Release of ^{3}H-5-HT from the hypothalamus of neonatal and adult animals is shown graphically in Fig. 3. The rate of spontaneous secretion of the monoamine in neonatal animals was higher than in adults. The reaction to depolarization approached in magnitude the level observed in adult rats, and was distinctly Ca^{++}-dependent in character.

Our findings agree with the results of Nomura's investigation [7], which revealed high affinity of whole brain synaptosomes for 5-HT, and also depolarization-induced Ca^{++}-dependent secretion of labeled 5-HT, noradrenalin, and dopamine from whole brain sections from 18-day rat fetuses, and also with the findings of Barachovsky and Bradford [1], who studied 3-day cultures of whole brain of 17-day fetuses.

By the 17th day of prenatal development, controlled secretion of serotonin from hypothalamic nerve fibers thus becomes possible in response to depolarization. The response of neonatal animals to the depolarizing signal becomes the same as that of adult animals.

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TEMPERATURE STABILIZING EFFECT OF TOCOPHEROL ON RHODOPSIN IN THE PRESSURE OF FATTY ACIDS STUDIED BY DIFFERENTIAL SCANNING CALORIMETRY

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Hydrolysis products of phospholipids by phospholipase A_2, namely free fatty acids and lysophospholipids, together with lipid peroxidation (LPO) products, are factors modifying the properties of the lipid bilayer of membranes and also of a number of integral membrane proteins [6, 12]. The writers showed previously that free fatty acids (but not lysophospholipids), and also LPO products can lower the thermostability of the visual pigment rhodopsin in photoreceptor membranes [4, 10, 11]. It was also found that α-tocopherol (TP) has a protective action against thermal denaturation of rhodopsin, which is manifested during LPO only after preliminary introduction into the membranes, whereas in the case of free fatty acids, it is manifested on the addition of TP either before or after the fatty acid [4, 10].

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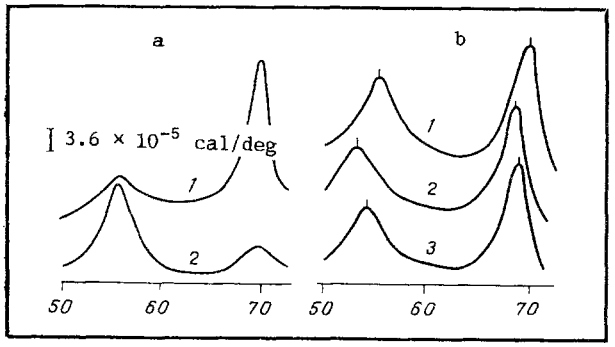

Fig. 1. Thermograms of different preparations of a suspension of bovine PRM. a) Effect of decolorization of PRM on heat absorption curves: 1) dark preparation of PRM, 2) decolorized preparation of PRM; b) effect of 2.5 moles % of arachidonic acid and 5 moles % of TP on heat absorption curves; 1) control preparation of PRM; 2) control preparation of PRM + arachidonic acid; 3) control preparation of PRM + arachidonic acid + TP. Abscissa, temperature (in °C).

Explanation of the mechanisms of the protective action of TP is important because a disturbance of the conformational stability of the visual pigment, taking place in the presence of LPO products and free fatty acids within a temperature range close to physiological may be the cause of the "dark noise" in the visual information processing channel [1, 5].

For these reasons, in the present investigation in order to study denaturation temperature transitions of the visual pigment rhodopsin, and also of its colorless form opsin, and their changes in the presence of free fatty acids and TP, we used a direct method of recording thermal denaturation of membrane proteins, namely differential scanning calorimetry.

