CHANGES IN PERIPHERAL BLOOD, LIVER, AND SPLEEN DURING EXPERIMENTAL LONG-TERM ADMINISTRATION OF MODIFIED HEMOGLOBIN

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Development of a plasma expander and oxygen carrier based on hemoglobin is an urgent problem in modern transfusiology. The use of solutions of native hemoglobin is made difficult by its rapid excretion from the blood stream, its high affinity for oxygen, and its unfavorable action on function of the kidneys, liver, and other organs [1, 4, 5, 15]. Preparation of high-molecular-weight derivatives of hemoglobin, known as polyhemoglobins (PHb), by polycondensation of hemoglobin with glutaraldehyde has enabled the circulating time in the blood setream to be substantially increased [1, 6, 9], and additional modification of the allosteric center of hemoglobin, pyridoxal-5-phosphate, with the formation of pyridoxylated PHb (P-PHb) has led to an improvement in its gas transport function [5, 9]. The problem of the highest degree of tolerance of hemoglobin preparations by experimental animals remains unsolved, as regards both variation of the volume of solutions injected and their concentration. Information on this matter is contradictory [4, 5, 11]. In particular, it has been shown that PHb solutions can be tolerated in a dose of 2.6 g/kg body weight [1, 11].

The aim of this investigation was to study the after-effects of intravenous injection of concentrated solutions of PHb into animals in doses of 10-15 g/kg, and also solutions of P-PHb in a dose of 1.8 g/kg.

EXPERIMENTAL METHOD

The PHb solutions had a concentration of 100 g/liter, a mean molecular weight (M) of 250,000, and affinity for oxygen (P_{50}) of 10-13 torr; they were injected in a dose of 25 ml/kg daily for 4 days (dogs) or 6 days (rats, rabbits). P-PHb solutions having a reverse oxygenation regulator were used at a concentration of 10 g/liter (M – 200,000 D), P_{50} = 23-29 torr. Solutions of P-PHb were injected in a volume of 30 ml/kg 6 times at intervals of 24 h. The experiments were carried out on 40 noninbred albino rats weighing 250-300 g, from 60 chinchilla rabbits weighing 2.5-3.0 kg, and on 20 male dogs weighing 12-17 kg. The data were subjected to statistical analysis by the use of parametric and nonparametric methods [2].

EXPERIMENTAL RESULTS

The effect of PHb solutions on the blood and hematopoiesis was studied by observing the time course of the parameters 1 and 30 days after a course of injections (Table 1).

After injection of solutions of P-PHb no changes were observed in the hematologic parameters. After injection of concentrated solutions of PHb, all the species of animals developed leukocytosis, relative lymphocytopenia, a raised ESR, and reticulocytosis, which disappeared during the next 30 days. These changes evidently reflected the transient stimulation of the bone marrow by loading with high doses of PHb..

These same doses led to hypoproteinemia on account of the globulin fraction and to a corresponding increase in the A/G ratio and in the concentration of organ-specific enzymes in the liver (Table 2). Injection of PHb was accompanied by an increase in the relative weights of the liver, kidneys, and spleen, as a proportion of body weight, in the animals of

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TABLE 1. Parameters of Peripheral Blood of 20 Rats, 40 Rabbits, and 10 Dogs (M ± m) during Infusion of PHb and P-PHb

