EFFECT OF NIFEDIPINE AND RIODIPINE ON BIOCHEMICAL PROCESSES IN INTACT ERYTHROCYTES

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Cardiovascular drugs which are antagonists of calcium ions, belonging to various chemical groups, are widely used in medical practice. They include derivatives of 4-aryl-1,4-dihydropyridines (1,4-DHP). Members of this series used in clinical practice include Nifedipine (Fenigidin, Adalat), Nicardipine, Nitrendipine, Nimodipine, and Riodipine (Foridon). Riodipine was synthesized in the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR [4]. Riodipine is not inferior to Nifedipine and Nicardipine in its vasodilator activity, but it is less toxic. The hypotensive and antianginal activity of 4-aryl-1,4-DHP can be explained by their ability to block the action of calcium channels. This action is supplemented by inhibition of phosphodiesterase activity, which increases the concentration of cyclic AMP in the myocardial cells [6]. The practical importance of the derivatives of this series necessitates a comprehensive study of their biochemical properties. It has been shown that Riodipine is distributed throughout the cell membranes and can bind with model lipid membranes [1], and like Nifedipine, it influences the structural organization of erythrocyte membranes [5]. This has stimulated trials of these compounds on processes connected with destruction of membranes, such as hemolysis of erythrocytes.

In this investigation we determine the effect of Riodipine and Nifedipine on acid hemolysis of erythrocytes and on the concentrations of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) in intact erythrocytes.

EXPERIMENTAL METHOD

Hemolysis was produced with hydrochloric acid by the method in [7]. Erythrocytes were isolated from blood from healthy human blood donors by washing three times in 0.9% NaCl, followed by centrifugation for 10 min at 1,000 g. The residue of erythrocytes was diluted 1,000 times (by volume with 0.9% NaCl solution and a suspension was obtained containing on average $6 \cdot 10^3$ cells/ml. The optical density of the suspension at 576 nm was close to 1.0. To 2.4 ml of the diluted erythrocyte suspension in a spectrophotometry cuvette 0.025 ml of a $5 \cdot 10^{-3}$ M solution of the test substance in ethyl alcohol was added. The sample was incubated for 20 min at 37°C. Hemolysis was induced by adding 0.1 ml of a 0.005 N solution of HCl (HCl concentration in the cuvette 0.002 N). The kinetics of the changes in optical density at 576 nm was recorded on a "Hitachi 557" spectrophotometer. The inhibitory effect (IE) was assessed as the relative lengthening of the 50% hemolysis time:

$$IE = \tau/\tau_0,$$

where τ is the 50% hemolysis time with the test substance, and τ_0 the same without the substance. The ATP concentration was determined by the "LKB Wallac" technique with standard reagents; luminescence was recorded on an "LKB 1250" luminometer. The erythrocyte suspension was prepared and incubated with the substance as described above. After incubation, to 1 ml of the erythrocyte suspension was added 1 ml of a 10% solution of TCA, and the sample was filtered, and diluted 100 times with 0.1 M Tris-acetate buffer containing 2 mM EDTA (pH 7.75). To 0.7 ml of the buffer solution was added 0.2 ml of the basic reagent (luciferin–luciferase), and the background luminescence (B) was recorded. Next 0.1 ml of the

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TABLE 1. Effect of Nifedipine and Riodipine on Acid Hemolysis of Erythrocytes and on ATP and 2,3-DPG Concentrations

State of erythrocytes	Control	Nifedipine $5\cdot10^{-5}$ M	Riodipine
Acid hemolysis (IE)	1,0±0,1	1,9±0,1	1,3±0,1
ATP concentration in erythrocytes, 10^{-3} moles·liter^{-1}	1,48±0,10	1,25±0,08	1,13±0,08
Concentration of 2,3-DPG in erythrocytes, percent	100	110	101

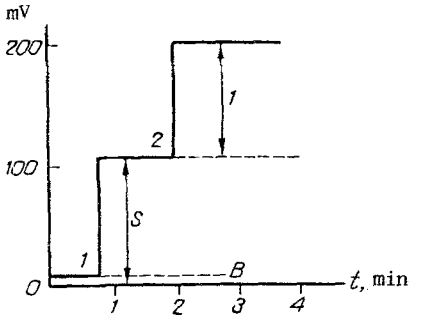

Fig. 1. Determination of ATP concentration in erythrocyte suspension: 1) addition of sample, 2) addition of lATP standard. Abscissa, time (in min); ordinate, intensity of luminescence (in mV).

