Influence of Nonsteroid Antiinflammatory Drugs on Osmotic Resistance of Erythrocytes

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Measurement of the osmotic resistance of erythrocytes (ORE) is one of the simplest methods of testing membranotropic effects of drugs. The literature available demonstrates a dose-dependent effect of drugs on hypotonic lysis of erythrocytes. In particular, local anesthetics, antidepressants, bile acids, and tranquilizers have been established to enhance ORE in concentrations of no more than 1 mmol/liter, which are considered to be therapeutic, and to lower it in concentrations one order of magnitude higher [4,5].

However, the effect of nonsteroid antiinflammatory drugs (NAID) on ORE is not claerly understood. An increased ORE induced by NAID has been previously reported, drawing the conclusion that they have a membrane-stabilizing effect [2,3]. The aim of our study was a more detailed investigation of the possible mechanisms underlying the membranotropic influence of NAID, based on the effect of various concentrations of current NAID on ORE.

MATERIALS AND METHODS

Voltaren (Latvbiofarm, Latvia), surgam (Russell India, Ltd., India), and izamben (M. V. Lomonosov Chemico-Pharmaceutical Plant, Ukraine) were used in the experiments. The experiments were performed on freshly isolated murine erythrocytes. The mice (weighing 20-22 g) were decapitated, and 1 ml of blood was collected in a glass with 50 ml incubation solution containing 142 mM NaCl and 10 mM sodium-phosphate buffer (pH 7.4) under constant mixing with magnetic stirring to prevent the blood from clotting. The erythrocytes were then sedimented by centrifugation at 3000 g for 10 min and resuspended in the incubation solution.

ORE was determined as described previously [4]. An aliquot of the stock erythrocyte suspension was added to test solutions of different ionic strength containing different concentrations of the drugs, so that the optical density at 720 nm of the control suspension of erythrocytes in an isotonic medium was 1.2 units. Samples without NAID served as a control. The samples were incubated at 37°C for one hour, followed by light scattering redetermination at 720 nm. The erythrocytes were then centrifuged at 3000 g for 10 min, and the content of hemoglobin in the supernatant was measured spectrophotometrically at 410 nm. The total content of hemoglobin in the erythrocytes was determined in samples completely hemolysed with H_2O.

ORE was expressed as the ionic strength of the solution which induced 50% hemolysis. This index was calculated after Litchfield and Wilcoxon [1] from 9 independent measurements of points on the characteristic curve of erythrocyte lysis. The data were processed statistically using the Student t test at the significance level $p \leqslant 0.05$.

RESULTS

ORE was increased in samples where the concentration of surgam or voltaren was 0.2-0.4 mmol/

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TABLE 1. Effect of NAID on Calculated Ionic Strength of the Solution (NaCl, mmol/liter) Which Induce 50% Lysis of Erythrocytes during 1-Hour Incubation at 37°C

Experimental conditions	Concentration of drug, mmol/liter						
	0	0.1	0.2	0.4	0.8	1.2	1.6
Control	77 (64.2-92.4)						
Surgam		77 (64.7-91.6)	74 (60.7-90.3)	72 (60.5-85.7)	90 (74.4-108.9)	94 (72.3-122.2)	–
Voltaren		75 (62.0-90.7)	74 (61.2-89.5)	73 (62.9-84.7)	72 (61.5-84.2)	78 (65.5-92.8)	91 (74.6-111.0)
Izamben (63.9-90.4)	(64.9-88.9)	76 (70.3-86.6)	76 (64.7-91.6)	78 (68.4-88.9)	77 (64.9-88.9)	78	76

Note. Numbers in parentheses are confidence intervals of the indexes ($n=9$, $p \leq 0.05$).

liter or 0.1-0.8 mmol/liter, respectively, whereas higher concentrations of these drugs lowered ORE (Table 1). Moreover, both surgam and voltaren induced hemolysis of the erythrocytes even in an isotonic medium (Fig. 1).

The data obtained from simultaneous recording of haemoglobin outflow from the erythrocytes and decrement of light scattering point to some differences in the membranolytic action of voltaren and surgam. In the presence of surgam, the erythrocyte membrane becomes more permeable for hemoglobin, and then, with higher concentrations of the drug, lysed completely, which was manifested as a drop of light scattering (Fig. 1). At the same time, under the influence of voltaren the light scattering of the samples decreased simultaneously with the hemoglobin loss from the erythrocytes. The concentrations of voltaren and surgam inducing 50% loss of hemoglobin from the erythrocytes and their confidence intervals (in parentheses) were 2.2 mmol/liter (1.75-2.77) and 2.8 mmol/liter (1.47-5.32), respectively. The light scattering of the erythrocyte suspension decreased twofold for concentrations of voltaren of 2.0 mmol/liter (1.86-2.15) and of surgam of 4.3 mmol/liter (2.39-10.3).

In the study of the effect of NAID on hypotonic lysis of erythrocytes, the light scattering of the erythrocyte suspension was found to decline correspondingly to the hemoglobin loss from the erythrocytes. Therefore, in Table 1 we present just the ORE index calculated from the hemoglobin outflow from the erythrocytes.

