Na^+/H^+ Countertransport and Calcium Exchange in Peripheral Blood Cells of Healthy Subjects and Patients with Chronic Heart Failure

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A comparative study is performed of Na^+/H^+ exchange and Ca^{2+} mobilization in erythrocytes and platelets of patients with stage I-II chronic heart failure caused by dilative cardiomyopathy and ischemic heart disease. A significant rise in the Na^+/H^+ exchange rate is found in the cells of chronic heart failure patients, which correlates with an elevated erythrocyte and platelet concentration of Ca^{2+} and an increased "calcium" response of platelets to inductors. The findings testify to a certain functional relationship between various cation- transporting cellular systems whose change in properties upon chronic heart failure can play an important pathogenic role.

Key Words: *Na^+/H^+ exchange; Ca^{2+} exchange; chronic heart failure*

Changes in the transport of individual cations across the cell plasma membrane and on the intracellular level play an important pathogenic role in various pathologies [3,4]. Blood cells, erythrocytes and platelets in particular, on the one hand present a suitable model for the study of ion transport in the cells of cardiac and vascular tissues [4,10], and on the other are one of the principal factors mediating the development of rheological and microcirculatory disturbances in cardiovascular diseases, including chronic heart failure (CHF). In this connection it is of interest to study the processes of ion transport and mobilization which determine the functional activity of blood cells in various pathologies [13].

We previously reported changes in the nature of ion transport and mobilization in blood cells of CHF patients, notably a rise in the platelet concentration of Ca^{2+} ions [2,7], and changes in the Na^+/H^+-ATPase activity of platelets [8]. Recently we published data on an increased rate of Na^+/H^+ exchange in the erythrocyte plasma membrane in patients with CHF [9] which is similar to that observed during hypertension [4], diabetes mellitus [12], dyspeptic disorders [5], and some other pathologies. There is some evidence in the literature of a possible link between the Na^+/H^+ countertransport rate and intracellular calcium activity, as well as the possible participation of Na^+/H^+ exchange in the process of platelet activation [14].

In the present study an attempt was made to compare indexes of amyloride-sensitive Na^+/H^+ countertransport of the erythrocyte plasma membrane with mobilization and calcium ion exchange in erythrocytes and platelets of CHF patients and healthy donors.

MATERIALS AND METHODS

Thirty-four patients with stage I-IIB CHF (26 males and 8 females aged from 31 to 65 years) and 27

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TABLE 1. Indexes of Erythrocyte Na^+/H^+ exchange and Ca^{2+} Mobilization in Erythrocytes and Platelets of Healthy Donors and CHF Patients ($M \pm m$)

Index	Healthy subjects (n=27)	CHF patients				
		total (n=34)	DCMP (n=20)	IHD (n=14)	I-IIA (n=22)	IIB (n=12)
Na^+/H^+ exchange rate (μ H^+ per liter of cells per min)	106.6±3.34 (76.2–146.2)	186.4±6.75 (107.6–248.4)	197.6±5.28 (166.5–248.4)	157.1±8.73 (107.6–245.4)	169.6±8.32 (107.6–245.4)	201.6±9.8 (116.4–248.4)
			$p<0.001$		$p<0.002$	
Erythrocyte $[Ca^{2+}]_i$ ($\times 10^{-4}$ M)	5.03±0.04 (4.71–5.34)	5.85±0.07 (5.21–6.73)	6.02±0.08 (5.26–6.73)	5.62±0.09 (5.21–6.48)	5.81±0.09 (5.21–6.53)	5.93±0.1 (5.38–6.73)
			$p<0.002$		n.s.	
Platelet $[Ca^{2+}]_i$ ($\times 10^{-3}$ M)	214.4±3.99 (174.7–242.8)	323.1±7.43 (208.3–386.2)	336.3±7.37 (277.8–386.2)	304.1±13.41 (208.3–377.4)	307.3±9.31 (208.3–377.4)	351.6±7.09 (299.2–386.2)
			$p<0.05$		$p<0.02$	

Note. All findings are significant with respect to the control ($p<0.001$); significant differences between groups are indicated; n.s. – nonsignificant.

healthy subjects (22 males and 5 females aged from 29 to 46 years) without signs of cardiac failure were enrolled in the study. Patients with a history of myocardial infarction (less than one year before), hypertension and symptomatic hypertension, diabetes mellitus, gastrointestinal disorders, liver and kidney diseases, and other chronic pathologies were rejected. Twenty patients developed dilative cardiomyopathy (DCMP), which was diagnosed by means of ventriculography and myocardium biopsy (16 males and 4 females, average age 41.7±2.4 years) and 14 patients developed ischemic heart disease (IHD) (9 males and 5 females, average age 43.2±1.6 years). Among the 20 patients with DCMP of stage I-IIA CHF was observed in 11 patients and stage IIB in 9 patients; among the 14 patients with IHD the same stages were found in 11 and 3 patients, respectively. All patients undergoing the study received no therapeutics 3-4 days prior to its initiation.

