TRANSHEMATION OF BOVINE AND HUMAN HEMOGLOBIN POLYMERS IN A SIMULATED CIRCULATORY SYSTEM

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Future research into the creation of artificial oxygen carriers on the basis of hemoglobin polymers (HP) obtained from bovine and human blood must depend on a solution to the problems of harmlessness of these preparations when given by massive intravenous injection [5]. Some of these problems are connected with the presence of toxic admixtures in the HP solution, such as stromal lipids, endotoxins, and highmolecular-weight fractions of HP [4]. Meanwhile hemoglobin (Hb) itself can cause damage to man and animals [7]. Both unmodified and polymerized (stabilized) Hb, if injected intravenously, is excreted in the course of time by the kidneys, but about half of the hemoprotein, although disappearing from the plasma, does not appear in the urine, i.e., it is taken up by the body [6]. The study of the possible metabolic pathways of an artificial oxygen carrier based on Hb in the blood stream is thus of great importance. The first step in this process is autooxidation: the conversion of Hb through autooxidation into the Met form, the content of which in the blood stream may reach 25% of the amount of the Hb preparation injected after circulating for only 2 h [3]. Met-Hb, when interacting with human serum albumin, takes part in a transhemation reaction with it: the transfer of heme from Hb to human serum albumin (HSA) [1] with the formation of methemalbumin – a colored complex of HSA with hemin.

The aim of this investigation was a quantitative study in vitro of the reaction of transhemation of artificial oxygen carriers, namely human HP modified by pyridoxal-5'-phosphate (PPHP) and bovine HP (BHP), by comparison with original human (HHb) and bovine (BHb) Hb under conditions simulating the situation in the blood stream, and to assess its contribution to metabolism of the preparations based on extraerythrocytic Hb.

EXPERIMENTAL METHOD

The hemoproteins for testing were converted into the met-form by treatment, in a concentration of 1.2 M as heme, with an excess of potassium ferricyanide, followed by exhaustive dialysis against a 0.15 M solution of NaCl, and then diluted to a concentration of 3 g/dl. The hemoprotein concentrations and completeness of conversion into the met-form were determined on a "CO-Oximeter-280" (IL, USA) and the pH of the solutions was corrected with Tris-buffer ("Serva"). The HSA was dialyzed against 0.15 M NaCl solution and its concentration adjusted to 12 g/dl; the protein concentration was determined spectrophotometrically by the biuret method. 0.25 ml of HSA solution (12 g/dl) was mixed with 0.25 ml of a solution of the test methemoprotein (3 g/dl), the pH adjusted to 7.40 ± 0.05 (at 37°C), and the mixture was incubated at 37°C for 3 and 24 h. The solution was diluted with 5 ml of 0.11 M acetate buffer and centrifuged at 3500 g for 15 min. The reaction mixture of methemoglobin and methemalbumin was fractionated by cation-exchange chromatography on "CM-Sepharose-CL-6B-FT" gel ("Pharmacia") in a two-step gradient. The percentage content of methemalbumin was determined from the ratio between the peaks on the chromatogram by numerical integration of the elution curve (Fig. 1).

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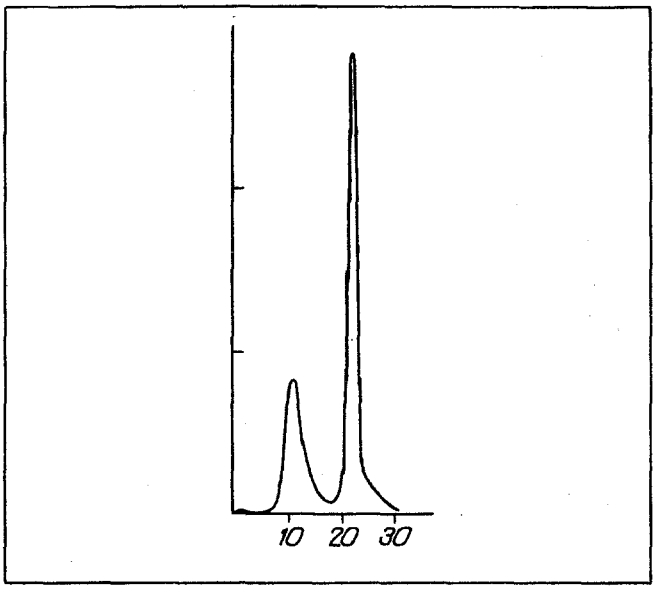

