POSTHYPOXIC CHANGES IN THE CEREBRAL CORTEX OF DOGS IN THE LATE RECOVERY PERIOD AFTER HYPOVOLEMIC HYPOTENSION FOR 4 HOURS

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On the 14th-21st day of the recovery period after hypovolemic hypotension lasting 4 h the total RNA level in the tissue of the gray matter of the brain fell by 20.9% and the DNA level by 13%. In the postmitochondrial supernatant the concentration of prealbumins fell by 26.5%, of α-globulins by 19.2%, and of γ-globulins by 59.8%; the concentration of albumins and β-globulins increased by 12.6 and 50% respectively. Activity of acid cathepsins rose by 50% and of acid phosphatase by 44%. Activity of total lactate dehydrogenase (LD) and glutamate dehydrogenase did not differ significantly from the control values. However, the LD isozyme spectrum showed a decrease in LD_{3+4+5} from 31.9 to 14.2%. Analysis of densitograms of electrophoresis in polyacrylamide gel showed changes in the physiocochemical properties of the protein molecules similar to denaturation in nature. The number of Purkinje cells in the cerebellar cortex was 41.3% below the control level.

KEY WORDS: *Hypovolemic hypotension; recovery period; cerebral cortex; nucleic acids; proteolysis; gel electrophoresis.*

The widespread use of resuscitation methods and intensive treatment in medical practice has raised the comprehensive study of the organism in the late recovery period to the status of one of the most important problems in modern resuscitation practice [6]. The study of posthypoxic changes in the CNS which determine the completeness or otherwise of recovery of the vital functions of the organism after resuscitation from terminal states is particularly interesting.

The object of this investigation was to assess the posthypoxic state of the cerebral cortex in dogs in the late postresuscitation period after hypovolemic hypotension lasting 4 h. For this purpose, the concentrations of nucleic acids and of total protein and its fractions and activity of lactate dehydrogenase (LD), glutamate dehydrogenase (GD), acid phosphatase, and acid cathepsins in the gray matter of the cerebral cortex were studied on the 14th-21st day after resuscitation. Histological investigation of the brain, with quantitative assessment of the changes taking place, was carried out at the same time.

EXPERIMENTAL METHOD

Experiments were carried out on 22 dogs of both sexes weighing 12-21 kg. After premedication with pantopon (8 mg/kg) the animals were bled quickly, in the course of 3-5 min, from the femoral artery under superficial pentobarbital anesthesia, with extensive use of local procaine anesthesia, until the arterial pressure had fallen to 40 mm Hg, at which level it was kept for 4 h. The arterial pressure was then restored by intra-arterial reinfusion of the blood. The total blood loss was 38 ml/kg. After 30 min or 1 h of the recovery period (when the central hemodynamics had become stabilized) dextran was injected intravenously in fractional doses [1].

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TABLE 1. Changes in Concentration of Nucleic Acids, Total Protein, and Its Fractions in Gray Matter of Cerebral Cortex of Dogs in Control and on 14th-21st Day of Resuscitation Period (M±m)

Index studied	Control	Experiment
Total RNA (in mg %)	11,0±0,8 (4)	8,7±0,6* (4)
DNA (in mg %)	5,4±0,3 (4)	4,7±0,25 (4)
Total protein (in mg %)	16,7±1,3 (6)	14,2±1,4 (4)
Protein fractions: postmitochondrial supernatant (in %)		
Prealbumins	5,33±0,36 (6)	4,08±0,54 (4)
Albumin	7,02±0,93 (6)	8,84±1,48 (4)
Postalbumins	15,13±0,72 (6)	17,77±1,11 (4)
Ceruloplasmin	10,36±0,64 (6)	9,58±1,16 (4)
Transferrin	12,0±1,08 (6)	12,63±0,44 (4)
α-Globulin	29,65±0,86 (6)	23,97±1,20* (4)
β-Globulin	10,60±1,86 (6)	20,10±1,54* (4)
γ-Globulin	9,87±2,59 (6)	3,97±0,80* (4)

Legend. Results differing significantly from control ($P<0.05$) marked by asterisk. Number of experiments shown in parentheses.

The biochemical and histological investigations were carried out on healthy dogs (control group, 14 animals) and on dogs in which hypovolemic hypotension had been produced, on the 14th-21st day after resuscitation (experimental group, 8 animals). The animals of the experimental group were indistinguishable from the control in their external appearance and behavior.

Brain tissue for biochemical tests was obtained by intravital punch biopsy through a burn hole. The operation of drilling the burn hole was carried out under superficial thiopental anesthesia.

The concentration of total RNA and DNA [10, 16] was determined in a homogenate of the gray matter of the brain tissue [10, 16], total protein was investigated in the postmitochondrial supernatant [13], and the protein fractions were separated by electrophoresis in polyacrylamide gel [3]. For quantitative protein analysis a densitometer (Carl Zeiss Jena, East Germany) was used. Tests were carried out at the same time to study the activity of the following enzymes: GD [16], total LD and its isozymes [15] at 340 nm, on the Era (USA) instrument, acid phosphatase [11], and acid cathepsins [9].

For morphological investigations the animals were killed by electric shock from the 127 V grid. Besides histological investigations of the cerebral cortex, the number of Purkinje cells in the cerebellar cortex was counted [8].