Blood in the amount of 10 ml was taken from the cubital vein in a lying position in the morning before meals and kept in plastic vials containing 1.0 ml of 3.8% sodium citrate. Platelet isolation and the addition of a Fura-2 fluorescent probe were performed by the method of Avdonin *et al.* described earlier [1]. Fura-2 fluorescence was recorded on a Hitachi-F3000 spectrofluorimeter at 37°C (excitation wavelength 350 nm, emission wavelength 500 nm). The concentration of ionized Ca^{2+} $[Ca^{2+}]_i$ in platelets was measured using formulas and K_d=224 nm, as described earlier [11]. Thrombin- and ADP-induced elevation of $[Ca^{2+}]_i$ in platelets was estimated by the difference between $[Ca^{2+}]_i$ (thrombin/ADP)-$[Ca^{2+}]_{ex}$ and expressed in percent.

After the platelet-enriched plasma had been taken, the pellet containing erythrocytes was kept at the temperature of thawing ice for not more than 4 h, after which the erythrocytes were sedimented by centrifugation at 1000 g for 10 min and rinsed three times in saline containing 5 mM sodium phosphate (pH 7.4). The Ca^{2+} ion content in hemolysates of washed erythrocytes was determined ionometrically by means of a Ca^{2+}-selective electrode (Beckman).

Na^+/H^+ exchange in erythrocytes was determined by the method described earlier [6] with slight modifications [5]. The kinetics of released ions was recorded on a pH-meter (Beckman-F-70), and the Na^+/H^+-exchange rate was calculated by the formula

$$(pH_1 - pH_2)B^{-1}m^{-1}t^{-1},$$

where $pH_1 - pH_2$ is the initial rate of pH change in amyloride-free and amyloride-containing medium, respectively; B is the buffer capacity of the incubation medium of 200 μ SITS (Sigma) (the proton micromole number required to change the pH from 8.0 to 7.0); m is the number of cells in the suspension (0.0001 liter); t is the incubation time (min).

SITS (50 mM) and amyloride (500 mM) solutions in dimethyl sulfoxide were used in the study. The SITS solution was prepared immediately before the experiment. Statistical analysis was performed using a standard statistical software package.

RESULTS

Table 1 demonstrates a reliable increase in the Na^+/H^+ exchange rate of erythrocytes even at the initial stages of CHF, while in patients with severe decompensation (stage IIB) this index is even

TABLE 2. Indexes of Ca^{2+} Exchange in Platelets after Thrombin and ADP Stimulation in Healthy Donors and CHF Patients ($M \pm m$)

$[Ca^{2+}]_i$ increment in platelets, (% of the initial value) after stimulation	Healthy subjects (n=27)	CHF patients				
		total (n=34)	DCMP (n=20)	IHD (n=14)	I-IIA (n=22)	IIB (n=12)
ADP, 5 μ	750.4±32.99 (436–1020)	964.1±44.22 (531–1594)*	1049.2±65.05 (531–1594)*	842.5±28.65 (586–997)*	845.4±38.69 (531–1315)*	1181.6±68.77 (875–1594)*
			$p<0.02$		$p<0.001$	
Thrombin, 0.5 unit per 1 ml of cells	1910.7±26.62 (1755–2236)	2804.4±60.06 (2056–3278)*	2957.6±64.01 (2056–3278)*	2585.6±86.16 (2186–3210)	2475.3±181.1 (2056–3278)	2983.2±81.47 (2256–3250)*
			$p<0.01$		$p<0.01$	

Note. An asterisk denotes significant differences from the control ($p<0.01$); the reliability of differences between the groups is indicated.

more pronounced. The concentration of ionized Ca^{2+} in erythrocytes is also significantly increased in CHF patients versus healthy subjects. The index tends to rise with increasing severity of cardiac failure.

The Na^+/H^+ countertransport rate and Ca^{2+} level in erythrocytes of CHF patients (irrespective of the main diagnosis or disease stage) were significantly higher than in the cells of healthy subjects. This may be attributed to the imbalance of intracellular ions, sodium and calcium in particular, observed in the majority of CHF patients, which was more pronounced in DCMP than in IHD patients (although the differences were not statistically significant). Patients with severe CHF (stage IIB) showed a significantly higher rate of Na^+/H^+ countertransport and Ca^{2+} content in erythrocytes compared with the patients at stages I-IIA ($p<0.05$).

The simultaneous increase in the Na^+/H^+ countertransport rate and $[Ca^{2+}]_i$ of erythrocytes was confirmed by the high correlation between these indexes observed both in healthy donors ($r=0.7767$, $p<0.001$; $y=3.921+0.0104x$) and in CHF patients ($r=0.6461$, $p<0.001$; $y=4.632+0.006\times \times 8x$). It is of interest to note that the division of CHF patients into groups depending on the main diagnosis revealed a statistically significant correlation between these parameters: for DCMP $r=0.5807$, $p<0.01$; $y=4.1082+0.0096x$; for IHD $r=0.5471$, $p<0.05$; $y=4.949+0.0043x$.

