POTENTIATION OF THE DAMAGING ACTION OF FREE FATTY ACIDS ON BRAIN SYNAPTOSOMES BY VITAMIN E DEFICIENCY

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Vitamin E protects biomembranes from lipid peroxidation (LPO) both *in vitro* and *in vivo* and in particular, in vitamin E deficiency [13]. However, inhibitors of free-radical oxidation, of varied chemical structure, cannot completely protect biomembranes from damage arising in the course of development of alimentary avitaminosis E in animals [4]. This means that besides activation of the LPO process there are also other important pathogenetic mechanisms which become activated in biomembranes in vitamin E deficiency. It has recently been shown that α-tocopherol (TP) can protect biomembranes from damage caused by accumulation of free fatty acids (FFA) in them [8], by forming stable complexes with the acids [9]. Since the development of alimentary avitaminosis E is accompanied by changes in the lipid composition of biomembranes [11] and, in particular, by an increase in their FFA content [7, 12], it was natural to suggest that one mechanism of injury to membranes in vitamin E deficiency is through an increase in their sensitivity to the action of FFA, which cannot be bound by concentrations of TP present in the membranes.

To test this hypothesis experimentally the effectiveness of the damaging action of FFA on synaptosomes, isolated from the brain of control and vitamin E-deficient animals was compared.

EXPERIMENTAL METHOD

Synaptosomes were isolated from the gray matter of the brain of control and vitamin E-deficient rats by the method in [10]. The protein concentration was determined by the method in [6]. The control and vitamin E-deficient animals were kept on the corresponding diets for 4 months, as described in [3]. Plasma vitamin E levels were 9.1 ± 0.1 and 0.8 ± 0.2 μg/ml for control and E-deficient rats respectively. Ca^{++} transport was assessed by the isotopic labeling method. Changes in the transmembrane potential (TMP) of the synaptosomes were recorded by means of the fluorescent probe 3,3-dipropyl-2,2-thiocarbocyanide by the method in [1]. TP, FFA, and di-S-C_3-(5) were added to the suspension of synaptosomes in alcoholic

TABLE 1. Fraction of Archidonic Acid on ^{45}Ca Transport into Brain synaptosomes of Control and E-Deficient rats (M ± m)

Experimental conditions	^{45}Ca accumulation, nmoles/mg protein	
	control	avitaminosis E
Resting medium	4,7±0,3	4,0±0,4
Depolarizing medium	6,8±0,5*	5,9±0,4
Resting medium + arachidonic acid (10 μM)	6,5±0,3*	14,5±0,9
Resting medium + arachidonic acid (100 μM)	14,0±0,7*	—
Depolarizing medium + arachidonic acid (100 μM)	7,2±0,5	—

Note. *P < 0.05 compared with resting medium.

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solution, the volume of which did not exceed 1% of the volume of the suspension. The effect of FFA on synaptosomes was studied in resting medium (20 mM HEPES, 145 mM NaCl, 1.5 mM KCl, 5 mM $NaHCO_3$, 1.3 mM $MgCl_2$, and 10 mM glucose), when TMP of the synaptosomes was —70 mV, and in depolarizing medium (0 mV), differing from the resting medium only in concentrations of KCl (145 mM) and NaCl (5 mM). The oleic, linoleic, and arachidonic acids used were from Sigma (USA), the HEPES, sucrose, glucose, and d-α-TP were from Serva (West Germany). The di-S-C_3-(5) was synthesized by A. Barronopo (USA). The remaining reagents were of Soviet origin and were at least of the chemically pure grade.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of arachidonic acid was compared on Ca^{++} transport into brain synaptosomes of control and E-deficient rats. Inflow of Ca^{++} was estimated by the accumulation of ^{45}Ca by the synaptosomes after incubation for 10 min with the isotope. In the absence of a fatty acid and during incubation in resting medium, significantly less ^{45}Ca was found to accumulate in the synaptosomes than in the depolarizing medium (Table 1). This fact is in good agreement with results obtained previously showing the existence of potential-dependent permeability channels for Ca^{++} [2, 5]. No significant differences in Ca^{++} accumulation by synaptosomes of the control and vitamin E-deficient animals could be found.

Incubation of the synaptosomes in resting medium in the presence of 10 μM arachidonic acid caused a 50% rise in the level of ^{45}Ca accumulation, but if the concentration of the acid was increased by an order of magnitude (up to 100 μM) Ca accumulation was increased by an order of magnitude (up to 100 μM) Ca accumulation was increased by a further twice. Incidentally, 100 μM of arachidonic acid does not increase Ca^{++} transport into synaptosomes in depolarizing medium.

Incubation of brain synaptosomes of vitamin E-deficient rats in resting medium with 10 μM arachidonic acid led to an increase in Ca^{++} transport, which was comparable in value with the action of 100 μM arachidonic acid on the synaptosomes of control animals. Thus arachidonic acid is a more effective activator of Ca^{++} transport into brain synaptosomes of vitamin E-deficient animals than of controls. Incidentally, the stimulating action of arachidonic acid on synaptic membranes of the rat brain depends on the membrane potential.

