Sodium-hydrogen exchange in erythrocytes of patients with acute deep venous thromboses

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Abstract. The rate of $\Delta\mu H^+$-induced Na/H-exchange in erythrocytes of patients with occlusive and with floating types of acute deep venous thromboses, and in control volunteers, was estimated. In patients with occlusive thrombi Na/H-exchange was revealed to be fourfold higher in comparison with patients with floating thrombi and with controls, while no difference was observed between the two latter groups.
Key words. Sodium-hydrogen exchange; erythrocytes; deep venous thromboses.

The adhesion of platelets to the injured venous wall and the resulting formation of platelet aggregates are believed to be important initiating mechanisms in venous thrombosis development [1].

It has been reported that in patients with essential hypertension and in spontaneously hypertensive rats, platelets show an enhanced aggregating capacity [2–4]. This platelet hyperreactivity in essential hypertension is associated with an increased rate of platelet membrane Na/H-exchange [5–7]. In primary hypertension, an increased rate of Na/H-exchange has also been reported for various other tissue cells, such as erythrocytes [8], white blood cells [9, 10], and smooth muscle cells [11, 12]; therefore, there is a widespread alteration of this ion transport system. In clinical studies, erythrocytes may be a more convenient object for the estimation of this carrier activity than platelets or vascular smooth muscle cells.

In erythrocytes, Na/H-exchange can be activated by creating a transmembrane proton electrochemical gradient ($\Delta\mu H^+$)[13]. In this paper we present the data for $\Delta\mu H^+$-induced Na/H-exchange in erythrocytes of patients with occlusive and with floating forms of acute deep lower limb venous thromboses.

Materials and methods

Samples of blood were obtained from the following study groups.

Volunteers: 7 male and 2 female volunteers (age range 42 to 60 years, mean age 49.5 ± 4.8 years; M ± SE) with a negative history of venous disease participated in the study as a control group.

Patients: A total of 22 patients treated for lower limb acute deep venous thrombosis in the 1st Moscow City Hospital Surgical Department were investigated. The thrombosis type was documented by phlebography: 8 patients (5 male, 3 female, aged 19 to 68 years, mean age 42.0 ± 5.5 years; disease duration from onset of symptoms to the investigation 21 ± 2 days; M ± SE) had the occlusive type of venous thrombosis; 14 patients (7 male, 7 female, aging 23 to 68 years, mean age 49.2 ± 4.9 years; the disease lasting for 18 ± 3 days; M ± SE) had floating thrombi. The patients did not develop symptoms of pulmonary embolism or lung infarction, nor did phlegmasia coerulea dolens ensue. All patients were treated with heparin, 7.5–10 thousand IU, subcutaneously three times a day, Troxevasin (Venoruton) 300 mg twice a day orally, and aspirin, 100 mg orally. The drug therapy was the same in both groups. None of them received digitalis, calcium channel blockers, hormones or diuretics.

Erythrocytes: The blood samples were collected in the early morning before the heparin injection, after an overnight interval, into tubes containing heparin (20–50 IU per ml blood), and kept ice-cold for 6–24 h. Erythrocytes were sedimented at 1000 g for 10 min and washed three times with saline solution containing 5 mmol/l sodium phosphate (pH 7.4).

Na/H-exchange: The method described by N. Escobales and M. Canessa [13] and modified by S. N. Orlov et al.[8] was used. 100 µl of packed erythrocytes were placed in 1.9 ml of medium consisting of (mM) 150 NaCl, 1 KCl, 1 $MgCl_2$, 10 glucose and incubated for 5 min at 37 °C. The pH value of the suspension was adjusted to 6.35–6.45 by the slow addition of 0.2 N HCl in 150 mmol/l choline-chloride. The acid equilibration lasted 45–60 s. A fast regulation of intracellular pH by the anion carrier (band 3 protein) was inhibited with 200 µmol/l 4,4′-diisothiocyanstilbene-2,2′-disulphonic acid (DIDS), and after 1 min the pH of the incubating medium was adjusted to 7.95–8.05 by addition of 0.02 N NaOH solution in 150 mmol/l choline-chloride. The alkalinization procedure took 30–90 s. The pH of the suspension never increased to 8 before DIDS addition. Every experiment was run in duplicate. Amiloride (0.5 mM) was added after DIDS. The kinetics of proton efflux were registered by means of a 91–95 electrode (Orion, USA) connected with a PHM-64 millivoltmeter (Radiometer, Denmark). The measurement of the initial rate of medium acidification lasted for 1 min, the pH of the medium changing from 8 to 7.8–7.95 at the end of the experiment. The rate of Na/H-exchange was determined as