diluted filtrate was added and luminescence (S) was measured. The internal standard was 0.01 ml of a 10^{-5} M standard solution of ATP (concentration in the medium 10^{-7} M), and luminescence (I) was measured. The ATP concentration in the erythrocytic suspension was determined by the equation (Fig. 1):

$$\text{ATP} = \frac{S-B}{I} \cdot 10^{-7}\ (M)$$

To determine the concentration of 2,3-DPG erythrocytes were isoalted from the donors' blood by triple washing in 0.9% NaCl solution. To 0.5 ml of the erythrocyte suspension was added 0.005 ml of a $5\cdot10^{-3}$ M solution of the test substance (concentration in the cuvette $5\cdot10^{-5}$ M). The sample was incubated for 20 min at 37°C. The 2,3-DPG concentration was determined by the method in [11] with standard reagents from "Sigma." Optical density at 660 nm was measured on a "Hitachi 557" spectrophotometer.

EXPERIMENTAL RESULTS

Physiologically active substances (local anesthetics, tranquilizers, anti-inflammatory agents, and other compounds), in a concentration of 10^{-8}-10^{-4} M, inhibit osmotic hemolysis of erythrocytes [9, 12]. We have shown that in the case of hydrochloric acid hemolysis (Table 1), Nifedipine and Riodipine inhibit this process, IE for Nifedipine being stronger. IE of Nifedipine on osmotic hemolysis has been demonstrated in [10].

It must be pointed out that the two compounds do not themselves induce hemolysis of erythrocytes (during preincubation). The absence of this hemolyzing ability is of great importance in view of their use in medical practice. Moreover, the erythrocyte-stabilizing activity of the compounds, especially Nifedipine, indicates the desirability of studying other aspects of their use. For example, when an artificial circulation apparatus is used, increased hemolysis of the erythrocytes takes place [8]. When the biological properties of Nifedipine are interpreted, it is also important to bear in mind its marked antioxidative properties, which have been demonstrated by experiments in vitro [10, 13].

The practical use of Nifedipine and Riodipine stimulated a study of their effect on some energy characteristics of erythrocytes. The results (Table 1) show that both compounds lower the ATP concentration in erythrocytes; Riodipine does so, moreover, to a greater degree than Nifedipine. Perhaps this may be connected with IE of their action on phosphodiesterase [6]. The increased cAMP concentration under these circumstances may also affect the ATP concentration under these circumstances may also affect the ATP concentration, by activating the hydrolases which catalyze the direct degradation of ATP to 5-AMP.

The normal physiological functioning of the erythrocyte is largely dependent on the concentration of 2,3-DPG, which, like ATP, is an allosteric regulator of the affinity of hemoglobin for oxygen [2]. The effect of the compounds on the 2,3-DPG concentration is given in Table 1 as a percentage of the control, since the 2,3-DPG concentration is different donors varied from $(1.6 \pm 0.2)\cdot10^{-3}$ to $(3.8 \pm 0.2)\cdot10^{-3}$ moles/liter. Nifedipine in all cases showed a tendency to increase the 2,3-DPG concentration, but these changes did not go beyond the limits of error. No changes were observed in the 2,3-DPG concentration as a result of an increase in the incubation time of 1,4-DHP with the erythocytes to 1 h.

Inhibition of erythrocyte hemolysis by Nifedipine and Riodipine is perhaps determined by their ability to interact with receptors on the erythrocyte surface. By protecting these physiologically important centers from the damaging action of protons, the 1,4-DHP derivatives delay the development of hemolysis. This suggestion relating to a possible mechanism of the erythrocyte-stabilizing action of 1,4-DHP derivatives, mediated through reception on the erythrocyte surface, is in agreement with similar ideas put forward in [3] regarding the erythrocyte-stabilizing action of other nitrogen-containing heterocyclic compounds (derivatives of pyrimidine, hydroxypyridine, and benzamidazole).

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