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MEDIUM MOLECULAR WEIGHT BLOOD PEPTIDES AS FACTORS MODIFYING ERYTHROCYTE MEMBRANES IN BURNS

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General ideas formulated previously on the mechanisms of involvement of medium molecular weight peptides (MMWP) in the process of burn autointoxication postulate a direct role of erythrocytes in the distribution of biologically active MMWP in the body fluids [5]. The efficacy of detoxication by transfusion of washed erythrocytes confirmed the validity of these suggestions [4]. At the same time, it was evident that direct proof of interaction between MMWP and erythrocytes was necessary. Obtaining such proof also is important in connection with analysis of the possible mechanisms of the change in morphological and functional characteristics of erythrocytes and their membranes in burns.

EXPERIMENTAL METHOD

Electrophoretic mobility (EPM) of the erythrocytes was measured by means of a horizontal chamber of Kharamonenko's design. EPM was measured in phosphate buffer (pH 7.4) at 22-24°C [3, 6]. Meanwhile, in some experiments the effect of MMWP on acid resistance of the erythrocytes was studied [2].

The investigations were conducted on noninbred male albino rats weighing 150-200 g. In all series of experiments the initial level of EPM of the erythrocytes was measured beforehand, and was 1.30 ± 0.02 $\mu \cdot V^{-1} \cdot sec^{-1} \cdot cm$ ($n = 107$). MMWP was obtained from blood plasma of intact and burned animals by the method in [1]. The fractions were numbered in order of elution from the column. Four concentrations of MMWP were used (from blood of intact animals — normal, from blood of burned animals — burn). Concentration 1 corresponded to the concentration of MMWP in plasma, whereas concentrations 2, 3, and 4 were 2.5, 5, and 10 times higher respectively than the MMWP level in the plasma of the experimental animals. For the control, erythrocytes were incubated for 4-6 h in plasma of intact animals, in phosphate buffer with

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TABLE 1. EPM of Erythrocytes (in $\mu \cdot V^{-1} \times sec^{-1} \cdot cm$) in 1st and 3rd Degree Burns (M ± m)

Time after burning, days	Degree of burn	
	I	III
Background	1,33±0,06	1,29±0,03
1	1,53±0,06*	1,81±0,07*
2	1,89±0,04*	1,85±0,06*
3	2,04±0,08*	1,96±0,06*
4	1,80±0,04*	1,98±0,03*
5	1,60±0,02*	2,01±0,09*
6	1,53±0,06*	1,94±0,02*
7—8	1,32±0,08	1,46±0,02*
9	1,35±0,07	1,51±0,03*
10—11	1,36±0,04	1,38±0,05*
12	1,27±0,04	1,37±0,04*
13	1,33±0,05	1,29±0,04

Legend. Here and in Table 2: *$p < 0.05$ compared with background.

TABLE 2. EPM of Erythrocytes (in $\mu \cdot V^{-1} \times sec^{-1} \cdot cm$) after Incubation for 4 h in Medium with MMWP (M ± m)

Fraction of MMWP	Concentration of MMWP			
	1	2	3	4
2: Normal	1,37±0,02	1,37±0,01	1,56±0,06*	1,62±0,02*
Burns	1,43±0,04*	1,47±0,04*	1,64±0,02*	1,73±0,07*
3: Burns	1,50±0,02*	1,50±0,02*	1,94±0,08*	1,91±0,04*
4: Normal	1,46±0,01*	1,43±0,01*	1,54±0,01*	1,51±0,02*
Burns	1,35±0,03	1,42±0,04*	1,40±0,02*	1,46±0,04*

heparin, and in physiological saline. In all cases EPM of the incubated erythrocytes was unchanged.

EXPERIMENTAL RESULTS

In the experiments of series I the time course of EPM of the erythrocytes in burns (1st and 3rd degree burns) was investigated. EPM of the erythrocytes was found to rise sharply on the day after burn trauma (Table 1). No significant differences were found in EPM of the erythrocytes depending on the severity of the burn. However, whereas in a 1st degree burn EPM of the erythrocytes returned to its initial value on the 7th-8th day after burn trauma, in the case of a 3rd degree burn, it did so only on the 13th day. The results are in good agreement with the time course of the clinical picture of burn toxemia. Incidentally, washing the test erythrocytes in phosphate buffer solution 3 times restored the initial value of their EPM.

An increase in the surface charge on the erythrocyte membranes in burn toxemia may be associated either with a membranotropic damaging action or with adsorption of MMWP. However, these processes are functional in character, as shown by the constantly exhibited effect of washing. The same effect is evidently produced also by extracorporeal hemoperfusion. To prove this hypothesis a series of experiments was carried out in which erythrocytes of intact animals were incubated in the plasma of rats with severe burns, on the 3rd day after trauma (EPM of the erythrocytes of the burned animals was 1.958 ± 0.008 $\mu \cdot V^{-1} \cdot sec^{-1} \cdot cm$). The cells were incubated at 37°C for 4 h. It will be clear from Fig. 1 that EPM of intact erythrocytes was significantly increased after only 1 h of incubation, and it continued to rise until the end of the experiment. Triple washing returned EPM of the erythrocytes to its initial level.

Thus in this case also a change in the surface charge of the erythrocyte membrane was functional in character, and it was evidently not accompanied by disturbance of integrity or permeability of the membranes.

