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BIOPHYSICS AND BIOCHEMISTRY

Influence of Activating Agents on the Membrane Potential of Lymphocytes in Patients with Hypertensive Disease

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There are few data concerning the membrane conception of hypertensive disease (HD). HD has been found to induce numerous changes in cell membranes, including alterations in ion permeability, activity of membrane-associated enzymes, physicochemical state, and in the structure of the membrane protein-lipid matrix [1-3,9]. Membrane defects, which have been demonstrated in a variety of blood cells, among them lymphocytes [3,5,8], are known to cause changes in the functional activity of immunocompetent cells and to promote the development of immune disorders in HD patients, as has been confirmed by previous studies [4,7].

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In view of this, the purpose of the present work was to study the changes in the membrane potential (MP) of the peripheral blood lymphocytes in patients with HD under the influence of activating agents.

MATERIALS AND METHODS

Twenty-five patients (males) with HD and 8 healthy person (males) aged 25 to 35 years were examined. Among the patients examined, stage I HD was diagnosed in 9 persons (36%) and stage II in 16 persons (64%). An HD duration of up to one year was detected in 4 patients (16%), from one year to 5 years in 12 patients (48%), and from 6 to 10 years in 9 patients (36%). In-depth clinico-instrumental and biochemical examination in a specialized cardiological department

made it possible to exclude symptomatic arterial hypertension. Persons with concomitant diseases were excluded from the study. Lymphocytes of the peripheral blood, obtained by ficoll-urografin gradient centrifugation in accordance with the conventional method [6], were used in the experiments. The cell count and the determination of cell viability were performed in a Fuchs-Rosenthal chamber using Trypan Blue. Measurements were carried out on freshly isolated cells. The MP of the lymphocytes was measured using a fluorescent potential-sensitive 1-aniline-naphthalene-7-sulfonate probe (ANS) on a Hitachi spectrophotometer (Japan). The ANS optimum excitation wavelength was found to be 380 nm, and the optimum radiation wavelength was 480 nm under the conditions of the experiment. The dye concentration in a cuvette was $3 \cdot 10^{-5}$ M. The MP value was expressed in relative units (r.u.). Fifty ml of ANS initial aqueous solution and 50 ml of lymphocyte suspension with a concentration of 10^6 cells/ml were added to a cuvette containing 1.5 ml of measuring medium (140 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM Na_2HPO_4, 1 mM KH_2PO_4, 4 mM $NaHCO_3$, 5 mM glucose, and 10 mM HEPES at pH 7.15) Then activating agents, namely phytohemagglutinin (PHA), concavalin A (Con A) - T-mitogens, and Ca^{2+}-ionophore A 23187 were added: PHA and Con A in a concentration ranging from 10 to 160 μM, and Ca^{2+}-ionophore A 23187 in a concentration ranging from 0.2 to 40 μM. The change (Δ) of the lymphocytes (the difference between the MP values before and after treatment with activating agents) was registered by the dynamics of ANS fluorescence intensity and was also expressed in relative units.

RESULTS

The drop of the lymphocyte MP in HD patients before treatment with activating agents (13.4±3.0 r.u) as compared to healthy persons (24.2±2.0 r.u., $p<0.05$) was found to be statistically reliable.

The data on the changes in the MP of lymphocytes in patients with HD as well as in healthy persons induced by PHA, Con A and A 23187 depending on the doses of the preparations are presented in Figs. 1 and 2. The addition of PHA, Con A and A 23187 to the lymphocyte suspension from healthy person led to the hyperpolarization of cells, and the higher the concentration, the greater the hyperpolarization. Maximum polarization was detected under the influence of A 23187, and minimum polarization in the case of PHA.

In patients with HD the hyperpolarization of the lymphocyte membranes influenced by Con A and A 23187 was reliably lower than in healthy persons (Fig. 1, *2*). In the case of PHA a certain tendency

