article submitted August 7, 1981.

 0007-4888/82/9303-0272$07.50

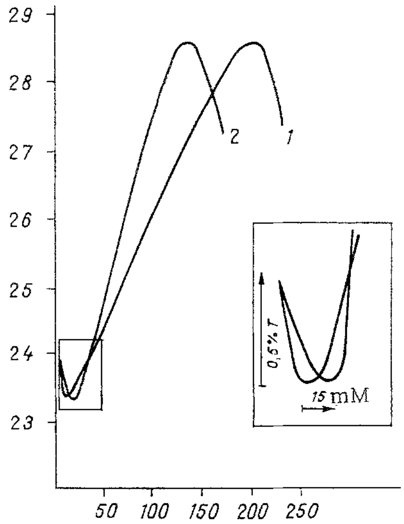

Fig. 1. Effect of thyroid state on transmittance of liposomes formed from phospholipids of liver mitochondria of normal (1) and hypothyroid (2) rabbits. Abscissa, concentration of potassium acetate in sample (in %); ordinate, transmittance of liposome suspension (in %).

TABLE 1. Effect of Thyroid State on Breakdown Potential and "Aggregation Parameter" in Liver Mitochondrial Liposomes

Parameter studied	Normal	Hypothyroidism
Breakdown potential, in mV	144,3±0,9	156,8±1,0
Aggregation concentration, mM $(NH_4)_2SO_4$	209,5±7,4	133,2±13,1

Legend. Mean values obtained with seven normal and seven thyroidectomized rabbits are shown.

the properties of the lipids in hypothyroid animals also consisted in a decrease in the total negative charge of their surface, as shown by a decrease in the concentration of ammonium sulfate necessary to start aggregation of lipid vesicles (Table 1).

The breakdown potential of phospholipid membranes depends on a number of factors, including the composition of the phospholipids present in the membrane system, the chain length and degree of unsaturation of the fatty acids, the viscosity of the bilayer, the surface charge, and the surface tension on the boundary with the aqueous phase [9]. All these factors make a concrete interpretation of the results of the present investigation difficult. It can be tentatively suggested that in this case changes in the index of unsaturation of the fatty acids in thyroidectomized animals with a low level of thyroid hormone [6] may to some degree determine the value of the breakdown potential. The fact that both an excess and a deficiency of thyroid hormones produces an effect in the same direction — an increase in the breakdown potential in both cases — will be noted. It can be postulated that the molecular mechanism is different in the two cases, although further research is needed to shed light on this problem. The reduced ability of the liposomes to counteract aggregation induced by $(NH_4)_2SO_4$ can be explained by a decrease in the fraction of negatively charged phospholipids in their total fraction. In particular, a decrease in the cardiolipid content in the liver mitochondria in hypothyroidism and recovery of its normal level after treatment with thyroid hormones have been reported [8]. Injection of thyroid hormones into animals with hypothyroidism leads to changes within a few days in the kinetics of lipid peroxidation by liver mitochondria [2] and corrects the character of dependence of mitochondrial enzyme activity on temperature [6, 7, 10]. The authors cited explain these phenomena by changes in synthesis of linoleic and certain minor highly unsaturated fatty acids [6]. In the present experiments hypothyroid rabbits also received a single dose of thyroxine (300 μg/kg body weight, intraperitoneally). Liposomes isolated from the mitochondria of these animals 41 h after injection of the hormone had the normal level of their breakdown potential restored (141.8 ± 0.35 mV) and their "aggregation parameter" largely so, to 184.3 ± 3.9 mM $(NH_4)_2SO_4$. In similar experiments when liposomes were isolated from mitochondria 24 h after injection of the hormone, no changes could be found in the electrical characteristics of the object compared with those of liposomes from hypothyroid animals used as the control. Incubation of liposomes in media with different thyroxine concentrations (10^{-7}-10^{-5} M) did not affect the electrical properties of these structures, and only in a concentration of 10^{-4} M was some increase observed in the breakdown potential with a decrease in the $(NH_4)_2SO_4$ concentration required to produce aggregation.

In hypothyroidism electrical stability of the liposomes prepared from phospholipids of liver mitochondria is thus increased. The increase is accompanied by a decrease in the negative charge on the surface of the liposomes. Perhaps differences in the electrical properties of the lipids observed in normal and hypothyroid animals may be due to changes in their phospholipid and fatty-acid composition which other workers have described [5, 9].

LITERATURE CITED

1. A. I. Marzoev, A. V. Kozlov, A. P. Andryushchenko, et al., Byull. Eksp. Biol. Med., No. 3, 36 (1982).
2. A. I. Marzoev, A. V. Kozlov, and Yu. A. Vladimirov, Byull. Eksp. Biol. Med., No. 3, 38 (1982).
3. O. M. Parnev, T. V. Puchkova, A. V. Putvinskii, et al., Abstracts Deposited in the All-Union Institute of Scientific and Technical Information, No. 5391 (1980).
4. E. J. Bligh and W. J. Dyer, Can. J. Biochem. Physiol., 37, 911 (1959).
5. I. Y.-D. Chen and F. L. Hoch, Arch. Biochem., 181, 470 (1977).
6. A. J. Hulbert, M. L. Augee, and J. K. Raison, Biochim. Biophys. Acta, 455, 597 (1976).
7. S. Jacovcic, H. H. Swift, J. L. Nicholas, et al., J. Cell Biol., 77, 887 (1978).
8. S. Ohki and D. Papahadjopoulos, in: Surface Chemistry of Biological Systems, New York (1970), pp. 155-174.
9. J. M. Shaw and F. L. Hoch, J. Mol. Cell. Cardiol., 9, 749 (1977).