ROLE OF THE KIDNEYS IN THE MECHANISM OF ERYTHRODIERESIS IN HEMOLYTIC (PHENYLHYDRAZINE) AND ACUTE POSTHEMORRHAGIC ANEMIA

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The degree of hemolysis was investigated in intact and nephrectomized rats after administration of phenylhydrazine. Hemolytic (phenylhydrazine) anemia in nephrectomized animals is manifested by a smaller decrease in the total erythrocyte count, the percentage of ^{51}Cr-labeled erythrocytes, and the intensity of the reaction for hemosiderin in tissues of the reticuloendothelial system. A lower degree of erythrodieresis was found in nephrectomized rats and after acute unreplaced blood loss. Perfusion of blood through the kidney of anemized rats leads to an increase in the plasma potassium concentration of the perfusion fluid, a decrease in the electrophoretic mobility and hemolysis time of the erythrocytes, an increase in their fragility, and a decrease in the proportion of protein fractions with molecular weights of between 74,500 and 27,000 in the erythrocyte stroma.

KEY WORDS: *Erythrodieresis; kidneys; anemia.*

Previous investigations have shown [4, 5] that nephrectomy weakens the hemolytic effect of phenylhydrazine. On the basis of these results a role of the kidneys was postulated in erythrocyte destruction.

In this investigation the degree of erythrodieresis in nephrectomized rats after injection of phenylhydrazine or acute blood loss and certain properties of the erythrocytes after perfusion of blood through the kidneys of animals with acute posthemorrhagic anemia were studied. The choice of this last model was determined by the increase in erythrodieresis during the first few hours after blood loss [1, 2, 7-9].

EXPERIMENTAL METHOD

Wistar rats were nephrectomized under ether anesthesia. Peritoneal dialysis was carried out with a solution of the following composition: NaCl 40 g, KCl 100 mg, $CaCl_2$ 50 mg, Na_2HPO_4 25 mg, glucose 7.5 g, penicillin 5 million units, streptomycin 1 million units, distilled water 4.75 liters. The duration of dialysis was 50-60 min, using the body weight as the control. The blood nonprotein nitrogen was determined colorimetrically. Acute posthemorrhagic anemia was induced by the loss of 40% of the blood volume from the jugular vein. The blood volume was determined with the aid of Evans' blue. A solution of phenylhydrazine hydrochloride (8 mg/100 g body weight) was injected intravenously [16]. The degree of hemolysis was judged from changes in the absolute erythrocyte count, the serum hemoglobin, the intensity of hemosiderin deposition in the tissues [15], and the lifespan of ^{51}Cr-labeled erythrocytes [12, 13]. Perfusion of the kidneys in situ was carried out by means of a device ensuring rigid fixation of the needle in the testicular or ovarian vein. Blood stabilized with solution TsOLIPK 7b (1:9) was injected into the renal artery at the rate of 9-10 ml/min. Blood was taken for testing from the renal vein 40-45 sec after the beginning of perfusion. Results obtained by perfusion of blood through the liver of the same animals were used as the control. The plasma potassium concentration [6, 11], electrophoretic mobility of the erythrocytes [10], and their acid resistance [3] were determined in blood en-

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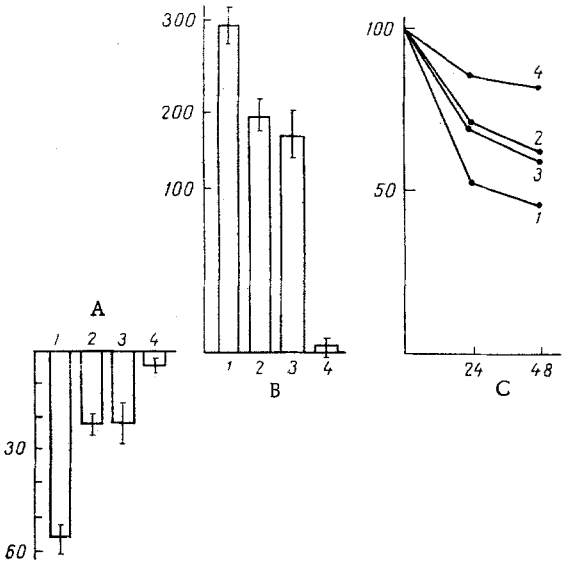