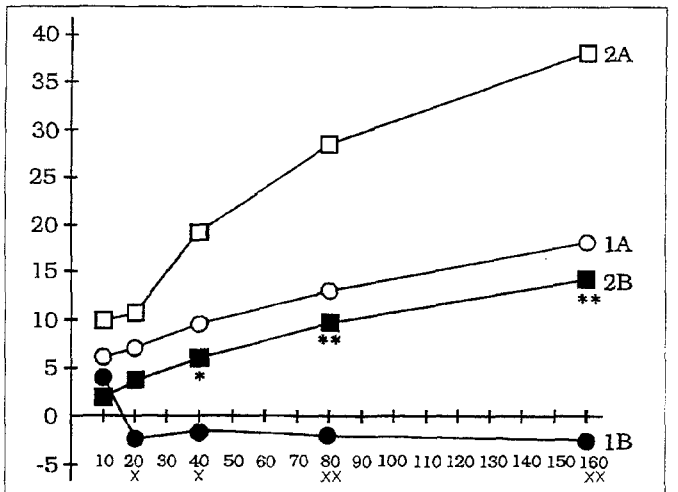

Fig. 1

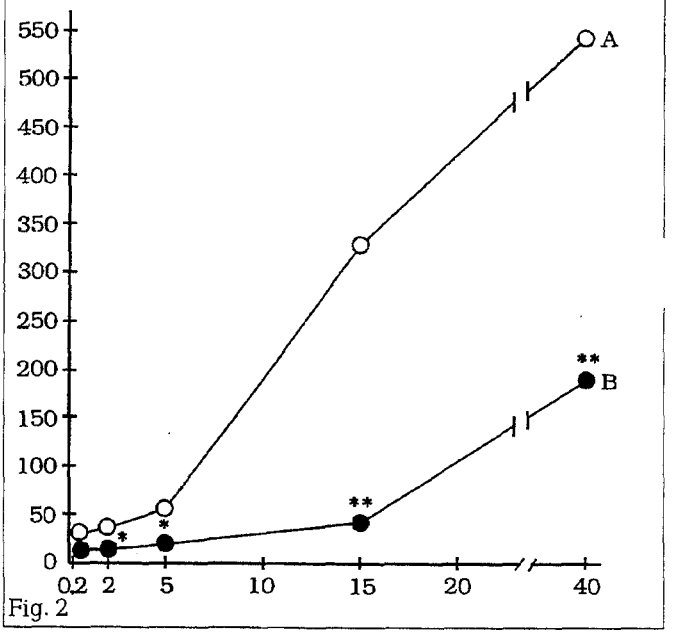

Fig. 2

Fig. 1. Changes in lymphocyte MP induced by various PHA (1) and Con A concentrations in healthy persons (*a*) and in HD patients (*b*). Abscissa: PHA and Con A concentration (in μM); ordinate: change (Δ) of MP (in r.u.). *: $p<0.05$; **: $p<0.01$ – differences are reliable between value in healthy persons and in patients with HD.

Fig. 2. Changes in lymphocyte MP induced by various concentrations of A 23187 in healthy persons (*a*) and in HD patients (*b*).Abscissa: A 23187 concentration (in μM); ordinate: change (Δ) of MP (in r.u.). *: $p<0.05$; **: $p<0.01$ – differences are reliable between values in healthy persons and in patients with HD.

was found toward a polarization of the cell membranes (Fig. 1).

Analysis of the changes in the lymphocyte MP in patients with HD depending on the stage and duration of disease revealed a tendency toward a decrease in the MP initial level as well as in the lymphocyte functional response to PHA and Ca^{2+}-ionophore as the HD duration increased.

Thus, in the patients with HD a reliable decrease in the MP level of the peripheral blood lymphocytes

as well as considerable changes in lymphocyte functional activity influenced by activating agents were found as compared to healthy persons.

The reduction of MP of blood cells and, in particular, the MP of lymphocytes in patients with HD is in accordance with the disturbances revealed in the cation-transport systems of these patients. [1,3].

It is reasonable to assume that the decrease of lymphocyte Ca^{2+}-ionophore sensitivity in HD patients is associated with an increased intracellular Ca^{2+} content due to both the enhancement of the passive Ca^{2+} permeability of the cell membranes and the disturbances in the Na^{+}-Ca^{2+} exchange [3,8,9].

These changes are undoubtedly of great importance in HD pathogenesis. The drop of blood cell MP and the increased intracellular Ca^{2+} content in patients with HD are assumed to be one of the essential factors, leading to an activation of the sympathetic, serotonic, and GABA-ergic systems as well as to an increased vascular reactivity [4].

The decrease of lymphocyte sensitivity to T-cell mitogens (PHA and Con A) attests to disturbances in the T-dependent immunity system in HD patients. The results obtained are in accordance with published data on a T-cell deficiency in animals with experimental arterial hypertension and, in particular, in spontaneously hypertensive rats [10].

Thus, the results of the investigations point to appreciable disturbances in the lymphocyte membranes and confirm the systemic nature of the membrane defects in this pathology.

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Detection of Cytoplasmic PCK BB in Nerve Cell Nuclei of Normal, Schizophrenic and Alzheimer Patients

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It has been demonstrated in our previous reports that in patients with mental diseases a depression of the activity of phosphocreatine kinase (PCK BB), which is one of the main enzymes of the brain's energy metabolism, is accompanied by a decrease of its content in extracts of cytoplasmic proteins from nervous tissue [1,2,6].

Additionally, it has been shown by the immunocytochemical method that normally PCK BB is located in the astrocyte cytoplasm and in indi-

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