Splitting up the group of CHF patients depending on the stage of cardiac failure demonstrated that patients with stages I-IIA exhibited a high positive correlation between parameters ($r=0.6455$, $p<0.002$; $y=4.576+0.0071x$), while the patients with stage IIB failed to show this correlation. This may be due to the disconnection between the functional activity of the systems responsible for Na^+ and Ca^{2+} ion transport in the erythrocytes of the patients with severe CHF stages, whereas at less severe stages this correlation was preserved, maintaining to some extent intracellular cation balance.

If we regard erythrocytes as a model of smooth muscle vascular cells and assume that Na^+/H^+ exchange occurs in the plasma membrane (which in fact it does not), we can arrive at the conclusion that Na^+/H^+ exchange plays a most important role in determining a stable level of ionized Ca^{2+} concentration in the cytosol of smooth muscle cells and ultimately determines the stability of the vascular tonus. Therefore, any factor promoting a rise in the intracellular Na^+ concentration, activated Na^+/H^+ exchange in particular, will provoke an elevation of the Ca^{2+} concentration in the cytosol via the Na^+/H^+ exchange and play a significant pathophysiological role in the development of chronic circulatory insufficiency. It is most likely that vascular tonus is very sensitive to changes in the intracellular pH, whose value is also regulated by Na^+/H^+ countertransport activity [13]. For instance, the vasodilatory effect of hypercapnia observed in various parts of the vascular bed may be due to a lowered intracellular pH. The nature of this correlation is still obscure and merits investigation. Published data have suggested that Na^+/H^+ exchange activity may be regulated either by Ca^{2+} intracellular activity by calmodulin, or by the effect of Ca^{2+} ions on phospholipid-dependent protein kinase (protein kinase C). However, no such correlation was found in a number of studies. It was demonstrated that mouse neuroblast treatment with A23187 Ca^{2+} ionophore at various concentrations in the presence of external Ca^{2+} did not change the rate of amyloride-sensitive Na^+ uptake. Using human lymphocytes it was shown that the calcium ionophore ionomycin had no effect on the Na^+/H^+ exchange rate. Our data are supported by other findings and indicate

that such a correlation occurs in the cells of normal subjects and CHF patients alike.

The mechanism of this correlation may be quite complex and the underlying causes are multifactor. There is some evidence in the literature about the participation of Na^+/H^+ countertransport in platelet activation by thrombin [14] accompanied by a dose-dependent increase in Ca^{2+} uptake and a rise in the cytoplasmic pH after stimulation. Thrombin-induced elevation of the pH was shown to decrease by 50% after preliminary treatment with amyloride (10^{-4} M) and was completely prevented with amyloride at a concentration of 10^{-3} M accompanied by partial inhibition of serotonin production by the cells. Other studies also showed the rise in Ca^{2+} intracellular concentration after thrombin stimulation to occur simultaneously with the activation of the Na^+/H^+ exchange rate [12], although the causes of this functional correlation were not elucidated

We obtained a significant positive correlation between the erythrocyte Na^+/H^+ countertransport rate and ionized Ca^{2+} platelet concentration in healthy donors ($r=0.7039$, $p<0.001$; $y=120.67+0.876x$) and CHF patients ($r=0.4913$, $p<0.001$; $y=213.4+0.6001x$). On the one hand, it was due to a positive high correlation between $[Ca^{2+}]_{ex}$ of erythrocytes and platelets in the control group ($r=0.8452$, $p<0.0001$; $y=3.021+0.0094x$) and in the patient group ($r=0.6359$, $p<0.001$; $y=4.0495+0.0056x$). On the other hand, it may indicate changes in Ca^{2+} exchange in the cells with accelerated Na^+/H^+ exchange, leading, first, to an increased mobilization of ionized Ca^{2+} in the cytosol of the latter, and, second, to an increase in platelet sensitivity to the inductor effect, mediating the changes in functional activity of the cells, and in particular aggregation activity. This interpretation is supported by the higher level of $[Ca^{2+}]_i$ increment stimulated by thrombin and ADP which was observed in the platelets of the patients with cardiac failure, whose Na^+/H^+ exchange rate and basal level of $[Ca^{2+}]_i$ were found to be significantly higher than the parameters calculated for the cells of healthy subjects (Table 2). The significant correlation found between the level of $[Ca^{2+}]_i$ increment of platelets stimulated by thrombin and the Na^+/H^+ countertransport rate of erythrocyte plasma membranes may also speak in favor of this ($r=0.7792$, $p<0.001$; $y=1531.29+7.037x$). The accelerated Na^+/H^+ exchange induced by the increase in cytoplasmic activated Ca^{2+} developing as a result of metabolic disturbances of the latter and mediated by the calmodulin effect may offer another possible explanation.

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