Decreased Sensitivity of Adenylate Cyclase to Sodium Ions in Lymphocytes of Patients with Arterial Hypertension

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Patients with arterial hypertension showed decreased sensitivity of isoproterenol-stimulated adenylate cyclase of mononuclear lymphocytes toward sodium ions compared with normotensive donors. Ouabain, an inhibitor of Na,K-ATPase, increased the half-maximal inhibition of adenylate cyclase activity by sodium ions in the cells of healthy subjects and did not change enzyme sensitivity to sodium in the lymphocytes of hypertensive patients.

Key Words: *sodium; adenylate cyclase; lymphocytes; arterial hypertension; ouabain*

Changes in the production of different neurotransmitters and hormonal factors, as well as alterations in the cellular mechanisms of hormonal signal transmission via membrane receptors and in the transport of calcium and monovalent cations are typical features of hypertension [4,14]. Progression of the disease is attended by a decrease in adenylate cyclase (AC) affinity to catecholamines in peripheral blood lymphocytes [8] and a rise in the intracellular concentration of sodium ions in erythrocytes and lymphocytes [3,11]. Interaction of α- and β-adrenergic receptors with ligands is modulated allosterically by monovalent cations ($Na^+ > Li^+ > K^+$), while changes in the receptor affinity to catecholamines correlate with the changes in the intracellular sodium concentration [10,12,13]. Sodium exerts an inhibitory effect on AC activity [7]. Data on the phosphorylation of the α-subunit of Na,K-ATPase by cAMP-dependent protein kinase A and protein kinase C suggest the existence of multiple bonds between the Na,K-ATPase system of active transport of monovalent cations and the cAMP system [6]. Our previous studies demonstrated that the Na,K-ATPase inhibitor ouabain modulates AC activity in the lymphocytes of healthy donors [1] and alters cyclase sensitivity to sodium ions [2]. The purpose of the present study was to investigate the inhibitory effect of sodium on AC activity in the lymphocytes of patients with arterial hypertension (AH) compared with the cells of normotensive donors.

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MATERIALS AND METHODS

Studies were performed on freshly isolated mononuclear peripheral blood lymphocytes taken from healthy donors and patients with pronounced AH. Cells of 11 normotensive males aged 47±2 years and of 10 males aged 49±3 years with stage IIA of AH according to the classification of Myasnikov (mean arterial pressure 130±3.3 mm Hg) were used in the study. The patients received no medication for 2 weeks prior to the study. For the isolation of lymphocytes blood was taken at 09:00 h from the ulnar vein (from a fasting patient) lying down. The fraction of mononuclear lymphocytes was isolated on a Ficoll-Verographin gradient according to a modified technique [2,5].

Basal and isoproterenol-stimulated (100 μM) AC activity was determined in lymphocyte homogenates by the method of Salomon [15] with some modifications [1]. α^{32}P-ATP (Amersham and Izo-

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TABLE 1. AC Activity of Lymphocytes ($M \pm m$)

Group of patients	AC activity, pmol cAMP/mg protein×min		
	basal	isoproterenol-stimulated	% stimulation
Normotensive donors	5.45±1.28	7.56±1.70	41.9±7.46
Hypertensive patients	6.02±1.58	7.67±2.00	29.0±4.28

TABLE 2. Half-maximal dose (ID_{50}) of Sodium Inhibition of Isoproterenol-Stimulated Activity of AC

Group of patients	ID_{50} for sodium, mM	
	without ouabain	with ouabain
Normotensive patients	36.4±3.06	64±8.29*
Hypertensive patients	70.3±5.45**	70.6±6.58

Note. *$p<0.01$, **$p<0.005$ compared with normotensive donors without ouabain.

top, specific activity 380-1850 GBq/mmol) was used as substrate. For analysis of the inhibitory effect of sodium ions on AC activity 20-100 mM NaCl were added to the incubation medium. The effect of ouabain (10 μM) was evaluated by applying it to the cell homogenate. The half-maximal dose of inhibition of AC activity (ID_{50}) by sodium ions was evaluated. The results are presented as the arithmetic mean and standard deviation. Statistical analysis of the data was performed according to Student's t test.

RESULTS

No statistically significant differences were observed in the comparative analysis of the basal and isoproterenol-stimulated AC activity and enzyme activation by isoproterenol in the lymphocytes of normo- and hypertensive patients (Table 1). AC activation by isoproterenol tended to decrease in the cells of stage IIA AH patients. AC stimulation by isoproterenol during AH of stage IIA was shown to be significantly lower than in normotensive donors [8]. The degree of AC activation by catecholamines probably diminishes as AH progresses.

The half-maximal inhibitory concentration (ID_{50}) of sodium ions for AC of lymphocytes of healthy donors (Table 2) corresponded to ID_{50} for cells of various origin, ranging from 20 to 40 mM according to published data [9]. ID_{50} for AC of lymphocytes of AH patients proved to be twice as high as in the control (Table 2), which may indicate a decreased sensitivity of lymphocyte AC to sodium ions during AH. Changes in enzyme sensitivity to sodium are observed at earlier stages of the disease.

Ouabain caused an almost two-fold increase in ID_{50} for AC of lymphocytes of normotensive donors (Table 2). In the cells of AH patients ouabain did not alter the ID_{50} level of sodium ions for AC. A study of the effect of ouabain on sodium inhibition of lymphocyte AC activity demonstrated that this cardiac glycoside changes ID_{50} only during isoproterenol activation of the enzyme via the β-adrenergic receptor without altering the direct activation of the catalytic subunit of AC or its activation via G_s-protein [2].

The content of an endogenous ouabainlike factor is known to increase in the plasma as AH progresses [4]. The effect of this factor is probably accompanied by an increase in the intracellular concentration of sodium ions in blood cells [3,11], and this increased sodium content may lower the affinity of the β_2-adrenoceptors and coupled AC for catecholamine stimulation [8]. Along with this, studies of ouabain suggest that the Na,K-ATPase inhibitor is able to reduce AC sensitivity to sodium ions in a manner which is not yet understood.

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