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

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Fat-soluble vitamin A (retinol) is a component of the natural antioxidative system of the cell. According to the membrane theory of the action of vitamin A [6], retinol can penetrate into the hydrophobic zone of biomembranes and interact with lecithin-cholesterol monolayers on the phase boundary, thereby inducing structural changes in the lysosomal membranes, leading to increased permeability of the membranes for water and ions.

Membrane effects of retinol have been linked in some investigations with its effect on the phospholipid composition of mitochondrial and lysosomal membranes [3].

Since vitamin A is used as a labilizer of lysosomal membranes [1, 2, 4], it is essential to study its effect on the electrophysiological characteristics of the cardiomyocyte, for the action of retinol on membrane permeability may be indirect rather than direct.

We have very little information on the effect of retinol on the phospholipid membrane of cardiomyocytes, and it is therefore interesting, by using the method of intracellular derivation of transmembrane potentials, to study the effect of retinol on the resting membrane potential (RP) and on action potential (AP) generation in ventricular fibers of the frog.

EXPERIMENTAL METHOD

Experiments were carried out on preparations of the isolated heart of the frog *Rana temporaria*. Transmembrane AP of the ventricular myocardium were recorded intracellularly with the aid of "floating microelectrodes." Microelectrodes with a resistance of 5 to 15 MΩ were filled with 3 M KCl. The preparation was incubated at room temperature in a bath containing Ringer's physiological saline, pH 7.2-7.4.

Daily for 2 weeks, the frogs were given injections of large (40 μg/ml) and small (1.5 μg/ml) doses of an ampul solution of fat-soluble vitamin A in oil (retinol acetate) intramuscularly.

The necessary concentrations of retinol for the intraperitoneal injections were obtained by diluting the ampul preparation in sterile olive oil when required in accordance with regulation (42-1087-77) of the Ministry of Health of the USSR. Since the doses of retinol were envisaged to be in volumes of 1 and 0.3 ml, in the two control groups of experiments intact frogs were given daily intraperitoneal injections of 1 or 0.3 ml of sterile olive oil for 2 weeks. In the 3rd group (control experiments) intact rats received injections in accordance with the same schedule, but of 1 ml of Ringer's physiological saline.

EXPERIMENTAL RESULTS

The data given in Table 1 show that intraperitoneal injection of a large volume (1 ml) of olive oil into frogs is undesirable, for compared with the control (injection of 1 ml of Ringer's solution) it causes significant fall of RP of the heart muscle fiber by 3.66%, a fall in the amplitude of AP by 5.7%, and in the magnitude of the overshoot by 24.41%. In frogs receiving injections of 0.3 ml of olive oil no significant differences in RP and AP were observed compared with the control (Table 1).

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TABLE 1. Effect of Large and Small Doses (LD and SD) of Vitamin A and Olive Oil on Excitability of Ventricular Fibers of Frog Myocardium ($M \pm m$)

Test substance	Dose	RP, mV	Amplitude of AP, mV	Overshoot, mV	Duration of AP, msec	Heart rate	Number of experiments
Ringer's solution	1 ml	92,8±1,4	102,6±1,4	9,79±0,58	697,9±13,9	34±0,1	14
Olive oil	SD 0.3 ml	92,4±0,95	101,6±1,3	9,14±0,83	725,7±12,7	34,3±1,85	7
	LD, 1 ml	89,4±1,3*	96,8±1,2*	7,4±0,62*	676±10,5	37,5±0,5	14
Vitamin A	SD, 1.5 µg/ml	90,6±0,86	92,4±0,77**	1,8±0,25**	561,1±12,3**	34±1,5	18
	LD, 40 µg/ml	66,8±1,25**	69,4±1,29**	2,6±0,54**	435,6±10,8**	37,7±1	18

Legend. *) Values differing significantly from control — 1 ml of Ringer's solution ($p < 0.05$); **) values differing significantly from control — 0.3 ml of olive oil ($p < 0.05$).

In the subsequent experiments, parameters of AP and RP observed during the action of a small dose of olive oil were taken as the control.

Under the influence of large doses of vitamin A a significant decrease in RP of the cardiomyocytes by 27.71% and also the following significant changes in the parameters of AP were observed: the steepness of rise of the leading edge of the potential (phase 0) was appreciably reduced, the amplitude of the spike was reduced by 31.69%, the overshoot was reduced by 71.55% or was absent altogether, the plateau of AP was significantly shortened, and the rate of development of terminal repolarization (phase 3) was reduced.

The duration of AP was shortened by 39.98% (Table 1).

Small doses of vitamin A did not affect RP, but the following significant changes were observed in the parameters of AP compared with the control: the spike amplitude was reduced by 9%, the overshoot was reduced by 80.31%, the plateau was shortened, the duration of AP was reduced by 22.7%, and the steepness of rise of the leading edge of the potential was reduced a little (Table 1).

The decrease in the steepness of rise of the leading edge of AP under the influence of retinol is evidence of a decrease in the rate of depolarization of the cardiomyocyte membrane in phase 0 as a result of possible inhibition of the first sodium channels, leading to a decrease in density of the first sodium current, flowing inside the cell.

We know that during AP generation two inputs of calcium ions are observed in myocardial fibers: fast, connected with the terminal part of phase 0 of the potential, and slow, corresponding to the plateau (phase 2) of the potential [5, 7, 8].

Reduction of the overshoot and shortening of the plateau, or in some cases even their complete absence, are evidence that large doses of retinol significantly inhibit both the fast and the slow input of calcium ions inside the cell during its depolarization.

The slowing of repolarization of AP (phase 3) indicates weakening of the outflow of potassium ions from the cell along the electrochemical gradient to the exterior, as a result of possible inhibition of the first sodium channels, leading to a decrease in density of the first sodium current, flowing inside the cell.

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The slowing of repolarization of AP (phase 3) indicates weakening of the outflow of potassium ions from the cell along the electrochemical gradient to the exterior, as a result of possible inhibition of potassium channels. Despite this slowing of repolarization, the duration of AP nevertheless was considerably reduced due to shortening of the plateau.

Lowering of the membrane potential of the myocardial fibers under the influence of high doses of retinol indicates damage to the cardiomyocyte membrane, causing inhibition of activity of membrane-bound enzymes, notably Na,K-ATPase and Ca-ATPase, which play the leading role in active cation transport and maintenance of the transmembrane potential. The electrogenic capacity of the cationic pumps, especially the Na,K-pump, are disturbed.

This investigation showed that large and small doses of vitamin A give rise to qualitatively identical effects.

The main effect of large doses of vitamin A is to cause a marked decrease in excitability of the cardiomyocyte membrane due to inhibition of the function of the Na, K, Ca-systems of the cell.

When the properties of vitamin A are studied, a fact to be taken into consideration is thus that, by altering membrane permeability for the contents of the cells, it evidently has a primarily direct action on the surface membrane of the cell, disturbing its permeability.

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