PLATELET Na^+/H^+ EXCHANGE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Essential hypertension in man and hereditarily determined hypertension in rats are characterized by disturbances of transmembrane transport of ions, especially Na^+, found in blood cells, smooth muscle cells of vessels, cardiomyocytes, and so on [5, 9, 12]. Numerous investigations conducted mainly on blood cells of patients with essential hypertension and of spontaneously hypertensive rats (SHR) have shown that disturbances of transmembrane Na^+ transport may be linked to a certain extent with Na^+/Na^+-antitransport, the velocity of which was found to be increased [5, 9, 12]. However, under physiological conditions this transport system cannot bring about changes in the Na^+ concentration inside the cell [7, 9, 13]. Proof has been obtained in recent years that increased Na^+/Na^+-exchange in patients with essential hypertension and in SHR indicates changes in activity of Na^+/H^+-exchange [7, 13]. In our previous investigations [1, 6] and in those by other workers [11, 14] the important role of this exchanger in the regulation of intracellular pH (pH_i), Na^+, and Ca^{2+}, cell growth and proliferation, cell metabolism, the formation of the cellular response to stimulation, and reabsorption of Na^+ in the renal tubules, has been established and is of great importance in the pathogenesis of arterial hypertension.

The aim of this investigation was to study activity of Na^+/H^+-exchange in platelets of SHR and normotensive Wistar-Kyoto (WKY) rats.

EXPERIMENTAL METHOD

Experiments were carried out on 10 SHR in the stage of stable high blood pressure (BP). The age of the animals was 20 weeks, their body weight 320 ± 10 g, and their BP 171 ± 5 mm Hg. The control consisted of 10 normotensive WKY rats of the same age (body weight 331 ± 12 g, BP 125 ± 7 mm Hg). Under ether anesthesia 6 ml of blood was taken from the abdominal aorta of the rats and mixed with anticoagulant (in mM): sodium citrate 97, citric acid 78, glucose 111 (pH 4.0) in the ratio of 6:1. Platelet-rich plasma (PRP) was obtained by centrifugation of blood for 15 min at 120g. The pH of the PRP was 6.8. All experiments were carried out at room temperature in the course of 2-3 h.

Activity of Na^+/H^+-exchange in SHR and WKY rat platelets was determined by the method in [13]. Platelets (10^8 cells in 1 ml) were added to a medium in which NaCl was replaced by an equimolar concentration of Na^+ propionate (in mM): Na^+ propionate 138, KCl 5, KEPES 10, glucose 5, pH 6.7; osmolarity, measured on a "Knauer" semimicro-osmometer (West Germany), was 275 milliosmoles/kg. In this medium the sodium propionate is in equilibrium with the undissociated form (propionic acid), which passes through the membrane into the cell and induces cytoplasmic acidification which, in turn, activates Na^+/H^+-exchange. As a result of this the extracellular Na^+ enters the cell in exchange for H^+ ions, and under these circumstances osmotically bound water enters the cell together with Na^+ ions, causing swelling of the cell, whose volume increases [7, 10, 14]. The volume of the platelets was measured on a 147C platelet analyzer (Sweden) and recorded on an X-Y writer. Measurements of volume were made every 30-40 sec and the velocity constant of Na^+/H^+-exchange in the initial region of the curve showing the change in cell volume, and the final volume of the platelets after the cells had been kept for 5 min in medium with sodium propionate were determined. The cell volume was calculated from the curve of distribution of latex particles 2 μm in diameter.

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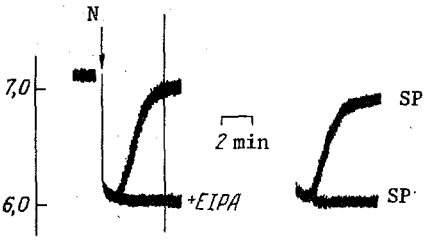

Fig. 1. Changes in pH_i in rat platelets under the influence of 25 μM nigericin (N) and sodium propionate (SP). Ordinate, changes in pH_i (in conventional units). Bottom curves – effect of 20 μM EIPA (specific inhibitor of Na^+/H^+-exchange).

To prove that Na^+/H^+-exchange was in fact activated in platelets in propionate medium experiments were carried out in which the cytoplasmic pH (pH_i) was directly recorded. For this purpose the PRP was incubated with 2 μM of the fluorescent probe BCECF-AM ("Molecular Probes," USA) for 30 min at 37°C. Platelets loaded with the probe were separated from PRP by centrifugation and resuspended up to a concentration of $3 \cdot 10^8$ cells/ml in control medium (buffer solution containing NaCl instead of sodium propionate). Fluorescence was measured on a "Hitachi 650-60" spectrofluorometer (Japan) at room temperature. The wavelengths of excitation and emission were 505 and 535 nm, respectively, and the slit width 5-8 nm. The value of pH_i was calculated by the method in [15].