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

where ΔpH_1 and ΔpH_2 are the initial rates of medium acidification in the absence (ΔpH_1) and in the presence of amiloride (ΔpH_2), b is the buffer capacity of the incubating medium, m is the volume of erythrocytes, and t is the time of incubation. The buffer capacity was determined

by titration of 1.9 ml of supernatant obtained after incubation and sedimentation of the cells with HCl and NaOH solutions ranging from pH 6.8 to 8.0. This parameter varied from 0.3 to 0.6 μEqH^+ per pH unit (mean, 0.45 μEqH^+ per pH unit).

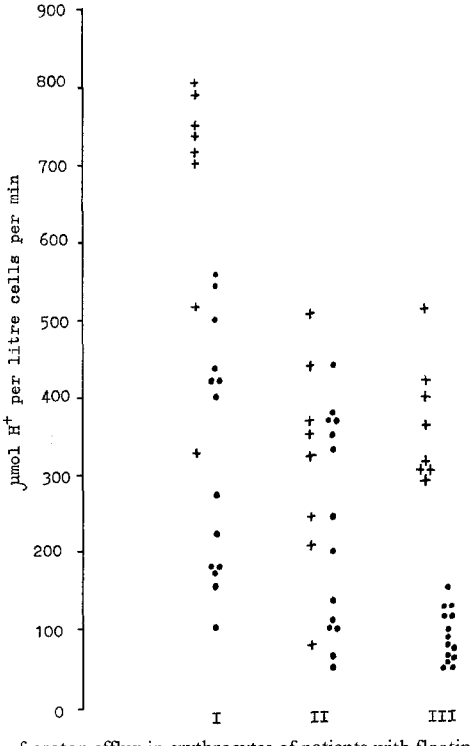
Results

The data obtained reveal significant differences in Na/H-exchange rates in erythrocytes of patients with the occlusive type of venous thromboses, in comparison with those from patients with floating venous thrombi and from the control group (see table and fig.). The total proton efflux rate, as well as the amiloride-insensitive and amiloride-sensitive proton effluxes in patients with floating thrombi did not differ from those in healthy subjects.

In patients with occlusive venous thrombi, the mean rate of total proton efflux was twice as large as in patients with floating thrombi and in controls ($p < 0.005$). No differences were observed between the rates of amiloride-insensitive efflux. The mean rate of amiloride-sensitive proton efflux, i.e. Na/H-exchange, in patients with occlusive venous thromboses was 361 ± 27.8 $\mu mol\ H^+$ per l cells per min, which was fourfold higher than in patients with the floating type of venous thromboses and in controls ($p < 0.0001$).

Study group	Total efflux	Amiloride-insensitive efflux	Amiloride-sensitive efflux (Na/H-exchange)
	$\mu mol\ H^+$ per liter cells per min		
1 Control (N = 9)	310.0 ± 41.0	221.8 ± 47.8	87.8 ± 12.0
2 Floating thrombi (N = 14)	326.7 ± 42.2	224.8 ± 39.1	90.5 ± 8.7
3 Occlusive thrombi (N = 8)	671.1 ± 58.3	314.4 ± 48.6	361.2 ± 27.8
$p_{1,2}$	NS	NS	NS
$P_{1,3}$	<0.005	NS	<0.0001
$P_{2,3}$	<0.005	NS	<0.0001

The rates of proton efflux in erythrocytes of patients with occlusive and floating venous thromboses and control volunteers; M ± SE.



The rates of proton efflux in erythrocytes of patients with floating (dots) and occlusive (crosses) types of deep venous thromboses; I – total efflux, II – amiloride-insensitive efflux, III – amiloride-sensitive efflux (Na/H-exchange). • = 1; + = 2.

