EFFECT OF α-TOCOPHEROL ON THE PHYSICOCHEMICAL PROPERTIES OF LIVER CELL MEMBRANES OF ADRENALECTOMIZED RATS

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One manifestation of the disturbance of metabolism after adrenalectomy is activation of lipid peroxidation (LPO) reactions [2]. This activation may be both the cause and the effect of disturbance of the physicochemical properties of biological membranes. It can be tentatively suggested that these disturbances take place also after adrenalectomy and play a definite role in lowering the resistance of the body to unfavorable factors.

In the present investigation the fluorescent probe pyrene was used to study structural changes in nuclear and microsomal membranes of rat liver cells after adrenalectomy and the effect of α-tocopherol (TP) on these changes. TP is known to increase the resistance of the body to extremal factors, and the mechanism of its protective action is linked not only with ability to prevent activation of LPO, but also with its effect on the state and reactivity of the adrenal cortex [5].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 170-220 g in the winter and spring. Bilateral adrenalectomy and the mock operation on the control animals were performed 8 days before the investigation. The rats were kept in individual cages, and half of them received 4 mg TP acetate daily (in a 10% oily solution) for 7 days after the operation, whereas the other half received the same volume of sunflower oil. The rats were killed by decapitation and the content of TP [9], and LPO products — diene conjugates [4] and malonic dialdehyde (MDA) [8] — in the liver was investigated. Microsomal membranes [3] and nuclei of the liver cells were isolated [6]. The microviscosity of the lipid component was investigated by means of the fluorescent probe pyrene by determining the efficiency of excimerization of the probe; the wavelength of the exciting light was 334 nm, that of fluorescence of the monomers 372 nm, and of the excimers 470 nm [1]. Protein-lipid interactions were judged from the efficiency of quenching of tryptophan fluorescence at 330 nm by pyrene [1]. The Hitachi MPF-4 spectrofluorometer (Japan) was used, the diameter of the cuvette was 0.5 cm, and the protein concentration 0.2 mg/ml. The working capacity of the rats was estimated as the maximal duration of swimming in water at 30°C.

EXPERIMENTAL RESULTS

The experiments showed that adrenalectomy increases the viscosity of the lipid component of the microsomal and nuclear membranes of the liver cells, as shown by a marked decrease in the efficiency of pyrene excimerization (Fig. 1). The increased efficiency of quenching of tryptophan fluorescence by pyrene in suspensions of the test membranes reflected a shortening of the distance between proteins and lipids and, consequently, an increase in the packing density of the membranes (Fig. 2). These changes in membrane structure could be the results of activation of LPO [1]. In fact, the MDA content in the liver of the adrenalectomized rats was increased when very small differences were present in the level of primary LPO products, namely diene conjugates (Table 1). Incidentally, not only was the TP concentration in the liver of the experimental rats not reduced, but it actually showed a tendency to rise (Table 1). The working capacity of the experimental animals was reduced by 1.7 times ($p < 0.05$).

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TABLE 1. Content of TP and LPO Products in Liver of Control and Adrenalectomized Rats Receiving Normal Diet and Diet with Added TP (M ± m)

Parameter	Group of animals				p
	control	control + TP	adrenalectomized	adrenalectomized +TP	
TP, mg/100 g liver	1,32±0,06 (15)	4,1±0,08* (20)	1,58±0,06*** (17)	5,2±0,1 (12)	$p_{1-2,\ 3-4} < 0{,}001$ $p_{1-3} < 0{,}05$
Diene conjugates, D 233 nm/mg liver lipids	0,95±0,07 (14)	0,89±0,04 (14)	1,28±0,05*** (9)	1,04±0,09 (8)	$p_{1-3} < 0{,}01$
MDA, nmoles/g liver	27,0±2,0 (10)	17,7±1,8* (10)	46,1±3,8*** (8)	23,0±0,7 (8)	$p_{1-2,\ 1-3,\ 3-4} < 0{,}001$

Legend. Number of animals given in parentheses.

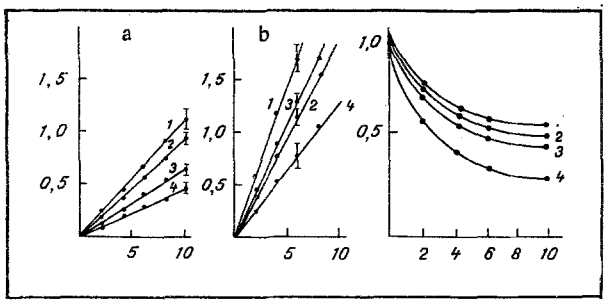

Fig. 1 Fig. 2

Fig. 1. Effect of TP on excimerization of pyrene in lipids of nuclear (a) and microsomal (b) membranes of liver cells of control and adrenalectomized rats. Abscissa, pyrene concentration (in μM); here and in Fig. 2, ordinate, efficiency of pyrene excimerization. Here and in Fig. 2: 1) control animals receiving TP; 2) adrenalectomized rats receiving TP; 3) control animals; 4) adrenalectomized.

Fig. 2. Decrease in intensity of fluorescence of tryptophan residues of nuclear proteins on the addition of pyrene. Ordinate, efficiency of quenching of tryptophan residues (in relative units).

Addition of TP to the diet was followed by its accumulation in the liver of the control and experimental rats (Table 1). In the control animals the level of LPO products fell, in the adrenalectomized animals its rise was prevented. The viscosity of the membrane lipids of the control and experimental rats was reduced by TP; the efficiency of pyrene excimerization in membrane suspensions from adrenalectomized rats receiving TP was the same as in the control, but in the microsomal membranes it was higher (Fig. 1). Investigation of the efficiency of quenching of tryptophan fluorescence by pyrene showed that TP prevented changes in this parameter caused by adrenalectomy and reduced its value a little in the control animals (Fig. 2). Thus in the latter some "loosening" of the packing of the membranes was observed, a process which increases the mobility of the protein molecules and may contribute to increased activity of membrane-bound enzymes. Addition of TP to the diet increased the working capacity of the control rats by 1.6 times ($p < 0.01$) and of the adrenalectomized rats by 2.2 times ($p < 0.01$), and it thus prevented the fall of this parameter produced by adrenalectomy.

It must be emphasized, in conclusion, that activation of LPO and disturbance of the physicochemical properties of the nuclear and microsomal membrane of the liver cells took place in adrenalectomized rats in which the TP content was unchanged. Addition of extra TP to the diet of these rats prevented activation of LPO and the associated increase in microviscosity of the membrane lipids and disturbances of protein-lipid interactions. This evidently contributed to the full restoration of the animals' working capacity. The mechanism of the protective action of TP may perhaps be connected with acceleration of recovery of the production of corticosteroids, which participate in the stabilization of the structure of biological membranes [7].

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