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MICROCALORIMETRIC STUDY OF ERYTHROCYTE MEMBRANE SUSPENSION FROM NORMAL SUBJECTS AND PATIENTS WITH ESSENTIAL HYPERTENSION

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KEY WORDS: essential hypertension; erythrocytes; membrane; calorimetry.

It has recently been shown both in patients with essential hypertension [7] and in the experimental model of this disease, namely rats with spontaneous genetic hypertension [1, 6], disturbances of the functions of the erythrocyte membrane (its permeability for monovalent cations, binding and ATP-dependent transport of calcium) are based on a substantial disturbance of its structure. One line of further research into this problem is the discovery of the components of the membrane that are responsible for the membrane defect described above.

It was accordingly decided to study erythrocyte membranes from patients with essential hypertension by means of differential scanning microcalorimetry. Blood from patients with a clinically established diagnosis of essential hypertension, stage III-II according to the WHO classification (six men and five women, aged 30-56 years, BP 160-190/100-120 mm Hg) and blood from normotensive patients (six men and seven women aged 30-50 years, BP 110-130/70-90 mm Hg) were used.

The method of obtaining erythrocyte ghosts was described previously [2]. The total protein concentration was determined by Lowry's method [5]. In every case 5 mM Na-phosphate buffer, pH 7.4, was used as the solvent. The protein concentration in the suspension of erythrocyte ghosts was 3-5 mg/ml. Microcalorimetric investigations were carried out on a DASM-1M high-sensitivity differential scanning instrument (Special Design Office, Biochemical Center, Academy of Sciences of the USSR), the design and principle of operation of which have been fully described previously [8]. The working volume of the measuring cell was 1 ml. The rate of heating was 1°C/min. The sensitivity of the calorimeter for heat capacity at this rate of heating was 5×10^{-5} J/°C. To obtain a base line, both calorimetric cells were filled with solvent. High reproducibility of the base line enabled the specific heat of the membranes to be recorded with an error of not more than 5%. The accuracy of temperature recording was ±0.1°C.

A microcalorimetric recording of the change in excess heat capacity of the heated human erythrocyte membrane suspension (normal) in a protein concentration of 4.55 mg/ml is given in Fig. 1. The heat uptake contour of the suspension of erythrocyte ghosts is complex (multistage) in appearance. Some workers have made a detailed study of the five most clearly defined thermoinduced transitions in erythrocyte membranes [3, 4, 9, 10]. The elementary components of the contour which we distinguished in a pure form are named in accordance with the terminology suggested by the authors cited above, allowing for the thermal irreversibility of each transition.

The procedure of obtaining elementary contours was as follows. The suspension was first heated to 45°C, which is close to the temperature maximum of the first presumptive elementary transition (A-transition). The suspension was then cooled to 10°C (the temperature at the beginning of heating), after which the membranes were heated at the same rate to 51°C — a

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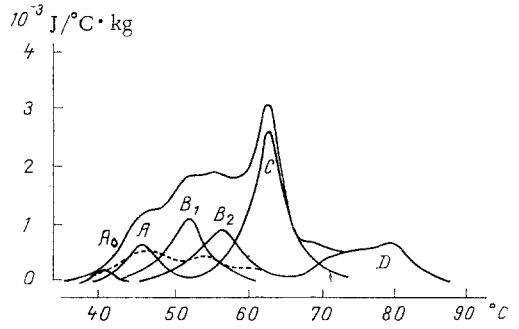

Fig. 1. Specific heat of erythrocyte membrane suspension as a function of temperature. Letters denote elementary components of the complex contour of irreversible transitions (continuous line). Broken line denotes conversions.