Experimental animals	Period of investigation	Hb, g/liter	RBC, 10^6, μl^{-1}	L, 10^3 μl^{-1}	P, 10^3, μl^{-1}	R, ‰	ESR, mm/h	SN, %	PN, %	E, %	B, %	M, %	Ly, %
Rats	Before infusion	150±3	5,3±0,9	8,9±0,2	—	30±2,7	4±13	1,3±0,5	22±4	1,7±0,5	absent	6±0,4	70±2,1
	Same	147±5	6,0±0,24	7,4±0,75	621±70	22±4	5±1,6	1,0±0,2	32±5	5,6±0,82	»	5±1,0	56±6
	After course: 1 day												
	P-PHb	157±5	5,7±0,1	9,3±0,7	—	32±2,2	4±0,5	2±0,7	20±3	2,3±0,1	»	7±0,2	68±1,7
	PHb	125±5	5,0±0,35	15,4±1,1*	895±87*	31±11	11±2,7	4±0,5*	37±4	2,3±0,5*	»	7±0,9	49±4
	30 days												
	P-PHb	160±1,5	6,0±0,2	8,5±0,75	—	31±16	5±0,2	1±0,8	25±2	2±0,2	»	6,3±0,8	67±4,9
	PHb	162±3	5,2±0,07	8,3±0,48	803±85	24±2	3±0,04	1±0,3	25±4	4,2±1,2	»	5±0,8	66±5
Rabbits	Before infusion	128±4	4,2±0,17	7,2±0,8	136±20	19±3	4±0,5	5±1,8	32±6	1,4±0,3	0,7±0,4	4±0,9	57±8
	Same	125±4	4,8±0,29	8,5±1,24	680±189	19±4	6±2,9	5±0,4	34±5	1,2±12,5	0,4±0,0	4±1,3	55±5
	After course: 1 day												
	P-PHb	130±3	4,0±0,2	8±0,7	139±11	20±2	5±0,2	4,3±0,8	31±3	1,1±0,2	0,7±0,4	5±0,3	60±3
	PHb	114±12	4,1±0,22	11,8±1,0	430±113	35±7,5	6±2,2	4±0,9	44±10	0,8±0,22	0,4±0,2	6±1,9	44±10
	30 days												
	P-PHb	137±8	4,9±0,72	7,4±0,93	142±9	20±3	4±0,9	3,4±12	27±5	1,3±0,43	0,8±0,4	6±1,9	63±5
	PHb	149±19	0,2±1,64	9,2±0,75	228±27*	19±4,6	3±0,67	1±0,4	34±4	0,8±0,22	0,8±0,4	4±0,9	60±5
Dogs	Before infusion	170±5,1	5,7±0,4	6,3±0,3	350±70	2±1,7	4±1	7,5±2,1	52±5,3	8,3±0,1	absent	7,8±1	23±2,3
	Same	168±6	5,8±0,3	5,9±0,6	385±116	3±1	3±0,4	7±2,4	56±3	3,8±1,29	0,3±0,1	14±1,6	20±2,7
	After course: 1 day												
	P-PHb	167±3,4	5,9±0,2	7,5±0,1	326±30	3±1,1	5,2±2	7,0±1,1	55±6	6,7±0,9	absent	8,3±1,6	20±2,0
	PHb	171±5	5,6±0,22*	19,1±6,1	276±68	1,3±1,1	4,1±6,0	13±7,3	68±2	1,2±0,6	0,1±0,1	8±2,2	9±4,3*
	30 days												
	P-PHb	169±4,7	5,2±0,5	7,0±0,5	370±50	3±1	4,5±0,4	6,9±2,7	54±3,7	7,2±2,8	absent	8±2,5	22±1,9
	PHb	152±6	4,9±0,17	12,1±0,9	302±3	2±1,99	5±3,1	8±1,6	53±4	7,8±1,55*	»	7±1,3	24±4,9

Legend. Here and in Table 2, asterisk indicates values differing significantly ($p < 0.05$) from initial data. Hb) Hemoglobin, RBC) red blood cells, L) leukocytes, P) platelets, SN) stab neutrophils, PN) polymorpho-nuclear neutrophils; E) eosinophils, B) basophils, M) monocytes, Ly) lymphocytes, R) reticulocytes, ESR) erythrocyte sedimentation rate.

TABLE 2. Changes in Some Parameters of Protein and Carbohydrate Metabolism in Blood of Rabbits (n = 20) after Loading with PHb and P-PHb (M ± m)

Parameter (serum concentration)	PHb		P-PHb	
	initially	1 day after course	initially	1 day after course
TP, g/dl	9,0±0,28	6,7±0,19*	8,6±0,18	8,5±0,30
A, g/dl	5,2±0,10	5,1±0,06	4,7±0,15	4,0±0,13
G, g/dl	3,8±0,26	1,6±0,19*	5,0±0,32	4,5±0,23
A/G	1,4±0,08	3,5±0,42	0,9±0,11	0,9±0,07
Gl, mM	4,0±0,15	4,5±0,5	4,2±0,10	4,7±0,40
NPN, mM	25,1±1,2	27,3±0,9	25,5±1,3	26,8±0,8
U, mM	4,7±0,3	4,4±0,29	4,6±0,28	4,4±0,31
AST, μmoles/ml·4	0,30±0,07	1,0±0,10*	0,38±0,05	0,70±0,09*
ALT, μmoles/ml·4	1,34±0,12	2,60±0,40	1,50±0,14	1,70±0,29
ALD, units/liter	4,9±0,30	10,2±0,57	4,3±0,65	5,6±1,78
ALP, units/liter	91±6	140±9	97±7	121±8*