In the experiments of series III erythrocytes of intact animals were incubated in physiological saline (pH 7.4) with the addition of different fractions of MMWP in four different concentrations (Table 2). The acid resistance of the incubated erythrocytes was studied at the same time. Fraction 2 (normal) in low concentrations (1 and 2) affected neither EPM of

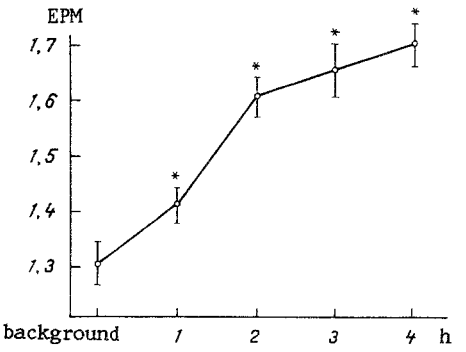

Fig. 1. Changes in EPM (in $\mu \cdot V^{-1} \cdot sec^{-1} \cdot cm$) of intact erythrocytes during incubation in plasma of burned animals ($M \pm \sigma$). *$p < 0.05$ compared with background.

the erythrocytes nor their resistance to the hemolytic agent. In concentration 3 this fraction significantly increased EPM at the 3rd hour, whereas in concentration 4 it increased it significantly after the 1st hour. In high concentrations these MMWP also accelerated acid hemolysis of the erythrocytes.

Fraction 2 (burn) in concentrations 2, 3, and 4 significantly increased EPM of the erythrocytes after the 1st hour and reached highest values toward the 4th hour of incubation. In concentration 1, EPM was increased significantly but only toward the 4th hour. The rate of hemolysis of the erythrocytes was increased only when a higher concentration of MMWP was used.

Fraction 3 (normal), with incubation for 4 h, did not change EPM of the erythrocytes, but in high concentrations it accelerated hemolysis of the erythrocytes (a shift of the erythrogram to the left).

Fraction 3 (burns), on the other hand, had an extremely strong action, increasing EPM of the intact erythrocytes in all concentrations used after only 1 h of incubation, and by the 4th hour the absolute values of EPM of the erythrocytes became comparable with EPM of erythrocytes of burned animals at the height of burn toxemia. This fraction also increased the rate of hemolysis in the highest concentration only.

Fraction 4 (normal) in all concentrations increased EPM of the erythrocytes significantly, but only very slightly, in the 1st hour. During incubation EPM continued to rise. This fraction had the same effect as fraction 4 (burns) on the rate of hemolysis.

Fraction 4 (burns) had an unusual action: in concentrations 2, 3, and 4 it significantly increased EPM of the erythrocytes only after 4 h of incubation, but in the lowest concentration, it did so by the 1st hour (1.427 ± 0.02 $\mu \cdot V^{-1} \cdot sec^{-1} \cdot cm$). However, on further incubation EPM of the erythrocytes (concentration 1) gradually returned to its initial level. This same fraction, in high concentrations, lowered resistance to the hemolytic agent, but in low concentrations it had no significant action.

Triple washing in all cases without exception returned EPM of the erythrocytes to its initial level, thereby confirming our suggestion that MMWP are adsorbed on erythrocyte membranes, thereby substantiating the clinical application of extracorporeal hemoperfusion and infusion of washed erythrocytes.

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DEVELOPMENT OF HEART FAILURE FOLLOWING TRANSVALVULAR CATHETERIZATION OF THE LEFT VENTRICLE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Systemic hypertension is one of the main etiological factors in the development of congestive heart failure [4]. Pfeffer et al. [8] showed that the cardiac output of spontaneously hypertensive rats (SHR) is lower at the age of 25 weeks than at 13 weeks. However, these data were obtained under acute experimental conditions, which may have differed in their effect on the circulatory system of animals of different ages [11].

The aim of this investigation was to compare systemic hemodynamic parameters in unanesthetized SHR and normotensive rats (NTR) of different ages, by the method of radioisotope-labeled microspheres.

EXPERIMENTAL METHOD

Experiments were carried out on SHR (of the Okamoto—Aoki line, from the Heidelberg University colony, West Germany) and Wistar—Kyoto NTR. An arterial catheter was implanted in the abdominal aorta through the femoral artery. After 1-2 days, under pentobarbital (40 mg/kg) or ether anesthesia, the left ventricle was catheterized through the right carotid artery. In one series of experiments the left atrium was catheterized. To do so, under artificial respiration and under open chest conditions a catheter was introduced into the auricle of the left atrium. The peripheral ends of the catheters were brought out on the animal's back and fixed in the interscapular region. The hemodynamic parameters were studied by the use of radioactive isotope-labeled microspheres [1]. The cardiac catheter (located in the left ventricle or left atrium) was used to inject the microspheres, the arterial catheter to take blood and

TABLE 1. Hemodynamic Parameters in 5-month-old NTR and SHR 3 and 24 h after Catheterization of Left Ventricle under Pentobarbital Anesthesia

Parameter	Time after operation, h	NTR ($n=5$)	SHR ($n=6$)
Mean BP, mm Hg	3	123±4	171±5*
	24	100±7	138±9*
HR, min^{-1}	3	300±16	315±10
	24	312±15	315±13
CI, ml/min/100 g	3	31,3±4,0	26,6±2,3
	24	32,6±3,4	29,1±2,7
TPVR, mm Hg/ml/min 100 g	3	4,22±0,54	6,58±0,38
	24	3,18±0,39	4,85±0,33

Legend. *$p < 0.05$ compared with corresponding value for NTR.

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