Effect of Hyperbaria on Acetylcholinesterase of Human Erythrocytes

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It is shown that a helium pressure of 100 kgs/cm² (9.81 MPa) inhibits acetylcholinesterase of erythrocytes within nonsaturation concentrations of the substrate and has no effect within saturation conditions. This effect is assumed to be one of the possible mechanisms of biological action of hyperbaria.

Key Words: *hyperbaria; acetylcholinesterase; erythrocytes*

The study of acetylcholinesterase (ACE) in erythrocytes (acetylcholine hydrolase EC 3.1.1.7) under hyperbaric conditions is very promising for a number of reasons. First, since ACE is a membrane enzyme, its kinetic characteristics may be related to the membrane surrounding, some parameters of which change considerably under hyperbaric conditions [6]. Second, the mechanism of action of the enzyme has been studied at length, and an important part in the formation of the enzyme-substrate complex is known to be played by ionic and hydrophobic interactions [2] which among other weak interactions are the most pressure-susceptible [8,12]. Therefore, the kinetic properties of ACE may be considered an appropriate test object for studying the effect of hyperbaric conditions on biological systems. Third, since the biochemical properties of ACE in erythrocytes and in the brain of mammals are very similar [1], the study of ACE from human erythrocytes (much more accessible for experiments than the brain) makes it possible to assess the effect of hyperbaric conditions on ACE in the brain. The fact that acetylcholine is an inhibitory transmitter in the brain, and that the role of inhibitory synapses in the development of the high pressure nervous syndrome (HPNS) is now being widely discussed [9] makes studies of the properties of ACE under hyperbaric conditions more and more important. Here we studied the effect of hyperbaria on ACE of human erythrocytes.

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MATERIALS AND METHODS

The activity of ACE was determined as described earlier [10]. Heparinized whole blood from fingertips was centrifuged in a hematocrit centrifuge; 20 μl erythrocyte mass was added to 2 ml 0.01% saponin solution, incubated at 20°C during 5 min, and placed in the cold (0-4°C); the obtained hemolysate was used within one working day. One hundred microliters of hemolyzed erythrocytes were added to 1600 μl working solution of 5,5'-dithiobis(2-nitrobenzoic) acid (DTNB), containing 0.33 mM DTNB, 0.0003% quinidine sulfate, and 0.1 M phosphate buffer, pH 8.0, and incubated at 37°C during 10 min, after which the reaction was initiated by adding 100 μl of the substrate acetylcholine iodide. The changes of transmission of the solution were recorded in a 1-cm cuvette at 410 nm during 1-2 min.

The study was performed in a high-pressure chamber equipped with quartz lamps, a thermostat, and a magnetic stirrer. The test sample was placed in a standard 1-cm cuvette and constantly agitated. Helium compression was carried out during the 10-

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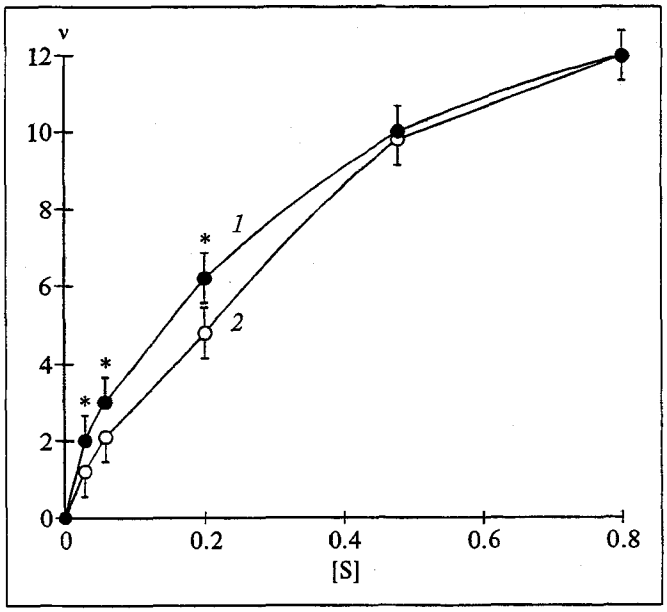

Fig. 1. Effect of helium pressure on ACE activity in human erythrocytes. Here and in Fig. 2: *v*: rate of reaction, $\times 10^6$ cells/sec; [*S*]: concentration of substrate, mM; *1*) control; *2*) helium pressure 100 kgs/cm^2 (9.81 MPa); an asterisk denotes $p=0.02$.

min incubation (from the 2nd to the 5th min), the temperature of the sample being increased by no more than 1°C and returned to 37°C 1-2 min after termination of the compression. The reaction was initiated by the addition of the substrate solution using a built-in magnet-operated sampler. The control experiments were carried out in a sealed helium-ventilated chamber at atmospheric pressure. The experiments at normal and elevated pressures were randomly alternated. The results were compared using the Student *t* test.

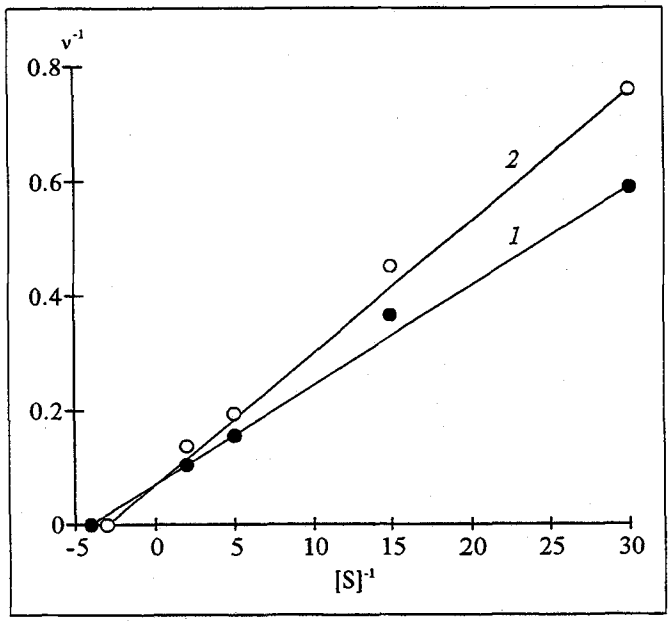

Fig. 2. Lainuiwer–Burke linearization.