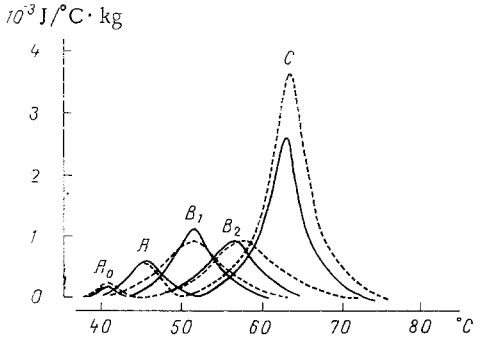

Fig. 2. Elementary irreversible components of heat absorption contour of suspension of erythrocyte membranes from normal individuals (continuous line) and patients with essential hypertension (broken line).

temperature close to the maximum of the second transition (B_1 transition). The suspension was then cooled again to the initial temperature before heating, after which it was heated to 56°C, and so on. By using a computer technique, the contours were subtracted successively: from the first heat absorption, recorded up to the temperature of the maximum of the first transition, the contour obtained as a result of the second scanning was subtracted, from this second contour thus obtained during the third heating was subtracted, and so on, after which, assuming the symmetry of the elementary contours, their complete profile was built up.

It will be clear from Fig. 1 that by the suggested method it was possible to distinguish at least four elementary irreversible heat transitions, associated with heat absorption of protein components of the membrane (A, B_1, B_2 and C transitions), within the temperature range from 35 to 70°C. In view of the lack of information on the nature of the temperature transitions in the region of higher temperatures (B transition) the remaining elementary transitions were not obtained, although it will be evident that the D transition consisted of at least three elementary transitions. Figure 1 also shows a completely reversible change of heat absorption (broken line), evidently associated with phase transitions in lipids of the erythrocyte membrane, and an irreversible transition in the region of 41°C, called the A_0 transition.

Comparison of the temperature dependence of the heat capacity of the erythrocyte membranes from normotensive subjects and patients with essential hypertension revealed the following distinguishing features (Fig. 2).

In erythrocyte membranes from patients with essential hypertension the maxima of the B_2 and C transitions were shifted into the region of higher temperatures, and the B_1 transition was significantly widened. These results demonstrate a disturbance of packing of the protein molecules in the membrane in hypertension on account of a disturbance of protein–lipid interaction, in agreement with data obtained previously during an investigation (probing) of the region of the annular lipids with the aid of pyrene [7].

For the transition in erythrocyte membranes from patients with essential hypertension an almost 30% increase in the enthalpy of the temperature conversions were observed, evidently a sign of the high protein content of these membranes, corresponding to the C transition. It must be pointed out in this connection that according to data published by other workers [9, 10], the C transition is due to thermal conversion of the transmembrane protein in band 3, i.e., the protein responsible for anionic transport through the membrane. Further research will fill in the details of the molecular nature of this band, and also of the components responsible for B_1 and B_2 transitions, for which the changes in enthalpy of heat conversion were less marked.

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REGULATION OF PYRUVATE TRANSPORT IN MITOCHONDRIA BY THYROID HORMONES

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Thyroid hormones are known to regulate the sensitivity of target cells to insulin and the insulin concentration in the blood serum [7]. The writers showed previously that the action of insulin on metabolism is mediated, at least partially, by changes in the activity of a cytoplasmic glycolipopeptide, which has been called insulin-dependent cytoplasmic regulator (IDR) [2]. Hormonal control of gluconeogenesis in the liver may be effected at the level of pyruvate transport from cytosol into mitochondria [9]. IDR is an endogenous inhibitor of pyruvate transport [10].

The object of this investigation was to study the role of thyroid hormones in the regulation of carbohydrate metabolism at the level of the pyruvate carrier.

EXPERIMENTAL METHOD

Rat liver mitochondria were isolated in 0.3 M sucrose containing 5 mM Tris-HCl, pH 7.4. Ca^{++} transport into mitochondria was measured by an ion-selective Ca^{++}-sensitive electrode, using a pH-metric method, by studying the kinetics of Ca^{++}/H^{+} exchange in the presence of phosphate as penetrating anion. Swelling of the mitochondrial suspension was measured from the change in optical density at 540 nm. Cytosol was obtained by centrifugation of homogenates of rat liver and diaphragm, prepared with mitochondrial isolation medium in the ratio of 1:1, at 30,000 g. To obtain the thermostable fraction the cytosol was heated for 7 min at 95°C,

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