injection of the preparations had the opposite effect, similar to that observed in the cerebral cortex. In the cerebellum, no significant effect on the cGMP level was discovered by either of the preparations studied. There was only a tendency for the level of this nucleotide to fall under the influence of adenosine. It may be that adenosine influences the bioavailability of calcium through a change in the activity of the guanylate-cyclase system [7].

The experiments showed that cAMP and cGMP at the level of the cerebral cortex have reciprocal relationships with one another, whereas in the hippocampus, hypothalamus, and cerebellum, this is not observed. Adenosine is known to be metabolized quite rapidly in the body by the enzyme adenosine deaminase to inosine and hypoxanthine. Since inosine is a ligand of benzodiazepine receptors [1, 4], and can also bind with adenosine receptors [7, 8], it can be tentatively suggested that the changes observed in the cyclic nucleotide levels are connected with the action both of adenosine itself and of its metabolites.

Thus adenosine, by modifying the activity of the cyclase systems, can interfere with the fine mechanisms of functioning of neuron populations.

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INCREASED Na^+/H^+ EXCHANGE IN ERYTHROCYTES OF HYPERTENSIVE PATIENTS

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In the mid-1970s the first data were obtained to show an increase in passive (i.e., unconnected with Na, K-ATPase function) permeability of the erythrocyte membrane of hypertensive patients [14] and spontaneously hypertensive rats (SHR) [6] for monovalent cations. These observations have now been repeated in several laboratories and they are associated with an increase in the rate of translocation of the following carrier ions: 1) Na^+,K-cotransport (furosemide-inhibited component of transmembrane Na^+ and K^+ transfer); 2) Na^+/Na^+ antitransport, which is activated 2-4-fold by substitution of Li^+ for Na^+, and which is accordingly calculated as the difference between the rate of Li^+ outflow into medium containing and not containing Na^+ [7].

Comparatively recently an Na^+/H^+ antitransport (exchange) system responsible for regulation of the intracellular pH, and blocked by amiloride and its derivative [8], has been identified in the plasma membranes of cells of a number of tissues. It has been shown that the rate of working of this transmitter is increased many times over under the influence of compounds causing activation of protein kinases of tyrosine type (growth factors) or a phosphoinositide response (α-adrenoreceptor agonists, some peptide hormones), and it is accompanied by alkalification of the cytoplasm and in some cases, by DNA duplication and tissue proliferation [11].

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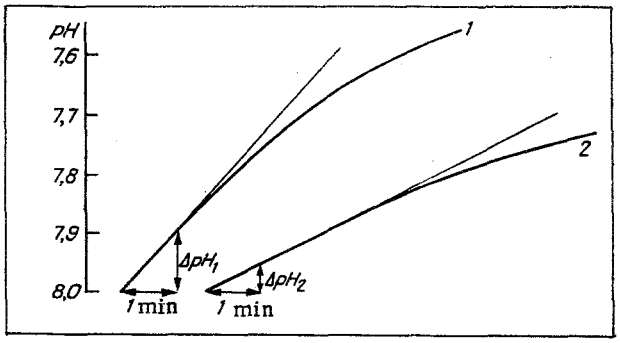

Fig. 1. Kinetics of proton outflow from human erythrocytes. 1) Control, 2) incubation medium contains 0.5 mM amiloride.

TABLE 1. Rate of Outflow of H^+ from Erythrocytes of Hypertensive Patients (M ± m)

Group tested	Without amiloride	With amiloride	Amiloride-inhibited component (Na^+/H^+ exchange
	μmole H^+/liter cells/min		
Control	310,0±41,0	221,8±47,4	87,8±12,0
Hypertensive patients	339,6±31,0	172,7±24,8	166,8±17,4*

Legend. Here and in Table 2: *p < 0.05.

Na^+/H^+ exchange in rat (but not human) erythrocytes can be activated by compression of the cells [4]; this procedure is more effective in erythrocytes of SHR than of normotensive rats [5]. Acidification of the cytoplasm ($\Delta\mu H^+$-induced Na^+/H^+ exchange) is another way of activating Na^+/H^+ exchange [9]. This method was used in the present investigation to study the rate of Na^+/H^+ exchange in the erythrocytes of hypertensive paitents.

EXPERIMENTAL METHOD

The group of hypertensive patients was made up of 11 men and 3 women aged from 39 to 58 years (average age 51.2 ± 1.5 years). The average duration of the hypertension was 8.5 ± 1.9 years (from 1 to 28 years) and the maximal arterial pressure (BP) was: systolic 215 ± 7 mm Hg, diastolic 120 ± 4 mm Hg, compared with the normal BP (systolic 144 ± 5 mm Hg, diastolic 82 ± 2 mm Hg). Stage II hypertension, according to the WHO classification, was diagnosed in eight patients, in three patients the course of hypertension was complicated by the development of myocardial infarction not less than 2 years before the present investigation, and three patients had cerebrovascular disturbances. During the investigation there were no signs of circulatory failure, the patients were not receiving cardiac glycosides, and no diuretics were taken, at least during the 2 days before the investigation. The diagnosis of essential hypertension was made after an investigation comprising stage 1 of a two-stage program for the investigation of patients with arterial hypertension.