EXPERIMENTAL METHOD

Changes in intracellular pH_i in platelets of control rats are given in Fig. 1. Both during the action of 25 μM nigericin (a K^+/H^+-ionophore) and when the cells were added to medium with Na^+ propionate, initial acidification of the cytoplasm took place, but later pH_i gradually returned to its initial level. This was due to activation of N^+/H^+-exchange, for the use of EIPA, a specific inhibitor of this exchanger [16], prevented the rise of pH_i (Fig. 1).

In medium containing NaCl or K^+ propionate, no changes took place in volume of either the WKY or the SHR platelets, at least in the course of 5-10 min; moreover, no differences were found between the volume of the cells in the two groups of animals (Table 1). Meanwhile in medium containing Na^+ propionate, the volume of the platelets was significantly increased. That the changes observed in platelet volume were in fact the result of activation of Na^+/H^+-exchange was shown by data obtained when this process was suppressed. Suspending the platelets in medium with Na^+ propionate and 20 μM EIPA did not lead to any changes in platelet volume (Table 1).

In medium with Na^+ propionate the increase in volume of the SHR platelets was greater than of WKY. The final volume of the platelets and the velocity constant in SHR were increased by about 30-50 ($p < 0.05$). Hence it can be postulated that activity of Na^+/H^+-exchange was increased in SHR.

The results of these investigations thus indicate that activity of Na^+/H^+-exchange is higher in SHR than in WKY platelets. Similar results were obtained previously in SHR lymphocytes [7] and in platelets from patients with essential hypertension [13]. The authors cited consider that these changes may be connected with an increase in the number of molecules of the exchangers or an increase in the velocity of exchange.

Activation of Na^+/H^+-exchange leads to several important consequences, which play an important role in the pathogenesis of arterial hypertension in SHR. First, investigations [5] have shown an increase in the intracellular Na^+, which is evidently connected also with the work of the antiporter, for in response to its activation the intracellular Na^+ level rises. Second, by changing pH_i, Na^+/H^+-exchange may modulate the action of several vasoactive compounds, hormones, intracellular enzymes, etc., which participate in the regulation of vascular tone, for an optimal pH_i is required for manifestation of their intracellular effect [10, 14].

TABLE 1. Changes in Final Volume and Velocity of Activation of Na^+/H^+-Exchange in WKY and SHR Platelets (M ± m)

Experimental conditions	Experimental animals	Number of animals	Maximal volume, μm^3 (5 min)	Rate of activation, $\mu m^3/sec \cdot 10^{-3}$
Na propionate	WKY	(10)	5,6±0,4	5,1±0,1
	SHR	(10)	9,3±0,4*	6,3±0,1*
NaCl or K propionate or Na propionate + EIPA	WKY	(10)	0	0
	SHR	(10)	0	0

Legend. *$p < 0.05$ compared with WKY.

Third, there is evidence of interconnection between Na^+/H^+-exchange and Ca^{2+}-transport in cells, including platelets. The present writers [6] and others [11] showed previously that activation of Na^+/H^+-exchange initially requires a small increase in intracellular Ca^{2+}, and in turn, the activated Na^+/H^+-exchange facilitates the full entry of Ca^{2+} into the cell. It can accordingly be suggested that the increase in the intracellular Ca^{2+} concentration in SHR platelets, discovered in a number of investigations [3, 5], including our own [2], is connected with activation of Na^+/H^+-exchange in these cells. If this takes place in vascular smooth muscle cells (VSMC) of SHR (and platelets are an adequate model of these cells), activation of Na^+/H^+-exchange in the latter will lead to a rise of the intracellular Ca^{2+} and, to an increase in vascular tone. Yet another important consequence of activation of the Na^+/H^+-antiporter in VSMC in arterial hypertension is evidently its participation in the mechanisms of development of their hypertrophy (see above), leading together with other factors to an increase in vascular resistance [4, 8]. Fifth and finally, if activation of Na^+/H^+-exchange takes place in cells of the renal tubules, this mechanism may lie at the basis of the increased Na^+ reabsorption and readjustment of kidney function in the presence of a raised BP. However, since Na^+/H^+-exchange in SHR and in patients with essential hypertension has been studied only in blood cells, it is too early to say whether the changes discovered are primary or secondary with respect to arterial hypertension and connected with it by other membrane and/or neurohumoral shifts.

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