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EFFECT OF HIGH AND LOW DOSES OF VITAMINS A AND E ON FROG CARDIOMYOCYTE EXCITABILITY

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KEY WORDS: combination of vitamins A and E; frog cardiomyocytes; action potential; cell membrane permeability.

Lipid-soluble vitamins A (retinol) and E (tocopherol) are components of the natural antioxidant system of the cell. Vitamin A is known as a labilizer of lysosomal membranes, vitamin E (depending on its concentration) as a labilizer and stabilizer of these membranes [1-4].

The aim of this investigation was to study the electrophysiological characteristics of cardiomyocytes exposed to the action of a combination of these vitamins. It was assumed that, being lysosomotrophic, tocopherol and retinol ought to influence the properties of the cell membrane, and specifically its ionic permeability.

EXPERIMENTAL METHOD

Preparations of the isolated frog (*Rana temporaria*) heart were used. Transmembrane potentials of the ventricular myocardium were recorded intracellularly by means of "floating" microelectrodes: the resting potential (RP) and action potential (AP). Microelectrodes with a resistance of between 5 and 15 MΩ were filled with 3 M KCl. The preparation was incubated at room temperature in a bath containing Ringer's solution, pH 7.2-7.4. Daily for 2 weeks the frogs were given intraperitoneal injections of retinol (40 μg/ml) and tocopherol (400 μg/ml) simultaneously, or they were given smaller doses (1.5 and 16 μg/ml) in olive oil (not more than 0.3 ml). The necessary concentrations of the vitamins were obtained by diluting ampul preparations in sterile olive oil ex tempora in accordance with the USSR Pharmacopoeia (42-1087-77). In control experiments, intact frogs received injections of 0.3 ml of the solvent (sterile olive oil or 1 ml Ringer's solution) by the same schedule.

EXPERIMENTAL RESULTS

Table 1 shows that the RP level and parameters of AP after injection of 0.3 ml of olive oil did not differ from those after injection of 1 ml of Ringer's solution.

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TABLE 1. Effect of High and Low Doses of Retinol and Tocopherol on Excitability of Ventricular Fibers of Frog Myocardium ($M \pm m$)

Substance	Dose	n	RP, mV	Amplitude of AP, mV	Overshoot, mV	Duration of AP, msec
Ringer's solution (control)	1 ml	14	98,2±1,4	102,6±1,4	9,79±0,58	679±13,9
Olive oil (control)	0,3 ml	7	92,4±0,95	101,6±1,3	9,14±0,83	725,7±12,7
Combination of vitamins A and E	1,5±16 μg/ml	15	89,7±1,4	94,3±1,26*	5,4±0,69*	612±7,3*
	40±400 μg/ml	15	62,3±2,3*	82,1±2,1*	20,1±1,1*	572±9,8*

Legend. *$p \leq 0.05$ denotes significant differences from control (olive oil).

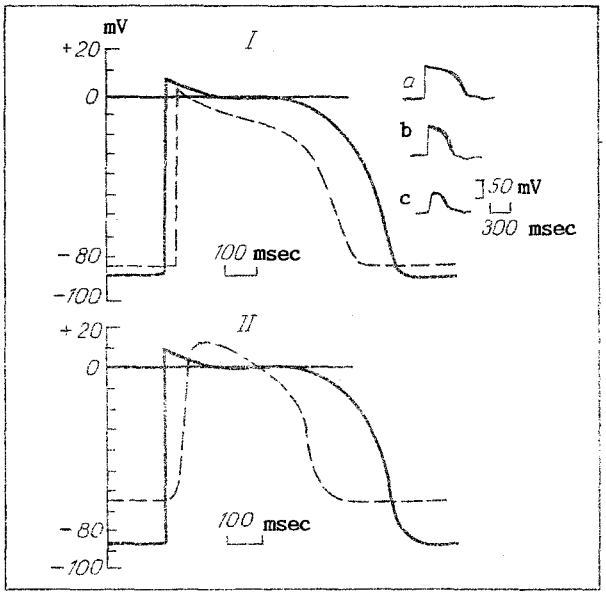

Fig. 1. Effect of low (I) and high (II) doses of vitamins A and E on electrical activity of the frog heart. Continuous line) control; broken line) experiment. Top right: examples of traces of ventricular action potentials: a) control, b) low doses, c) high doses of vitamins. Horizontal lines at top indicate zero potential

High doses of vitamins A and E significantly reduced RP by 33% and the amplitude of AP by 19%; they increased the overshoot by almost three times, and reduced the duration of AP by 21% compared with the control. High doses of vitamins A and E affected the shape of AP: the rate of rise of the ascending phase of AP was reduced, the apex of AP had a smoother shape, the plateau was at a lower level and considerably shortened, and the steepness of the descending phase of AP (the repolarization phase) was increased (Fig. 1). No significant changes were observed in the heart rate under these circumstances.

Low doses of the vitamins did not affect RP of the cardiomyocytes. Unlike high doses, they did not change the steepness of rise of the ascending phase of AP, nor did they affect the rate of membrane depolarization. Injection of small doses of the vitamins significantly reduced the overshoot (by 41%), shortened the plateau, and reduced the duration of AP by 16%. The steepness of the descending phase of AP, i.e., the rate of repolarization, and also the heart rate were unchanged.

Lowering of the amplitude of AP and slowing of the depolarization phase during administration of high doses of vitamins A and E are evidence of inhibition of activity of the fast sodium channels in the cardiomyocyte membrane and of reduction of the density of the fast inward sodium current. Two inputs of Ca^{2+} into the cell were demonstrated by a bioluminescence method (aequorin): fast, connected with the O-phase of AP, and slow, corresponding to the AP plateau and to the time of developed tension [6, 7]. The duration of AP also is known to be determined by a current entering through the slow calcium channels and by outward potassium currents [5, 8]. The significant increase in the overshoot under the influence of high doses of the vitamins and the marked shortening of the plateau may be evidence of activation of the fast Ca^{2+} input into the cell in the

depolarization phase against the background of inhibition of the slow inward calcium current. The increase in steepness of the descending phase of AP indicates an increase in the rate of repolarization, i.e., activation of the outward potassium currents.

Potassium conduction is evidently activated even more through elevation of the intracellular Ca^{2+} level due to an increase in the inward fast calcium current.

Thus, the factors reducing the duration of AP under the influence of high doses of vitamins A and E are, first, blockade of the slow inward current through calcium channels and, second, strengthening of the outward potassium currents from the cell.

The action of low doses of vitamins A and E on the cardiomyocyte membrane was manifested mainly as inhibition of calcium conductance. High doses of the vitamins, unlike low doses, damaged the cardiomyocyte membrane, lowered the RP level, reduced activity of the membrane-bound enzymes, and disturbed the electrogenic capacity of the cationic pumps.

The investigation showed that a common feature of the action of high and low doses of vitamins A and E is their ability to block, to a greater or lesser degree, calcium conductance of the cardiomyocytes; large doses, however, can also inhibit fast sodium conductance.

It can be postulated on the basis of these results that a combination of vitamins A and E can act not only on lysosomal membranes, but also on the surface phospholipid membrane of cardiomyocytes, with which it acts mainly directly, modifying its ionic permeability.

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