The control group consisted of two women and seven men aged from 42 to 60 years (average age 49.5 ± 1.6 years) with normal BP, and not previously suffering from persistent hypertension. The patients were admitted to hospital for various diseases and, on the basis of their state of health, they were ready to be discharged at the time of the investigation. Two patients had infectious-allergic bronchial asthma, one had focal pneumonia, two had chronic bronchitis, two had peptic ulcer, one had chronic cholecystitis, and one patient had chronic colitis. Blood was taken from the cubital vein in the morning before breakfast into tubes containing heparin (20-50 U to 1 ml blood). The erythrocytes were washed with physiological saline containing 5 mM sodium phosphate (pH 7.4) to remove plasma. Packed cells were kept on ice for not more than 30 h.

Na^+/H^+ exchange in the erythrocytes was determined by the method in [9] with minor modifications. The 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. In some experiments protein kinase C acti-

TABLE 2. Effect of Protein Kinase C Activator (1 μM PMA) on Rate of Outflow of Protons from Human Erythrocytes (M ± m)

Conditions of testing	Without amiloride	With amiloride	Amiloride-inhibited component (Na^+/H^+ exchange)
	μmoles H^+/liter cells/min		
Control	380,0±26,2	277,6±33,3	102,4±14,9
PMA	588,0±25,0*	162,4±27,5	425,6±11,5*

Legend. Results of three investigations on pooled erythrocytes from eight control subjects are shown.

vator (β-phorbol-12-myristate-13-acetate – PMA) was added at the preincubation stage. After incubation of the cells for 5 min at 37°C and constant mixing the pH of the suspension was adjusted to 6.35-6.45 by the addition of 0.2 N HCl solution in 150 mM NaCl, after which an anionic transport inhibitor (200 μM 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid – DIDS) was added to the suspension and the pH of the suspension was increased by the addition of 0.05 N NaOH solution in 150 mM NaCl to 7.95-8.05. In some cases, before alkalification of the suspension, 0.5 mM amiloride was added. The kinetics of proton release was recorded on a PHM-64 pH-meter (Radiometer, Denmark) with a 91-15 electrode (Orion, USA). The rate of Na^+/H^+ exchange was calculated as:

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

Here ΔpH_1 and ΔpH_2 denote the initial rate of change of pH in the medium without and with amiloride respectively (Fig. 1); b denotes the buffer capacity of the incubation medium containing 200 μM DIDS (the number of micromoles of protons necessary to change the pH from 8.00 to 7.00); m denotes the number of cells in the suspension (0.0001 liter); t the incubation time (in min). Mother solutions of DIDS (50 mM) and amiloride (500 mM) in dimethylsulfoxide were used. The DIDS solution was made up immediately before the experiment.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that both the basal rate of H^+ outflow (first column) and the rate of this process in the presence of amiloride (second column) in erythrocytes of hypertensive patients did not differ from those in the control group. The amiloride-inhibited component of the rate of H^+ release in erythrocytes of hypertensive patients was virtually doubled and differed significantly from the corresponding control values.

Additional experiments showed that the amiloride-inhibited component was equal to the rate of increase of the H^+ outflow when the erythrocytes were transferred from sodium-free medium (130 mM choline chloride) into the incubation medium used in this work (data not given). This observation indicates that the twofold increase in the amiloride-inhibited component of the H^+ outflow in hypertensive patients reflects an increase in the rate of Na^+/H^+ exchange.

Unlike erythrocytes, the concentration of the anion exchanger in other blood cells inducing rapid equalization of the pH gradient is small. Accordingly, to produce transient acidification of the cytoplasm NH_4Cl or salts of weak acids passing through the membrane were used. The rate of Na^+/H^+ exchange in this case can be judged by the rate of increase in volume of the cells due to massive NaCl inflow. It has been shown by this method that the rate of $\Delta\mu H^+$-induced Na^+/H^+-exchange is approximately doubled in the lymphocytes of spontaneously hypertensive rats [10] and in platelets of patients with essential hypertension [12].

The increase in the rate of Na^+/H^+-exchange in primary hypertension may be due to the following causes: 1) an increase in protein kinase C activity [2, 15], induced, for example, by disturbance of polyphosphoinositide metabolism [1, 13]; 2) a change in the structural state of the membrane controlled by cytoskeletal proteins [3]; 3) an increase in the content of carrier molecules per unit area of membrane.

Evidence in support of the first hypothesis is given by the fourfold activation of Na^+/H^+-exchange (Table 2) by the protein-kinase C inducer PMA. Further investigations will show whether the increase in protein kinase C activity described previously in primary hypertension is the true cause of activation of Na^+/H^+-exchange. Even at this stage, however, it is clear that the increase in the rate of translocation of this carrier in smooth-muscle cells of blood vessels and epithelial cells of renal tubules may be a sufficient cause of activation of the servo mechanisms of long-term maintenance of a raised BP.

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