Legend. TP) Total protein (after Lowry [12]), A) albumin, G) globulins, Gl) glucose (after Hagedorn–Jensen [10]), U) urea (after Crocker [8]), NPN) nonprotein nitrogen (after Rapoport [13]), AST and ALT) activity of aspartate- and alanine-aminotransferase (by Reitman and Frankel's micromethod [14]), ALD) fructose-1,6-diphosphate aldolase activity (by micromethod of Anan'ev and Obukhova [3]), ALP) alkaline phosphatase activity (Biol test [7]).

all species, by signs of hemosiderosis of the liver, kidneys, and spleen, and of delymphatization of the lymph nodes, and by moderate degenerative changes in the myocardium, hepatocytes, and epithelium of the renal tubules, which disappeared 30 days after the last injection of PHb.

Infusion of P-PHb did not change the relative weights of the animals' internal organs. Preparations of the organs tested had the characteristic histological structure without any dystrophic changes.

Thus there is an undoubted connection between the quantity of PHb injected and the degree of the functional and pathomorphological changes immediately after the course, just as with hemoglobin [4]. Large doses of PHb induced the development of transient nonspecific reactive hepatitis with disturbance of the protein-synthesizing function of the liver and with increased permeability of the hepatocyte membranes. On infusion of large doses of PHb, blockade of the reticuloendothelial system of the liver and spleen is possible. Morphological changes in the spleen and lymph nodes and hypoglobulinemia are evidence of the risk of the immunosuppressive action of high doses of PHb (10-15 g/kg). Although these changes are temporary and disappear 30 days after injection of the PHb solution, nevertheless a careful study of chronic toxicity of concentrated preparations of PHb is essential. Meanwhile the injection of P-PHb into the blood stream in a dose of 1.8 g/kg body weight was shown to be harmless.

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FORMATION OF CATECHOLAMINERGIC STRUCTURES IN THE MEDIOBASAL HYPOTHALAMUS OF THE RAT

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Information on the formation of catecholaminergic (CAE) structures of the hypothalamus is very sparse and is confined mainly to the postnatal period of development. The fluorescence histochemical method of Falck and Hillarp can detect CAE neurons as early as the 2nd-3rd day of postnatal life [10, 12]. Progress achieved in recent years in immunocytochemistry has led to the discovery of neurons containing marker enzyme of catecholamine (CA) synthesis in the hypothalamus of 13- to 15-day fetuses [4, 13]. However, it has not provided the answer to the question whether these enzymes can catalyze CA formation in the prenatal period of development.

The aim of this investigation was to study the formation of CAE structures in the mediobasal hypothalamus (MBH) of rat fetuses by the more sensitive histofluorescence method with glyoxalic acid.

EXPERIMENTAL METHOD

CA in the hypothalamus of 16- and 18-day Wistar rat fetuses were determined by a fluorescence histochemical method using glyoxalic acid in the modification in [5]. To intensify the fluorescence, injection of pargyline (100 mg/kg), a monoamine oxidase inhibitor, was given to the pregnant rats 2 h before sacrifice. Control animals received an injection of reserpine (5 mg/kg). After decapitation, the brain was quickly removed, the MBH region isolated, and a fragment 1-3 μ thick was placed in a cryostat (−30°C). Serial frontal sections 10-25 μ thick were dried at room temperature on a slide and incubated for 1-3 min in a 1% solution of glyoxalic acid, made up in phosphate buffer (pH 7.4). The sections were dried with benzene (20-30 min) and kept in a drying cupboard at 80°C. They were then mounted in mineral oil and examined in the LYUMAM-IZ luminescence microscope.

EXPERIMENTAL RESULTS

On the 16th day of prenatal development, nuclear zones begin to be formed in MBH. At this stage small cells, with the characteristic green fluorescence of CA, were found along the 3rd ventricle. Terminal fields are found both in the periventricular and in the more lateral regions of MBH: axon terminals of varied caliber, mainly small and delicate (Fig.

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