Fig. 1. Elution curve of mixture of met-PPHP (1.5%) and HSA (6 %) after incubation at 37°C for 24 h. Column packed with "CM-Sepharose-CL-6B-FF" 12 × 100; elution rate 1 ml/min; separation carried out in two-step gradient: buffer 1) 0.11 M sodium acetate, pH 5.5; buffer 2) 0.4 M sodium acetate, pH 8.0; volume of sample 0.1 ml; detection at 410 nm. Abscissa, time (in min); ordinate, absorbance, in activity units.

TABLE 1

Original hemoprotein	n	Quantity of heme transferred to HSA, % of total quantity		Presence of residue
		after 3 h	after 24 h	
HHb	3	21,3±0,8	35,1±3,6	+
PPHP	3	24,4±1,3	34,5±3,3	+
BHb	3	19,1±0,6	28,8±1,2	—
BHP	3	19,3±1,1	27,6±1,2	—

EXPERIMENTAL RESULTS

As Table 1 shows, the hemoproteins studied were equally subject to the transhemation reaction, irrespective of species and of chemical modification. After only 3 h, up to one-quarter of the prosthetic groups had been transferred to HSA, rising to one-third after 24 h.

During incubation of HHb and its polymer PPHP with HSA, a white residue, insoluble in water, was precipitated. It was shown in [7] that under certain conditions specific denaturation of Hb takes place during the transhemation reaction of HSA and HHb, and the residue observed consists of precipitated denatured globin. The presence of a large quantity of precipitate during circulation of PPHP in the blood stream may cause microcirculatory disturbances, and the effect observed may be an obstacle to the use of PPHP as a blood substitute.

Neither BHb nor its polymer gave a precipitate on reacting under the same conditions with HSA, possibly due to greater stability of the globin in solution. This effect, i.e., removal of globin from the reaction mixture, can be explained in terms of the equilibrium character of the transhemation reaction and the higher methemalbumin concentration in the experiments with human hemoproteins than in those with bovine hemoproteins (for 24 h $p < 0.05$).

Thus, by considering autooxidation of Hb preparations in the blood stream (1-2% in 1 h) the contribution of transhemation to metabolism of HP can be estimated to be not less than 10-15% of the amount of the preparation injected, and about half of the quantity not eliminated with the urine. A direct biological evaluation of this effect is

prevented by the fact that the transhemation reaction is species specific for man, i.e., the plasma proteins of other mammals and, in particular, of laboratory animals such as dogs, rabbits, rats, cats, and guinea pigs, do not take part in it [2, 8].

Consequently, the transhemation reaction to HSA must play an important role in metabolism of an artificial oxygen carrier based on Hb.

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PREVENTION OF POSTISCHEMIC DISTURBANCES BY NEW ADENOSINE DERIVATIVES

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The study of methods of prevention and treatment of postischemic disorders has a relatively short history. Different approaches to the solution of this problem have been discussed [1, 2, 7], in particular: 1) the possibility of using artificial electron carriers, capable of relieving the load on the respiratory chain and of restoring oxidative phosphorylation; 2) the use of antioxidants, limiting the oxygen consumption mainly by inhibiting free (nonphosphorylating) oxidation and limiting the accumulation of lipid peroxidation (LPO) products; 3) the use of antifatigue agents, accelerating recovery processes, due both to their effect on mechanisms of protein synthesis and to the increased efficiency of tissue respiration, ATP production, and the more rapid utilization of products of anaerobic metabolism; 4) future prospects for a new class of antihypoxic agents based on adenosine. Stable adenosine analogs, namely allyladenosine (ALAD) and cyclohexyladenosine (CHAD), which are purine receptor agonists, have a marked antihypoxic and protective action in cerebral ischemia [8].

The aim of this investigation was to estimate the efficacy of these compounds as agents preventing postischemic disturbances associated with liver damage induced by ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on 120 male albino rats weighing 180-200 g. The experimental model was acute hypoxia of the liver, induced by compressing the hepatoduodenal ligament (HDL). Under ether anesthesia the liver was

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