EXPERIMENTAL RESULTS AND DISCUSSION

The results given in Table 1 show a marked decrease in the concentrations of total RNA (by 20.9%), total protein (by 15%), and DNA (by 13%) in the tissue of the gray matter of the brain on the 14th-21st day of the postresuscitation period after 4 hours of hypotension induced by massive blood loss. Electrophoresis of the postmitochondrial supernatant revealed 23 protein fractions. To assess the differences between the control and experimental samples the protein fractions were pooled into eight groups and identified by comparing their mobility with that of blood serum proteins [7]. A decrease in the concentration of prealbumins by 26.5%, of α-globulins by 19.2%, and of γ-globulins by 59.8% and an increase in the concentration of albumins and β-globulins by 12.6 and 50% respectively were found. Changes in the fractions corresponding to postalbumins, ceruloplasmin, and transferrin were not significant. The most significant changes in the protein fractions of the cerebral cortex in the late postresuscitation period were connected primarily with changes in the globulin fraction (Table 1). A shift to the right on the densitogram of the cerebral cortical proteins in the experimental group compared with the control (Fig. 1) indicates a change in the phys-

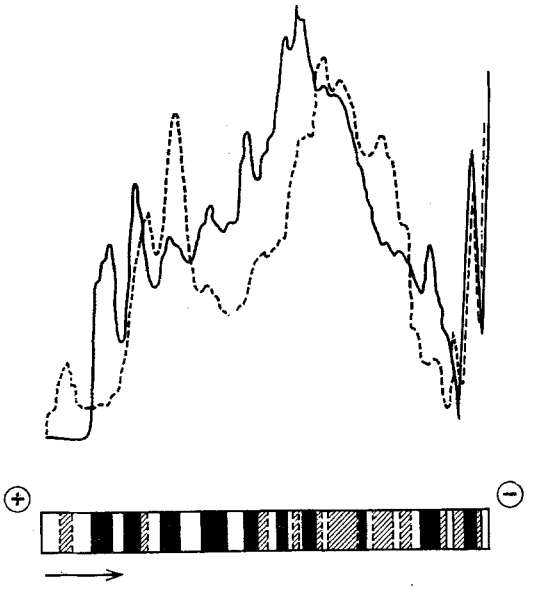

Fig. 1. Densitograms of proteins of postmitochondrial supernatant of gray matter of brain from dogs in initial state and 2 weeks after resuscitation: continuous curve is control, broken curve experimental.

icochemical properties of the protein molecules, analogous in nature to denaturation. After various types of injury, including hypoxic, denaturation changes are known to develop in the cell proteins [5]. A shift of the densitogram to the left indicates the appearance of low-molecular-weight products in the prealbumin fraction that were absent in the control.

During denaturation changes in the cell proteins the likelihood of attack of the intracellular proteins by proteases is increased [14], so that the conditions for intensification of proteolysis are created [2]. Activity of acid cathepsins increased by 50% (2.03±0.22 i.u. compared with 1.14±0.13 i.u./mg protein in the control; $P<0.05$) and activity of acid phosphatase by 44% (8.5±0.67 i.u. compared with 4.76±1 i.u./mg protein in the control; $P<0.05$). The high acid cathepsin activity in the tissue of the gray matter of the brain explains the decrease in the γ-globulin fraction (Table 1).

One result of the profound hypoxic changes in the cerebral cortex after prolonged hypovolemic hypotension [4] was the decrease observed in the content of LD_{3+4+5} isozymes (from 31.9 to 14.2%), whereas the total LD activity showed only a very slight change (2.72±0.7 and 3.35±0.6 i.u./mg protein in the control and experimental series, respectively). The change in the LD isozyme spectrum in the brain tissue was thus the result of degenerative and necrotic disturbances in the cortex in the postresuscitation period. This conclusion is confirmed by histopathological studies of the cortex on the 14th-21st day after resuscitation, when areas of necrosis and large numbers of cells with vacuolated cytoplasm were seen in all the animals investigated. As a result of quantitative analysis of Purkinje cells in the cerebellar cortex a decrease in their number in the late postresuscitation period from 2023±47 to 1188±38 ($P<0.05$) was found. Along with the decrease in the number of Purkinje cells, loss of the cells of the granular layer also occurred.

Neurological recovery of the animals in the postresuscitation period is evidence of preservation of the functional activity of the brain tissue. This also was confirmed by preservation of normal GD activity in the brain tissue (38.04±5.53 and 47.0±8.0 i.u./mg protein in the control and experimental series, respectively).

In the cerebral cortex of dogs recovering from prolonged hypovolemic hypotension profound degenerative changes largely connected with disturbances of protein and nucleic acid metabolism were thus found in the late resuscitation period. Denaturation of protein molecules and intravital proteolysis occupy an important place in the mechanism of these disturbances.

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THROMBOLYTIC ACTIVITY OF TERRILYTIN AND ITS EFFECT ON THE BLOOD CLOTTING SYSTEM

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In experiments *in vitro* after preliminary incubation of fibrinogen with terrilytin clot formation was retarded and subsequent lysis accelerated. Terrilytin *in vitro* lengthened the recalcification time, reduced thromboplastic activity and fibrinase activity and, at the same time, increased the fibrinolytic activity of blood plasma. In experiments on dogs roentgenovasography revealed considerable thrombolytic activity of terrilytin when injected intravenously into animals with experimental thrombosis of the femoral veins.

KEY WORDS: *Thrombosis; terrilytin; coagulation; thrombolytic activity.*

Reports have been published [2-5] of the high lytic activity of terrilytin in animals with experimental thrombosis.

During a continuation of these investigations the effect of terrilytin on venous thrombi and on the clotting system of the blood was studied.

EXPERIMENTAL METHOD

In the experiments of series I the effect of terrilytin was investigated on fibrin clots obtained from whole blood, plasma, and fibrinogen by the addition of equal volumes (0.1 ml) of a solution of thrombin with activity 12" (in plasma) to it. The solid clots formed under

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