EXPERIMENTAL METHOD

Photoreceptor membranes were obtained from bovine retina as described previously [1]. The protein concentration was determined by a modified Lowry's method [13]. The microcalorimetric measurements were made on the DASM-4 differential adiabatic microcalorimeter. The accuracy of recording the temperature of the specimens was 0.2°C. Thermal denaturation of rhodopsin in a suspension of photoreceptor membranes (PRM) was carried out in 0.1 M Na-phosphate buffer, 0.1 M NaCl, pH 7.4. The concentration of rhodopsin (opsin) in the PRM suspension was determined from the absorption spectrum, the molar coefficient of extinction at 500 nm being taken to be 42,000 cm/mole. Chromatographically pure alcoholic preparations of arachidonic acid ("Koch-Light," England) and D,L-α-tocopherol ("Serva," West Germany) were added to the PRM suspension; the ethanol concentration did not exceed 0.1-0.5%.

EXPERIMENTAL RESULTS

Typical thermograms of a suspension of bovine retinal PRM are given in Fig. 1. Two peaks will be observed on the heat absorption curve. The first and wider peak has a temperature maximum of $T_1 = 55.2°C$, the second with a maximum at $T_2 = 69.5°C$. Values of molar enthalpy of the transitions (ΔH) are given in Table 1. The first peak corresponds to thermal denaturation of the decolorized pigment (opsin), the second to that of the nondecolorized visual pigment (rhodopsin). This conclusion can be drawn from the fact that after decolorization of PRM the amplitude of the low-temperature peak rises sharply and that of the high-temperature peak falls correspondingly, i.e., one temperature peak is apparently "pumped" into the other. These observations are in agreement with known facts on the higher stability of the rhodopsins than of opsin [1, 9].

Addition of arachidonic acid to the PRM suspension caused a concentration-dependent shift of both peaks into the region of lower temperatures (Fig. 1b, curve 2; Fig. 2) and a simultaneous decrease in the value of ΔH. The action of the fatty acid rose in a straight line within the concentration range of 1 to 7 moles %, after which the curve reflecting this dependence reached saturation. It is interesting to note that in the initial part of the concentration curve no change

TABLE 1. Effect of Arachidonic Acid and TP on Thermal Denaturation of Opsin and Rhodopsin in Photoreceptor Membrane (M ± m)

Preparation	Opsin			Rhodopsin		
	T_m, °C	$\Delta t_{1/2}$ °C	ΔH, kcal/mole	T_m, °C	$\Delta t_{1/2}$, °C	ΔH, kcal/mole
Control (without addition of fatty acid or TP)	55,2±0,2	3,6±0,1	101,9±4,9	69,5±0,2	2,8±0,1	127,5±4,8
With addition of 2.5 moles % of fatty acid	53,4±0,3	3,9±0,2	89,5±3,5	68,2±0,2	2,9±0,1	117,7±3,5
With addition of 2.5 moles % of fatty acid and 5 moles % of TP	54,1±0,2	3,7±0,2	92,5±6,5	68,9±0.2	2,9±0.1	120,4±4,9

Legend. T_m) Temperature maximum of heat absorption.

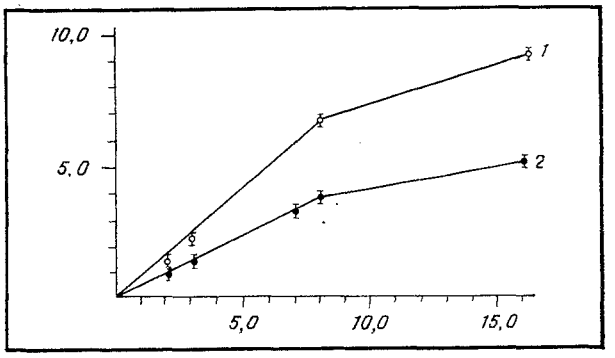

Fig. 2. Dependence of denaturation temperature of rhodopsin and opsin on arachidonic acid concentration in PRM. 1) Change in heat absorption peak of opsin, 2) change in heat absorption peak of rhodopsin. Abscissa, arachidonic acid concentration (in moles %), ordinate, temperature difference $T_1 - T_2$ (in °C), T_1) transition temperature of control peak, T_2) transition temperature in presence of fatty acid.

was observed in the half-width of the temperature transitions, whereas with higher concentrations of the fatty acid the half-width of the transition increased both for opsin and for rhodopsin.

