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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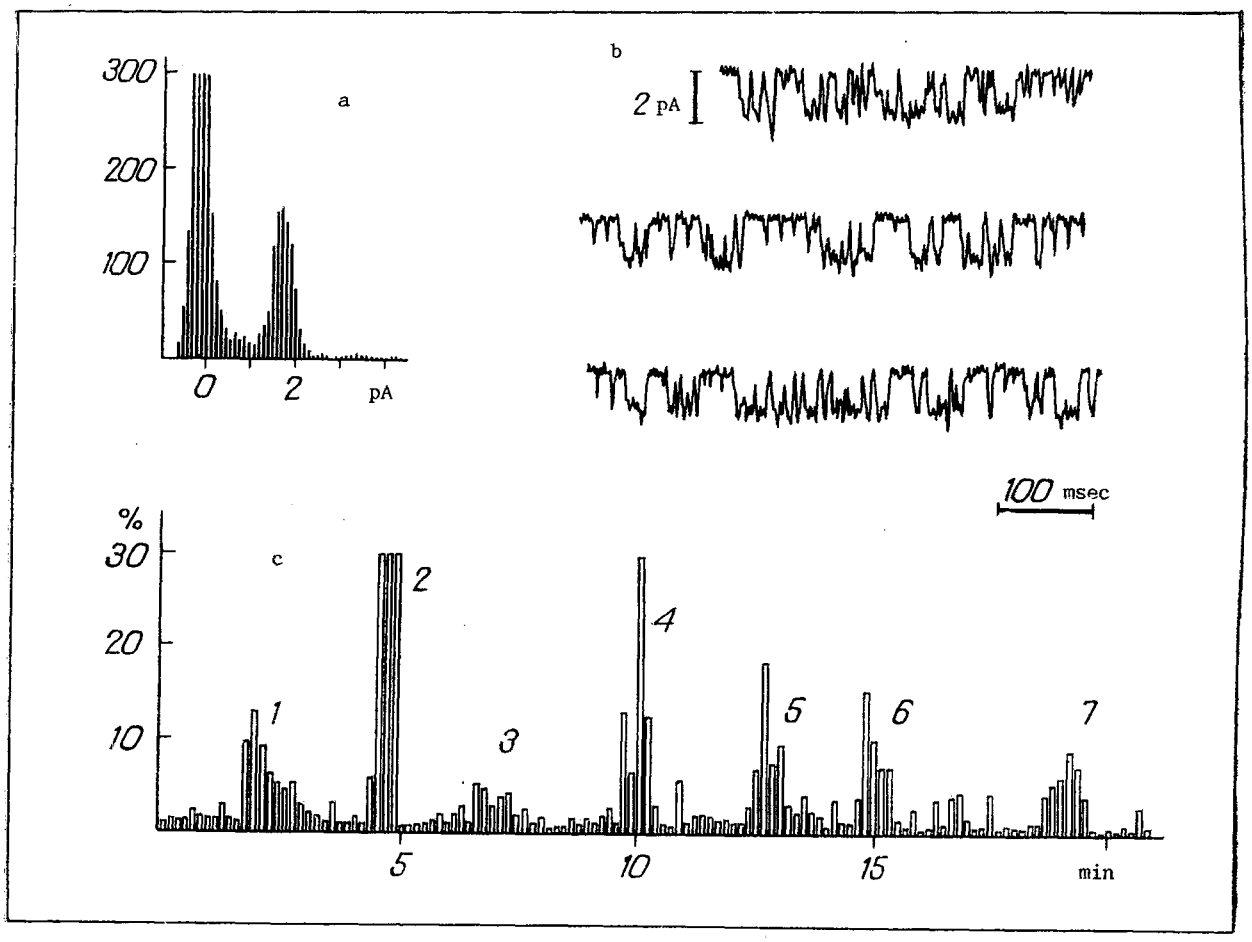

Fig. 1. General characteristics of the preparation. a) Distribution of amplitudes of open state of ion channels in fragment; b) examples of activity; c) histogram of duration of open state (bin width 10 sec). Substances applied: 1, 3) diazepam 1 μM, 2) GABA 1 μM (columns trimmed, true value 75%); 4, 6) GABA 0.5 μM; 5, 7) GABA 0.1 μM and diazepam 1 μM.

TEACl 10 mM, $MgCl_2$ 2 mM, $CaCl_2$ 1 mM, Tris HCl 28 mM, Tris 4.4 mM, pH 7.4. The electrodes were filled with a solution containing: NaCl 105 mM, TEACl 10 mM, Tris HCl 29 mM, Tris 23.6 mM, EGTA 8 mM, $CaCl_2$ 0.4 mM, $MgCl_2$ 0.8 mM, pH 7.4, and had a resistance of 20-40 MΩ. When necessary the slices were transferred by pipet to the recording chamber. Under the control of a binocular microscope, regions CA_1, CA_2, and part of CA_3 of the slice were destroyed by means of sharpened injection needles at the level of the pyramidal layer, and the part of the section containing the alveus and the stratum oriens was discarded. The preparation was washed from the syringe by a jet of filtered extracellular solution, and the dissected region of the section was oriented in a direction opposite to the movement of the recording electrode. The electrode was applied by means of a micromanipulator to the selected neuron and firmly pressed against it. Next followed a series of standard manipulations necessary to form a membrane fragment [4]. The experiments were carried out at room temperature (21-25°C). The activity recorded was digitized at a frequency of 4 kHz and stored in the memory of a computer.

