ACCELERATED SODIUM-PROTON EXCHANGE IN ERYTHROCYTES OF OCCLUSIVE THROMBOSIS PATIENTS

S. A. Polikarpov and S. N. Orlov

UDC 616.14-005.6-007.272-07:616.155.1-008.923.3

KEY WORDS: erythrocytes, occlusive thrombosis, Na/H exchange

An important stage in the pathogenes is of venous thrombosis is activation of platelets, which form the primary clot and trigger the coagulation stage of thrombogenesis [7]. Many investigations have been devoted to the study of the connection between the transmembrane ionic current and platelet function. It has been shown that Na/H exchange is directly related to signal conduction during activation of these cells and that an increase in the rate of translocation of the Na/H exchanger determines cellular hyperreactivity [1, 5, 8]. Thus differences in the course of venous thrombosis may be connected with the state of the Na/H exchange system.

We accordingly decided to study the rate of Na/H exchange in patients with various forms of acute venous thrombosis. Erythrocytes, in which the kinetic characteristics of this system remain virtually unchanged when blood is kept on ice for 40-60 h, were chosen as the test object.

EXPERIMENTAL METHOD

Altogether 22 patients with acute venous thrombosis in the system of the inferior vena cava were studied.

Group 1 consisted of five men and three women with occlusive venous thrombosis, aged from 19 to 68 years (42 ± 6). The duration of the disease from the time of appearance of the first symptoms until the taking of blood samples was 20 ± 2 days. Group 2 consisted of seven men and seven women with floating venous thrombosis, aged from 23 to 68 years (49 ± 5). The mean duration of the disease was 16 ± 3 days. All patients received antithrombotic treatment with heparin and disaggregating agents in standard doses. None of them received any cardiac glycosides, diuretics, calcium channel blockers, or hormones. The character of the venous thrombosis was established on the basis of phlebography.

The control group consisted of seven men and two women aged from 42 to 60 years (50 ± 5). The subjects were clinically healthy and had never suffered from venous pathology.

Blood from the cubital vein was collected in test tubes containing heparin (20-50 U/ml blood). Erythrocytes were washed free from plasma with physiological saline containing 5 mM sodium phosphate, pH 7.4.

Na/H exchange in the erythrocytes was determined by the method in [6] with modifications described by the writers previously [3]. Erythrocytes (100 μl) were added to 1.9 ml of medium containing 150 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$ and 10 mM glucose. After incubation of the cells for 5 min at 37°C with constant mixing, the pH of the suspension was adjusted to 6.35-6.45 by the slow addition of 0.2 N HCl in 150 mM NaCl, after which an anion carrier inhibitor (200 μM of 4,4'-di-isothiocyanostilbene-2,2'-disulfonic acid — DIDS) was added to the suspension and its pH increased with 0.05 M NaOH in 150 mM NaCl to 7.95-8.05. Each measurement was repeated with the addition of 0.5 mM amiloride before alkalification of the suspension. The kinetics of H^+ release was recorded on a PHM-64 pH-meter ("Radiometer," Denmark), with 91-95 electrodes ("Orion," USA). The velocity of Na/H exchange was calculated by the formula

$$(\Delta pH_1 - \Delta pH_2) \cdot b \cdot m^{-1} \cdot t^{-1},$$

Faculty of Biology, M. V. Lomonosov Moscow State University. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 1, pp. 35-36, January, 1991. Original article submitted May 3, 1990.

TABLE 1. Rate of Outflow of H^+ from Erythrocytes of Patients with Occlusive Venous Thromboses (M ± m)

Group of subjects	n	Without amiloride	With amiloride	Amiloride-inhibited component
		μmoles H^+/liter cells/min		
Control	9	310,0±41,0	221,8±47,4	87,8±12,0
Patients with floating thromboses	14	326,0±42,22	224,8±39,1	90,0±8,7
Patients with occlusive thromboses	8	671,1±58,3	314,4±48,6	361,2±27,8

where ΔpH_1 and ΔpH_2 denote the initial rate of change of pH in medium without amiloride and with amiloride respectively, b the buffer capacity of the incubation medium, containing 200 μM DIDS (the number of micromoles of protons required to change the pH from 8.0 to 7.0); m) the number of cells in suspension (0.0001 liter); t) the incubation time, in min. Mother solutions of DIDS (50 mM) and amiloride (500 mM) in dimethyl sulfoxide were used. The DIDS solution was made up immediately before the experiment.

EXPERIMENTAL RESULTS

The results in Table 1 show that the basal rate of H^+ outflow (first column), the rate of this process in the presence of amiloride (second column), and the amiloride-inhibited component of the rate in erythrocytes from patients with fluctuating venous thromboses and the control group were identical. In patients with occlusive thrombosis a significant increase was observed both in the basal rate of H^+ outflow (twofold) and the amiloride-inhibited component of proton outflow (fourfold) compared with the control and the floating thrombosis group.

Comparing these results with those obtained in patients with essential hypertension [3] shows that the basal rate of H^+ release and, indeed, the velocity of Na/H exchange in patients with occlusive phlebothromboses were significantly greater, whereas the amiloride-noninhibited component of the proton flow was virtually identical. This difference is evidently related to the clinical overdiagnosis of essential hypertension. The results are convincing evidence that an increase in the rate of total flow of H^+ ions from the cell in patients with occlusive venous thromboses is in fact connected with an increase in sodium-proton exchange, which probably is also responsible for functional changes in the cellular components of thrombogenesis.

The Na/H-exchanger plays the key role in various physiological processes in the cell, such as regulation of pH, changes in metabolism under the influence of various hormones and neurotransmitters, coupling of stimulus with response in excitable tissues, growth and proliferation of the cells, and so on [4]. An increase in the velocity of Na/H exchange in platelets is accompanied by strengthening of their aggregation properties and increased synthesis of thromboxane — a very powerful inducer of platelet aggregation and vasoconstrictor [2].

The increase in the rate of translocation of the Na/H-exchanger in patients with occlusive venous thromboses may prevent functional changes in the cellular components of thrornbogenesis responsible for the formation of this type of venous thrombosis.

LITERATURE CITED

1. G. M. Kravtsov, Z. V. Karagodina, S. N. Orlov, et al., Kardiologiya, No. 12, 49 (1983).
2. N. V. Mandrovnaya, Kh. M. Markov, I. E. Smirnov, et al., Kardiologiya, No. 12, 62 (1983).
3. S. N. Orlov, I. Yu. Postnov, N. I. Pokudin, et al., Byull. Éksp. Biol. Med., No. **9**, 286 (1988).
4. Yu. V. Postnov and S. N. Orlov, Primary Hypertension as Cell Membrane Pathology [in Russian], Moscow (1987).
5. A. E. Rozenberg and Kh. M. Markov, Kardiologiya, No. 12, 56 (1983).
6. N. Escobates and M. Canessa, J. Memb. Biol., **90**, 1 (1985).
7. V. V. Vakkar, Am. J. Surg., **150**, 1-6 (1985).
8. V. Valtier, P. Guicteney, M. Baudoin-Legros, and P. Meyer, J. Hypertens., **5**, 551 (1986).