Addition of TP to the PRM suspension within the concentration range 1-5 moles % caused no significant changes in the thermograms. On insertion of 5 moles % TP into the membranes followed by addition of 2.5 moles % of the fatty acid, the shift of both the low-temperature and the high-temperature peaks was less marked than with the action of the fatty acid alone (Fig. 1b, curve 3; Fig. 2). Meanwhile an increase was recorded in molar values of the enthalpy of transition ΔH, although they did not reach the control values, being depressed as a result of the action of the fatty acid (Table 1). The stabilizing effect of TP was exhibited about equally with both opsin and rhodopsin, although in absolute values, the "reverse shift" of the denaturation temperatures of opsin is higher (ΔT_1 = 1°C) than that of rhodopsin (ΔT_2 = 0.7°C). However, the relative shifts of the denaturation temperatures were virtually identical (50% of the value of the reduction of the denaturation temperature caused by addition of the fatty acid). It can be tentatively suggested that the stabilizing effect of TP is based on the formation of its complexes with free fatty acids, demonstrated previously [7]. Indirect proof of this is given by the fact that in cases when TP was mixed beforehand with the fatty acid in solution in ethanol and added in the form of this mixture to the PRM suspension, the protective effect reached 100%. It must also be emphasized that the protective action of TP was manifested only in the region of concentrations of fatty acids not exceeding 2-5 moles %, i.e., in the range of concentrations in which no change takes place in the cooperativeness of the transition, determined from the half-width of the peaks. It will also be noted that these amounts of free fatty acids accumulate in PRM in various pathological states (stress, hypoxia, degenerations, retinopathies, etc.), whereas under normal conditions their concentration does not exceed 0.5 mole % [6, 8].

The results agree with data published previously on the ability of TP to "restore" values of the thermal denaturation constants of rhodopsin, when sharply reduced as a result of the addition of free fatty acids [4]. The protective action of TP on resistance of other membrane proteins to thermal denaturation, when "labilized" by the action of free fatty acids, has been demonstrated on the example of Ca^{++}-ATPase in membranes of the sarcoplasmic reticulum [3] and of H^+-ATPase in mitochondria [2].

The results illustrate the polyfunctional action of vitamin E in biomembranes and are evidence of its ability to stabilize not only the lipid bilayer, but also membrane proteins. This effect of TP can probably be used to repair damage to the visual pigment rhodopsin and other membrane proteins in pathological states associated with activation of phospholipases A_2.

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PROPERTIES OF GABA-ACTIVATED CHLORIDE CHANNELS IN HIPPOCAMPAL SLICES

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Successful realization of the path clamp method, used for recording single ion channels, requires preparations containing membranes of the test object in the form accessible for direct visual monitoring and manipulation. During the study of the CNS, modern practice is limited to neurons isolated by treatment with enzymes, and nerve tissue culture. In both cases the nerve tissue may be subjected to conditions capable of inducing definite modifications of the structures to be studied [1]. The use of the model of brain slices and, specifically, of transverse hippocampal slices, well known in neurophysiology, as the test object would be a definite step in the direction of the study of native nerve tissue.

Although the suggested preparation is undoubtedly a universal material with which to study ion channels of nerve cells, in this investigation we limit our attention to the analysis of GABA-activated chloride conductance.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats aged 1-4 months. Transverse hippocampal slices 200-300 μ thick were incubated for 1 to 8 h in a salt medium of the following composition: NaCl 130 mM, KCl 5 mM, Na_2HPO_4 0.3 mM, KH_2PO_4 0.4, $MgCl_2$ 0.8 mM, $CaCl_2$ 1.2, $NaHCO_3$ 10 mM, glucose 5 mM, phenol red 10 mg/liter. Sections were placed on a Kapron grid, fixed in a 150-ml jar. The solution was stirred with a magnetic mixer and aerated with carbogen to pH 7.2-7.4. Recording was carried out in a salt medium of the following composition: NaCl 115 mM,

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