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EFFECT OF LARGE AND SMALL DOSES OF VITAMIN E ON EXCITABILITY OF FROG CARDIOMYOCYTES

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Fat-soluble vitamin E (tocopherol) is a component of the natural antioxidant system of the cell. According to the most widely held view [4] tocopherols protect unsaturated lipids of biological membranes against peroxidation. Since vitamin E, depending on its concentration, can exert both a labilizing and a stabilizing action on lysosomal membranes [1-3], it was decided to study the effect of different doses of tocopherol directly on the cell surface membrane itself.

By using a technique of intracellular recording of transmembrane potentials, we studied the effect of tocopherol on the resting membrane potential (RP) and on action potential (AP) generation in ventricular fibers of the frog heart.

EXPERIMENTAL METHOD

Preparations of the isolated heart of the frog Rana temporaria were used. Transmembrane potentials of the ventricular myocardium were recorded intracellularly with the aid of "floating microelectrodes." Microelectrodes with the resistance of between 5 and 15MΩ were filled with 3M KCl solution. The preparation was incubated at room temperature in a bath containing Ringer's solution, pH 7.2-7.4.

Daily for 2 weeks large (400 μg/ml) and small (16 μg/ml) doses of an ampul solution of fat-soluble vitamin E in oil (α-tocopherol) were injected intraperitoneally into the frogs. The necessary concentrations of tocopherol for intraperitoneal injection were obtained by diluting the ampul preparation in sterile olive oil as required, in accordance with data on substance 42-1087-77 in the Pharmacopoeia of the Ministry of Health of the USSR. In control experiments, 0.3 ml of the solvent (sterile olive oil, Table 1) was injected into intact frogs of group 1 in accordance with the scheme described above, and in the experiments of group 2, 1 ml of Ringer's solution was injected into intact frogs in accordance with the same schedule.

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TABLE 1. Effect of Large and Small Doses (LD and SD) of Vitamin E and Olive Oil (control) on Excitability of Ventricular Fibers of Frog Myocardium (M ± m)

Substance tested	Dose	RP	Amplitude of AP	Overshoot	Duration of AP	Number of experiments
Ringer's solution	1 ml	98,2±1,4	102,6±1,4	9,79±0,58	679±13,9	14
Olive oil	0,3 ml	92,4±0,95	101,6±1,3	9,14±0,83	725,7±12,7	7
Vitamin E	SD, 16 μg/ml	92,6±0,7	102,2±0,76	9,55±0,46	749±8*	20
	LD, 400 μg/ml	78,5±1,8*	97,8±2,2*	19,3±1,2*	579±20,8*	22

Legend. *$p \leq 0.05$: Mean differences from control within each group are significant.

EXPERIMENTAL RESULTS

The results are given in Table 1.

Under the influence of large doses of vitamin E a significant decrease was observed in RP of the cardiomyocyte membrane by 20% compared with the value of RP in the control experiments, taken as 100%, and also the subsequent changes in the parameters of AP compared with the control were increased: the amplitude of the spike and overshoot by 5 and 97%, respectively. However, no changes were found in the steepness of rise of the ascending phase of AP – phase 0. The duration of AP fell by 15% (Table 1, Fig. 1).

Small doses of vitamin E had no effect on RP. No difference was found in the steepness of rise of the ascending phase of AP, in the amplitude of AP or its overshoot, or in the steepness of rise of the ascending phase of the AP – the phase of terminal repolarization, compared with the control. However, the duration of AP was increased by 10% as a result of lengthening of phase 2 – the plateau phase (Table 1).

The absence of changes in the steepness of rise of the ascending phase of AP under the influence of both large and small doses of tocopherol indicate that the substance does not affect the rate of depolarization of the membrane and does not change the density of the fast, inward sodium current of the cell. By means of bioluminescence microscopy (aequorin) two entrances for Ca^{++} into the cell were demonstrated: fast, linked with the 0 phase of AP, and slow, corresponding to the plateau of AP and the time of the developed tension [6, 7]. The duration of AP is known to be determined by the inward current through the slow calcium channels and by the outward potassium current [5, 8].

The significant increase in the overshoot which we observed under the influence of large doses of tocopherol evidently took place due to an increase in the inward calcium current; meanwhile, the increase in the steepness of the descending phase of the AP indicates an increase in the rate of repolarization and, consequently, an increase in the

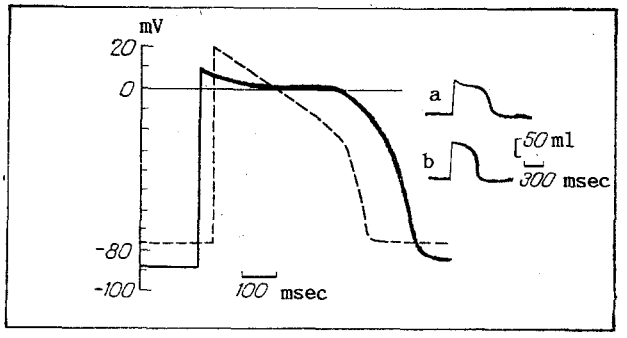

Fig. 1. Effect of large dose of vitamin E on electrical activity of frog heart. Continuous line – control, broken line – experiment. Examples of traces of ventricular action potentials shown on right: a) control, b) action of LD of vitamin E. Upper continuous line shows line of zero potential. Explanation in text.

outward K^+ currents. It can thus be tentatively suggested that large doses of vitamin E shorten the duration of AP as a result of an increase in the outward K^+ currents and not as a result of inhibition of the inward Ca^{++} current.

In the case of small doses of tocopherol the increase in the duration of AP evidently takes place through strengthening of the inward calcium current in the plateau phase.

Large doses of vitamin E can evidently exert a metabolite action on certain enzyme systems and, in particular, on adenosine triphosphatase, which is involved in active ion transport through the cell membrane.

Thus during the action of both large and small doses of tocopherol a qualitatively identical effect was obtained: the inward flow of calcium ions into the cell is increased. Small doses of tocopherol, unlike large doses, have no membrane-damaging action. It can be concluded that the effect of tocopherol on the permeability of components of the cell is evidently indirect: tocopherol interacts first with the surface membrane of the cell itself, modifying its permeability.

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