Discussion

The plasma membrane Na/H-exchanger plays an important role in various physiological processes such as regulation of intracellular pH, metabolic responses to hormones and neurotransmitters, stimulus-response coupling, and the control of cell growth and proliferation [14–16]. In patients with essential hypertension and in spontaneously hypertensive rats, increased rates of Na/H-exchange in erythrocytes [8] as well as in platelets [5–7] and vascular smooth muscle cells [11, 12] have been reported. This membrane alteration is thought to affect the physiological characteristics of the cells. Thus, in essential hypertension, increased Na/H-exchange rates are accompanied by enhanced aggregating capacities of platelets [2–4] and augmented contractility of vascular smooth muscle [17].

In this study, a marked increase in Na/H-exchange was found in erythrocytes of patients with occlusive deep venous thromboses compared with patients with floating thrombi and control volunteers. Previously-reported rates of this exchange in essential hypertensives are almost twofold lower than the values for patients with occlusive venous thrombi obtained in this study [8]. It can be suggested that the differences in Na/H-exchange that were shown in erythrocytes may also occur in other cells, including those involved in thrombogenesis. The nature and pathophysiological consequences of such a drastically increased Na/H-exchange in patients with this type of venous thrombosis are unknown. It might be suggested that the effect found in this study could be a predisposing factor for the development of a certain type of venous thrombosis.

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Expression of alpha-cardiac myosin heavy chain in mammalian skeletal muscle

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Abstract. We have investigated the reactivity of different human, rat and cat muscles to a monoclonal antibody directed against human α-cardiac myosin heavy chain. We have found that special fiber subpopulations of human masseter and extraocular muscles, as well as the bag fibers of human, rat and cat muscle spindles, were reactive to this antibody, indicating that these fibers expressed α-cardiac myosin heavy chain or a closely related isoform. This isomyosin was present in the spindle bag fibers at early fetal stages, whereas its expression in masseter and extraocular muscle fibers was not detected during the first 22 weeks of gestation. Our results add to the list of muscle proteins which are expressed in locations or at developmental stages other than those initially described, suggesting that a revision of the present nomenclature of the subgroups of myosin heavy chains might be considered in the future.
Key words. Masseter; extraocular muscles; muscle spindle; bag fibers; immunocytochemistry; human.

The myosin heavy chain (MHC) is the main component of the sarcomeric thick filament, and different MHC isoforms have been identified[1]. These MHCs are encoded by a large multigene family and their expression is tissue-specific and developmentally regulated[2]. Recent data suggest that some MHCs are expressed in tissues or at developmental stages other than those in which they were initially characterized: the adult human masseter has been shown to express fetal MHC[3], and an α-cardiac like MHC is present in nuclear bag fibers of rat muscle spindles[4]. Histochemical[5,6] and immunocytochemical[3,7] data suggest that the human masseter muscle has a complex myosin composition. The extraocular muscles also have a special MHC composition, including the expression of slow tonic MHC[8–11] and a tissue-specific fast MHC[1,11,12]. The possible expression of hitherto unknown MHC isoforms has not been excluded. We have therefore investigated the reactivity of a variety of muscles to a monoclonal antibody raised against α-cardiac MHC[13,14]. In this study we have demonstrated the expression of α-cardiac MHC in a subpopulation of extrafusal fibers, both in human masseter and extraocular muscles, and in the bag fibers of human, rat and cat muscle spindles.

Material and methods

Muscle samples from the extraocular muscles of two adults and from the masseter and the biceps muscles of four adults, three 7- to 9-week-old, one 3-month-old and four 4- to 7-year-old healthy human subjects were collected one to three days after death[15]. Masseter, extraocular and limb muscles were collected from human fetuses, obtained from legal abortions at 14-, 18-, 20- and 22 weeks of gestation. The investigation was approved by the Medical Ethical Committee of the University of Umeå. The diaphragm and the masseter muscles of two adult Sprague-Dawley rats and the masseter of an adult cat were collected under sodium pentobarbital anesthesia.

Muscles were rapidly frozen in propane chilled in liquid nitrogen. Serial frozen cross-sections were cut in a Reichert-Jung (Nussloch, Germany) cryostat at −20 °C. The sections for demonstration of myofibrillar ATPase activity after preincubation at pH 10.3, 4.6 and 4.3[16] were 8 μm thick, whereas sections for immunocytochemistry were 4–6 μm thick. The monoclonal antibody F 88.12F8 (Sera-lab, Sussex, England) was raised against human α-cardiac MHC and has been shown to bind specifically to this MHC in both rat and hu-