Unlike voltaren and surgam, izamben did not change ORE and did not induce lysis of erythrocytes in isotonic medium even in a concentration of 15 mmol/liter. This is possibly attributed to the fact that the izamben molecule is charged due to positively charged nitrogen atom in the pyridine ring, which may interfere with its penetration to hydrophobic areas of biomembranes.

The detected biphasic action of voltaren and surgam on ORE is not unique to NAID. Yasuhara et al. [4] believe that the analogous biphasic influence of bile acids, local anesthetics, and antidepressants on ORE is attributed to an increased "fluidity" of biomembranes. This mechanism probably applies to NAID as well. Owing to an increased mobility of the protein and lipid components, biomembranes become more rigid and less "fragile" during erythrocyte swelling in a hypotonic medium. This is, in fact, the main cause of the hypotonic lysis of erythrocytes. However, a considerable increase of the "fluidity" of membranes leads to their disintegration even in an isotonic medium.

Thus, the assumption reported previously concerning the membrane-stabilizing effect of NAID [2,3] does not completely reflect the essence of the

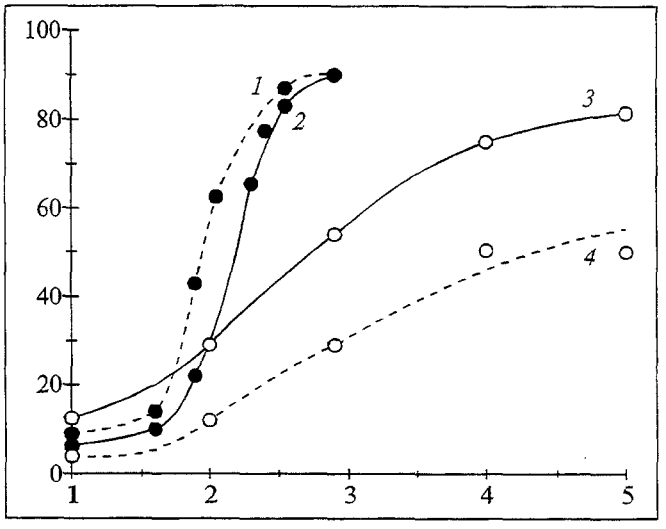

Fig. 1. Membranolytic effect of surgam and voltaren on erythrocytes. Erythrocytes were incubated in an isotonic medium at 37°C for one hour in the presence of different concentrations of surgam (open circles) or voltaren (dark circles). The loss of hemoglobin by erythrocytes (solid line) was expessed in % of 100% hemolysis induced with water. The decrement in light scattering of the erythrocyte suspension (dotted line) was expressed in % of the control 100% hemolysed sample.

membranotrotic effect of these drugs. The effects of NAID discovered by us may be of importance for understanding the mechanisms of their ulcerogenic effect. When the drugs are taken per os, the local concentration on the stomach wall may be close to or even exceed the membranolytic concentration. Surprisingly, there are no data concerning the ulcerogenic effect of izamben in the literature available.

REFERENCES

1. M. L. Belen'kii, *Elements Involved in Quantitating Pharmacological Effect* [in Russian], Leningrad (1963).
2. F. P. Trinus, *Farmakol. Toksicol.*, № 7, 55-62 (1972).
3. F. P. Trinus, N. A. Mokhort, and B. M. Klebanov, *Nonsteroid Antiinflammatory Drugs* [in Russian], Kiev (1975).
4. P. Seeman, *Pharmacol. Rev.*, **24**, 584-655 (1972).
5. H. Yasuhara, M. Tonooka, K. Kamei, *et al.*, *Toxicol. Appl. Pharmacol.*, **79**, 453-460 (1985).

Antiarrhythmic Effect of *Rodiola rosea* and Its Possible Mechanism

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Prophylaxis of ventricular arrhythmias is one of the most important problems of modern cardiology [11]. The possibility of preventing arrhythmias in experimental coronary occlusion by adaptation to brief periodic immobilization, physical loads, and high-altitude hypoxia has been shown in recent publications [5,9].

However, the possibility of preventing arrhythmias by administering *R. rosea* extract, which is known to exhibit a milder antistressor effect in comparison with physical means of adaptation [7], has not been discussed in the literature. We have earlier shown that preadaptation of animals, by administering a course of injections of *R. rosea* extract promotes the accumulation of enkephalins and prostacycline, which possess antiarrhythmic activity [4,14,16], in organs and tissues [3,4,8].

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Hence, it seemed of interest to investigate the antiarrhythmic activity of *R. rosea* preparation, as well as to study some possible mechanisms of this effect.

MATERIALS AND METHODS

Experiments were carried out on 183 male Wistar rats weighing 150-200 g. The animals were adapted by administering courses of injections (per os) of officinal preparation of *R. rosea* (8 days, a single dose of 1 ml/kg), which is a known adaptogen [10]. One day after the last session of adaptation, arrhythmias were simulated in the rats by intravenous injections of norepinephrine in a dose of 90 μg/kg [13] or of 10% $CaCl_2$ in a volume of 0.15 ml/100 g body weight [2]. The electrocardiogram (ECG) in the standard lead II was recorded during 5 min postinjection.

In separate series of experiments, 30 min before the simulation of arrhythmias, animals adapted as mentioned above received injections (0.5 mg/kg) of naloxone, which blocks the μ-opioid receptors

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