EXPERIMENTAL RESULTS

Membrane fragments obtained from neurons in area CA_1 and in the "outside out" configuration were used. GABA-activated chloride conductance was investigated with a membrane potential of —70 mV. Under the conditions of the potassium current block used in the investigation, this activity was identified by its appearance in response to GABA application. Altogether 57 membrane fragments were recorded (at least one application was made, the recording time exceeded 3 min), six of which preserved acceptable electrical parameters and responded to GABA application in the course of 15-20 min. Standard preparations withstood a voltage of between —100 and +50 mV and had a leakage

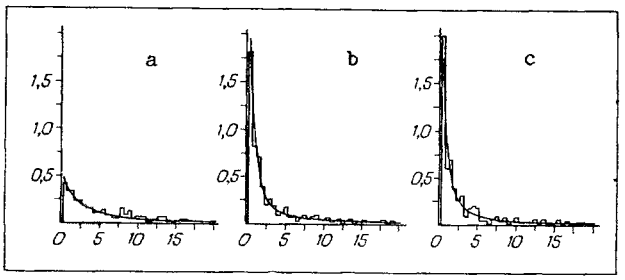

Fig. 2. Comparison of kinetic characteristics of ion channels. Characteristics normalized for number of events in 1 sec. Smooth curve shows approximation of three exponents. a) Insensitive preparation (GABA 2 μM, open state 5.6%, duration of bursts 10.9 msec); b, c) highly sensitive preparation; b) GABA 0.5 μM, open state 6.5%, duration of bursts 7.7 msec), c) (GABA 0.15 + diazepam 1 μM, open state 6.2%, duration of bursts 7.6 msec). Abscissa, duration of bursts (disregarding closures lasting less than 5 msec), bin width 0.5 msec; ordinate, frequency of events with duration corresponding to the given bin.

resistance of 20-40 GΩ. Kinetic parameters recorded during working of the channels were analyzed after manual correction of the zero level. Automated single-threshold discrimination of events at the 0.8 pA level was used.

Usually the membrane fragments had spontaneous activity, which varied widely from preparation to preparation and consisted of short (life 0.4 msec) single sudden changes of current. The total duration of the open state could reach 2.5%. The basic value of conductance of the channels studied in the open state was 26 pC (Fig. 1a). Additionally, on visual analysis it was possible to distinguish one or more extra sublevels [2], which usually had values of 12, 18, 23, and 32 pC. As a rule the probability of transition of a channel into one of these states was low and was uniformly distributed throughout the recording time; however; however, segments of the trace were noted in which one of the sublevels, most frequently 12 pC, became dominant.

During the investigation of evoked activity the main problem was the rapid inactivation of the response to application of the test substances, usually after only a few minutes. To minimize the effects due to this, each application was accompanied by rinsing of the preparation, and as a rule it did not exceed 1 min in duration. Besides the response of the membrane fragment to application of GABA, we were also interested in the character of the action of diazepam on this process. The standard experimental procedure is illustrated in Fig. 1c. First tests were made to see how these two substances act on the preparation separately, choosing a GABA concentration below the threshold for simultaneous opening of several channels. GABA was then applied together with diazepam, in this case a concentration being used which allowed the open state of the channels to be maintained for application of the mediator alone. Stable regions were then distinguished on the histogram of duration of the open state (Fig. 1c) and the kinetic parameters of the response of the preparation in these regions were determined. It was assumed that if diazepam changed the kinetics of the channel, this approach would allow the effect to be assessed quantitatively.

It must first be pointed out that at least two types of preparations were observed, differing mainly in their sensitivity to GABA. For instance, besides membrane fragments which responded to application of the mediator starting with a concentration of 10^{-7} μM, there were others for which this strength was below threshold. Differences also were observed in the quality of the response. Histograms of distribution of the duration of bursts of the open state of the channels in two membrane fragments are given in Fig. 2a, b. Spontaneous activity was absent in both cases. To excite activity with a closely similar duration of the open state, in one case (Fig. 2a) GABA was used in a concentration of 2 μM, whereas in the other case (Fig. 2b) the concentration was 0.5 μM. It will be clear that whereas this response of a preparation insensitive to GABA consisted mainly of bursts, in preparations highly sensitive to GABA much of the duration of the open state of the channels was associated with short openings. Considering that isolated openings of the channels are linked with unimolecular mediator—receptor interaction [3, 6], it can be tentatively suggested that differences between the two families distinguished, in addition to a quantitative difference in sensitivity to the mediator, are due to different relations between the efficiency of single- and double-ligand activation of conductance. The reasons for this state of affairs are not clear. According to subjective impressions, transition of channels into one of the states is determined by the external parameters, such as the membrane properties of the tested neurons, and concrete details of the fragment formation procedure, and does not reflect their native state.

The action of diazepam on GABA-activated chloride conductance consisted of an increase in duration of the bursts of activity and in the frequency of activation of the recorded channels. The presence of diazepam in the medium in a concentration of 1 μM maintained a level of activity of the channels, similar in the duration of the open state, with GABA in concentrations 3-4 times lower than those used when the mediator was applied alone. Analysis of activity under these circumstances against the background of diazepam showed a decrease in duration of the bursts in three experiments, an increase in one, and in one case, in our opinion, no changes were present (Fig. 2b, c). Thus the kinetic parameters of a given level of activity of the channels evidently do not depend on the use of GABA alone or of a mixture of GABA with diazepam, to obtain it. In other words, the addition of diazepam leads to increased efficiency of the action of GABA, which naturally is accompanied by a change in the kinetic parameters of activity, although these changes are not specific in character, for they can be simulated by a corresponding increase in the concentration of the mediator.

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