In the experiments of series II, by recording potential-dependent fluorescence (PDF) of di-S-C_3-(5) changes in TMP were studied in synaptosomes from the brain of control and E-deficient animals on the addition of FFA. It must be noted that the intensity of fluorescence in the suspension of synaptosomes in depolarizing medium was unchanged after the addition of fatty acids (arachidonic or linoleic) up to concentrations of $8 \cdot 10^{-6}$ M, and of TP up to 10^{-5} M. Addition of arachidonic acid to the suspension of synaptosomes in resting medium caused their depolarization (Fig. 1), manifested as an increase in the intensity of PDF of di-S-C_3-(5). Comparison of the action of arachidonic acid on syaptosomes of the control and and E-deficient animals revealed two effects: 1) Saturation concentrations of arachidonic acid causing the maximal increase in PDF were lower for the experimental than for the control group ($4 \cdot 10^{-6}$ and $6 \cdot 10^{-6}$ M, respectively); 2) the amplitude of the maximal change of PDF under the influence of saturating cpncentrations of arachidonic of the maximal change of PDF under the influence of saturating concentrations of arachidonic acid was lower in the experimental than in the control group. Under the influence of linoleic acid on synaptosomes under analogous conditions, these two effects were manifested even more clearly: saturating concentrations of linoleic acid were $4 \cdot 10^{-6}$ and $8 \cdot 10^{-6}$ M, respectively, and the amplitude of the maximal change in PDF differed by more than twice (Fig. 1). Starting from the quantitative differences in the action of arachidonic and linoleic acids on synaptosomes of the control and experimental groups (which may be due to differences in their ability to become incorporated into the membranes, differences in the rate of their metabolism by cyclo-oxygenases, or differences in their content of auto-oxidation products) it can be concluded that synaptosomal membranes of E-deficient animals are more sensitive than the controls to the action of exogenously added FFA, and are initially more severely damaged, for in resting medium they gave smaller changes in PDF, i.e., less marked depolarization. Assuming that disturbance of the organization of the synaptosomal membranes in vitamin E deficiency is caused by accumulation of LPO products and the action of FFA, uncompensated by TP, the choice between these two mechanisms of damage can be made on the basis of experiments with addition of TP *in vitro*. TP can interact with FFA, to form complexes with them, and thus, as it were, neutralizing their perturbing action in the membranes, but cannot avoid the modifying effect of LPO products already formed, since it interacts with rad-

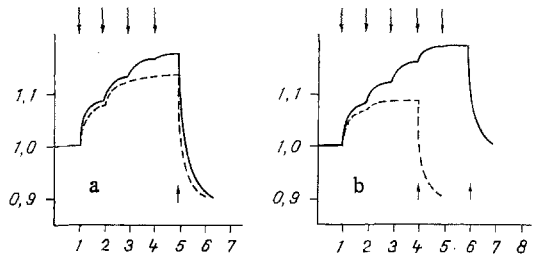

Fig. 1. Kinetics of change in PDF of di-S-C_3-(5), incorporated into membranes of synaptosomes isolated from the brain of control (continuous line) and E-deficient (broken line) rats under the influence of arachidonic (a) and linoleic (b) fluorescence (in relative units). Arrow pointing downward, times of addition of 10^{-5} M FFA; arrows pointing upward, times of addition of $5 \cdot 10^{-5}$ TP. Concentration of probe 10^{-8} M, of synaptosomes 0.3 mg protein/ml. Fluorescence of the probe was excited by light with a wavelength of 650 nm, recorded at 688 nm.

ical intermediates of LPO, and not with its molecular products [13]. It was shown that addition of TP to a suspension of synaptosomes from E-deficient animals, in concentrations exceeding that of the added FFA, not only restores the value of TMP (PDF of di-S-C_3-(5), but also induces considerable hyperpolarization. Thus damage to the synaptosomal membranes recorded in viataminosis E is based on a factor that is removable by exogenous TP, which differs from LPO products, and can be identified as an FA. In fact, as well be clear from Fig. 1, exogenously added TP abolishes damage caused by the action of FFA on brain synaptosomes from control and E-deficient animals (differences in the effectiveness of protective action of TP in the presence of linoleic acid can probably be attributed to a difference in the fatty acid–TP ratio, which was 1:1 for the control and 0.6:1 for the experiment.

To sum up the results it can be concluded that vitamin E deficiency increases the sensitivity of the synaptosomal membranes of the brain to the damaging action of FFA. This effect is reversible and is abolished by incorporation of additional quantities of exogenous TP into the membranes. The results confirm the hypothesis of universality of the stabilizing effect of vitamin E on biological membranes *in vivo*, through a mechanism of interaction of FFA, which was discovered previously in homogeneous systems (solutions) and in membrane fragments *in vitro* [8].

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