Fig. 1. Change (in % of initial level) in total number of erythrocytes (A), weight of spleen (in mg/100 g; B), and number of ^{51}Cr-labeled erythrocytes (C) in rats: 1) in animals with intact kidneys after injection of phenylhydrazine; 2) in nephrectomized rats after injection of phenylhydrazine; 3) in nephrectomized animals with peritoneal dialysis after injection of phenylhydrazine; 4) in nephrectomized rats undergoing no other treatment. Values of M ± m shown.

tering and leaving the organ. After washing twice in 2 volumes of phosphate buffer in a concentration of 310 milliosmoles at pH 8.0, the erythrocytes were lysed in 10-milliosmolar phosphate buffer (ratio 1:30) for 1 h. The resulting hemolysate was centrifuged at 5000g for 30 min, after which the stroma was washed four or five times in the same phosphate buffer. The resulting stroma was dissolved in 1% sodium dodecylsulfate solution (1:1) for 12 h. Electrophoresis of the stromal proteins was carried out in polyacrylamide gel [14] in the presence of sodium dodecysulfate as dissociating agent [17]. The relative percentages of the protein fractions were determined by densitometry. In the experiments of series I (40 rats) the degree of hemolysis induced by phenylhydrazine was determined in intact and nephrectomized rats; in series II (20 rats) the degree of erythrodieresis was determined in nephrectomized animals after acute posthemorrhagic anemia; in the experiments of series III (26 rats) changes in the properties of the erythrocytes and their stromal proteins were studied after perfusion of the rats with acute posthemorrhagic anemia through the kidney. Blood from normal rats was used for perfusion. Blood was taken 1-1.5 h before perfusion.

EXPERIMENTAL RESULTS AND DISCUSSION

The total erythrocyte count in the intact rats 48 h after injection of phenylhydrazine was reduced by 43% (from 58.5×10^9 to 33.2×10^9; $P < 0.001$). In the nephrectomized animals (nonprotein nitrogen 46.5 ± 0.7 mg %) this decrease was only 22% (from 57.6×10^9 to 46.4×10^9; $P < 0.001$). Peritoneal dialysis of the nephrectomized animals (nonprotein nitrogen 41.2 ± 0.5 mg %) did not change the degree of hemolysis.

The change in weight of the spleen and relative percentage of ^{51}Cr-labeled erythrocytes confirmed the difference in the degree of destruction of erythrocytes following injection of phenylhydrazine into intact and nephrectomized animals (Fig. 1). The reaction for hemosiderin was increased (mainly in the subcapsular zone) and the number of macrophages in the stage of incomplete erythrophagocytosis was higher in sections through the spleen of rats with phenylhydrazine anemia. In the lungs, dust-like (sometimes lumpy) masses staining for

hemosiderin were observed around the lymphoid formation. In the liver, solitary cells located at the periphery of the subcapsular lobules, but sometimes diffusely throughout the pulp, gave a positive reaction.

In the nephrectomized rats hemosiderin was distributed more uniformly in the spleen after injection of phenylhydrazine. Hemosiderin was not found in the lungs, and in the Kupffer cells of the subcapsular zone of the liver only traces of it were seen.

A lower intensity of erythrodieresis was found in nephrectomized animals with acute posthemorrhagic anemia. For instance, the proportion of ^{51}Cr-labeled erythrocytes, which were injected 2 h before anemization, in the circulation 24 h after bleeding was 57 ± 4%, falling to 49 ± 5% 48 h after bleeding. When ^{51}Cr-labeled autologous erythrocytes (taken at bleeding) were reinjected 2 h after anemization, the intensity of erythrodieresis was 70 ± 6 and 61 ± 5% respectively in the nephrectomized rats and 56 ± 5 and 43 ± 4% respectively in the controls.