RESULTS

For evaluation of the effect of helium pressure on the kinetic parameters of the ACE-catalyzed reaction, the rate of hydrolysis of acetylcholine iodide was measured for 5 concentrations of the substrate (to 0.85 mM). The reaction rate without and under pressure was compared for each concentration independently. As is seen from Fig. 1, 100 kgs/cm^2 helium (9.81 MPa) slows down the reaction within nonsaturation concentrations and has no effect as saturation is approached. The linear approximation in the Lainuiwer-Burke coordinates reveals a competitive type of inhibition under the elevated pressure, the Michaelis constant being increased 1.5-fold (from 0.33 to 0.5 mM) and the maximal reaction rate being unchanged (17.5×10^6 cells/sec).

Helium pressure leads to inhibition of the ACE-catalyzed reaction within nonsaturation concentrations of the substrate, while at high concentrations the rate of the reaction remains unchanged. The active center of ACE consists of two sites: anionic and esterase. The substrate binds specifically at the anionic site by ionic bonds between a positively charged nitrogen in a choline group of acetylcholine and hydrophobic interactions due to translocation of three methyl groups of acetylcholine to a hydrophobic surrounding of the anionic center of ACE. The acetate group specifically bound in the anionic center acetylcholine resides at the esterase site responsible for the cleavage of the ester bond.

A reduced specificity of acetylcholine binding with ACE, for instance, due to replacement of one or more methyl residues in the choline group of acetylcholine with hydrogen, resulted in a considerable inhibition of the reaction within nonsaturation concentrations of the substrate, the maximal reaction rate in excess of the substrate being unchanged [2]. Analogous changes of kinetic parameters of ACE were also observed in our experiments, and therefore we assumed that the helium pressure lowered the reaction rate within nonsaturation concentrations of the substrate due to weakening of the hydrophobic and ionic interactions between acetylcholine and the anionic site of ACE. Since the volume is known to increase by 12 ml/mol due to the formation of ionic bonds and by 22 ml/mol due to the transfer of methane to the hydrophobic phase [4], we can evaluate the decrease of the binding constant of acetylcholine and ACE corresponding to a hydrostatic pressure of 100 kgs/cm^2, i.e., we can verify our assumption.

The effect of pressure on the equilibrium constant (in our case, on the binding constant) is determined by the formula:

$$ln(K_2/K_1)=-\Delta V(P_2-P_1)/RT$$

where K_1 and K_2 are equilibrium constants at pressures P_1 and P_2, respectively, ΔV is the change in volume, R is the gas constant, and T the absolute temperature.

The maximal increase of the volume due to the formation of one ionic bond and the transfer of three methyl groups to a hydrophobic surrounding accounts for 70-80 ml/mol. The actual estimate may be somewhat lower, since the methyl groups are bound with nitrogen (i.e., shielded on one side from interaction with the solvent). Nevertheless, under the hydrostatic pressure of 100 kgs/cm^2 (9.81 MPa) the maximal possible decrease of the binding constant is 25-27% and, taking into account the actual estimates of volume change, this value is consistent with the obtained decrease of the reaction rate (15-20%).

Thus, the most likely cause of the reduced activity of ACE within nonsaturation concentrations of the substrate under elevated pressure is the weakened binding of the substrate in the active center of the enzyme. There appears to be no need to invoke speculation on the effect of pressure on the membrane lipid phase for interpretation of the observed phenomenon.

The biological significance of the observed changes may be viewed in the light of some clinical effects of ACE inhibitors. The mechanism of action of some anticholinesterase compounds, so-called reversible ACE inhibitors, resembles the disturbance of ACE functioning under hyperbaric conditions, and consists in hindered binding of the substrate at the anionic site of the enzyme, the esterase site of ACE being unaffected. It would be interesting to compare the specific effects of reversible anticholinesterase compounds with the clinical manifestation of symptoms under hyperbaric conditions. However, the clinical effects of reversible and irreversible anticholinesterase compounds are usually not distinguished. Nevertheless, the comparison of symptoms of poisoning with anticholinesterase compounds and clinical manifestations of HPNS [7] reveals a number of common features, among them myoclonia, nausea, dizziness, sleep disturbances, sleeplessness, slow-wave activity on the encephalogram, memory impairment, and respiratory and gastroenterological disorders.

The functional significance of ACE has been definitely determined only for synapses and neuromuscular terminals, where ACE utilizes the transmitter released during the previous pulse. It is possible that the time of the drop of miniature current of the end-plate on the postsynaptic membrane of the frog neuromuscular joint [5] may be attributed not only to increased miscoviscosity of membrane lipids, which prolongs the life-span of membrane channels, but also to ACE inhibition. Moreover, the abundance of ACE in the organism (it is present in erythrocytes, the lens, and in membranes of the axon and body of neurons [11]) points to other, as yet unknown functions. In this context, the reduced activity of ACE under hyperbaric conditions may be considered to be a primary effect of one of the primary mechanisms of action of hyperbaria.

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