The experiments of series III showed that after the passage of blood from normal rats to the kidney of anemized rats the plasma potassium concentration in the perfusion fluid was increased ($P < 0.001$). This could be the result of hypoxia of the kidneys, but after perfusion with plasma or physiological saline, no increase in potassium liberation was found. A more likely explanation is that the increased plasma potassium concentration in the perfusion fluid was due to the conversion of the erythrocytes into a prehemolytic state, manifested in particular by loss of potassium ions. This explanation is confirmed also by the decrease in electrophoretic mobility of the erythrocytes ($P < 0.001$), the change in the relative proportions of blood cells in groups with different fragility, an increase in their overall fragility, and a decrease in the duration of hemolysis. Parallel investigations of erythrocyte stromal proteins revealed a considerable decrease in fractions with mobility between 0.964 and 1.697 mm/min relative to human albumin and with a molecular weight of between 74,500 and 27,000.

Perfusion of the liver of the anemized rats also gave an increase in the plasma potassium concentration of the perfusion fluid. However, the electrophoretic mobility and acid resistance of the erythrocytes were unchanged in this case.

It can be concluded from the results of this investigation that the degree of intensity of erythrodieresis induced by phenylhydrazine and of posthemorrhagic erythrodieresis, developing during the first few hours after acute blood loss, is reduced in nephrectomized animals. The reason is evidently a decrease in the erythrophagocytic activity of the reticuloendothelial system and elimination of the effect of the kidneys on erythrocyte resistance. This last effect is manifested as conversion of the erythrocytes into a prehemolytic state during their passage through the blood vessels of the kidneys of the anemized animals. The possibility cannot be ruled out that a similar effect occurs also in kidney disease, thus giving nephrogenic anemia its hemolytic character.

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FUNCTIONAL STATE OF THE HYPOTHALAMO-HYPOPHYSEO-ADRENAL SYSTEM IN GUINEA PIGS EXPOSED TO RADIAL ACCELERATION

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The effect of an increased gravitational field on activity of the hypothalamo-hypophyseo-adrenal system was studied in experiments on guinea pigs. A single exposure led to activation of this system; however, during repeated exposure to radial acceleration the animals ceased to respond by an increase in the blood corticosteroid level, evidently on account of adaptation of the central components of the system and not of exhaustion of the adrenal cortex.

KEY WORDS: *Hypothalamo-hypophyseo-adrenal system; radial acceleration; corticosteroids.*

The study of the action of acceleration and the associated overloading on the neuroendocrine regulatory mechanism is an urgent problem in aviation and space medicine [2].

In this investigation the effect of single and repeated exposure to radial acceleration on the state of the hypothalamo-hypophyseo-adrenal system, the hormones of which have an important influence on the resistance of the body to unfavorable environmental factors [6, 7], was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 96 male guinea pigs weighing 320-400 g. In these animals, as in man, the main adrenocortical hormone is cortisol (17-HCS) [4, 8]. Its concentration was determined in the blood plasma [5] of the animals after they had been spun in a special centrifuge with a lever 60 cm long. Twenty minutes before exposure to radial acceleration (11g for 10 min) the guinea pigs received an intraperitoneal injection of methyldiazine (10 mg/kg), chlorpromazine (10 mg/kg), or physiological saline. Control animals received physiological saline over a period of 11 days. Blood samples were taken from the heart in the morning. The results were subjected to statistical analysis [1].

EXPERIMENTAL RESULTS AND DISCUSSION

The 17-HCS concentration in the blood plasma of the guinea pigs 90 min after a single spinning was much higher than in the control animals. During repeated acceleration, starting from the sixth day, the 17-HCS concentration was almost unchanged after spinning (Table 1).

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