EFFECT OF PROLONGED DEXTRAN INFUSIONS IN THE POSTRESUSCITATION PERIOD ON METABOLIC AND MORPHOLOGICAL CHARACTERISTICS OF THE BRAIN

V. L. Kozhura, G. N. Mirotvorskaya, L. V. Molchanova, and S. I. Pylova

UDC 616-036.882-085.
384-07:616.831-07

Infusions of dextran (10 mg/kg) during the 10 days of the postresuscitation period after hypovolemic hypotension lasting 4 h restored the normal concentrations of total RNA and of the rapidly moving protein fractions corresponding to prealbumins, albumins, postalbumins, ceruloplasmin, transferrin, and α-globulins. However, the high-molecular-weight protein fractions (β- and γ-globulins) remained considerably altered: The concentration of β-globulins was doubled whereas that of γ-globulins was reduced by 41%. The relative acid phosphatase activity increased in the postmitochondrial supernatant by 53%; an increase in acid phosphatase activity was found in the neurons, glia, and vascular endothelium. The number of Purkinje cells in the cerebellar cortex in the experimental series did not differ significantly from the control. The results emphasized the importance of using prolonged dextran influsions in the program of resuscitation measures for the treatment of patients with hypovolemic states.

KEY WORDS: hypovolemic hypotension; postresuscitation period; infusion of dextran; RNA; proteins; acid phosphatase in the cerebral cortex; cerebellar Purkinje cells.

It was shown previously that dextran infusion (20-30 ml/kg) during the first hour of the postresuscitation period after hypovolemic hypotension lasting 4 h improves the neurological recovery and the survival rate of the animals [2]. However, despite the outwardly complete recovery of functions of the CNS, all the animals showed severe degenerative changes, a low RNA concentration, and marked disturbances of the physicochemical properties of the protein molecules in the cerebral cortex and cerebellum [3]. The present writers suggested that longer administration of dextran infusions after replacement of the lost blood could minimize hypoxic brain damage.

The object of the present investigation was to study the possibility of using long infusions of dextran (for the first 10 days) in order to prevent irreversible changes formed in the CNS in terminal states and in the postresuscitation period.

EXPERIMENTAL METHOD

Experiments were carried out on 24 dogs of both sexes weighing 12-18 kg. After premedication with Pantopon (8 mg/kg) under superficial pentobarbital anesthesia supplemented by extensive local procaine anesthesia the animals were bled rapidly from the femoral artery for 5-8 min until the blood pressure reached 40 mm Hg, at which level it was maintained for 4 h. The total blood loss was 42 ± 5 ml/kg. Immediately after reinfusion of the blood, dextran was given by intravenous drip in a volume of 25-30 ml/kg body weight. Adequate management of the recovery period was verified by monitoring the pulse rate, the presence or absence of cardiac arrhythmias, and the dynamics of the pulmonary arterial pressure and the central venous and arterial pressure. Dextran infusion therapy (10 ml/kg) was then given on the 1st, 3rd, 5th, and 10th days after resuscitation.

The biochemical, histochemical, and histological investigations of the brain were carried out 14-28 days after resuscitation. The animals of the experimental group were indistinguishable in external appearance and behavior from the healthy dogs of the control group.

Laboratory of General Resuscitation, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. A. Negovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 12, pp. 668-671, December, 1978. Original article submitted November 28, 1977.

TABLE 1. Changes in Content of Total RNA and DNA and Protein Fractions in Gray Matter of Cerebral Cortex in Dogs of Control Group and on 14th-28th Day of Recovery Period, Treated With infusions of Dextran (M ± m)

Index	Control	Experiment
Total RNA, mg %	11,0±0,8 (4)	10,5±0,3 (4)
DNA, mg %	5,4±0,3 (4)	5,3±0,4 (4)
Protein fractions in postmitochondrial supernatant, %:		
Prealbumins	5,33±0,36 (6)	4,48±0,63 (3)
Albumins	7,02±0,93 (6)	7,95±0,79 (3)
Postalbumins	15,13±0,72 (6)	14,75±1,23 (3)
Ceruloplasmin	10,36±0,64 (6)	9,21±0,79 (3)
Transferrin	12,00±1,08 (6)	9,76±1,11 (3)
α-globulin	29,65±0,86 (6)	26,45±1,20 (3)
β-globulin	10,60±1,86 (6)	21,70±1,99* (3)
γ-globulin	9,87±2,59 (6)	5,70±0,34 (3)

Legend. 1) Asterisk marks results for which $P < 0.05$ compared with control; 2) Number of experiments given in parentheses.

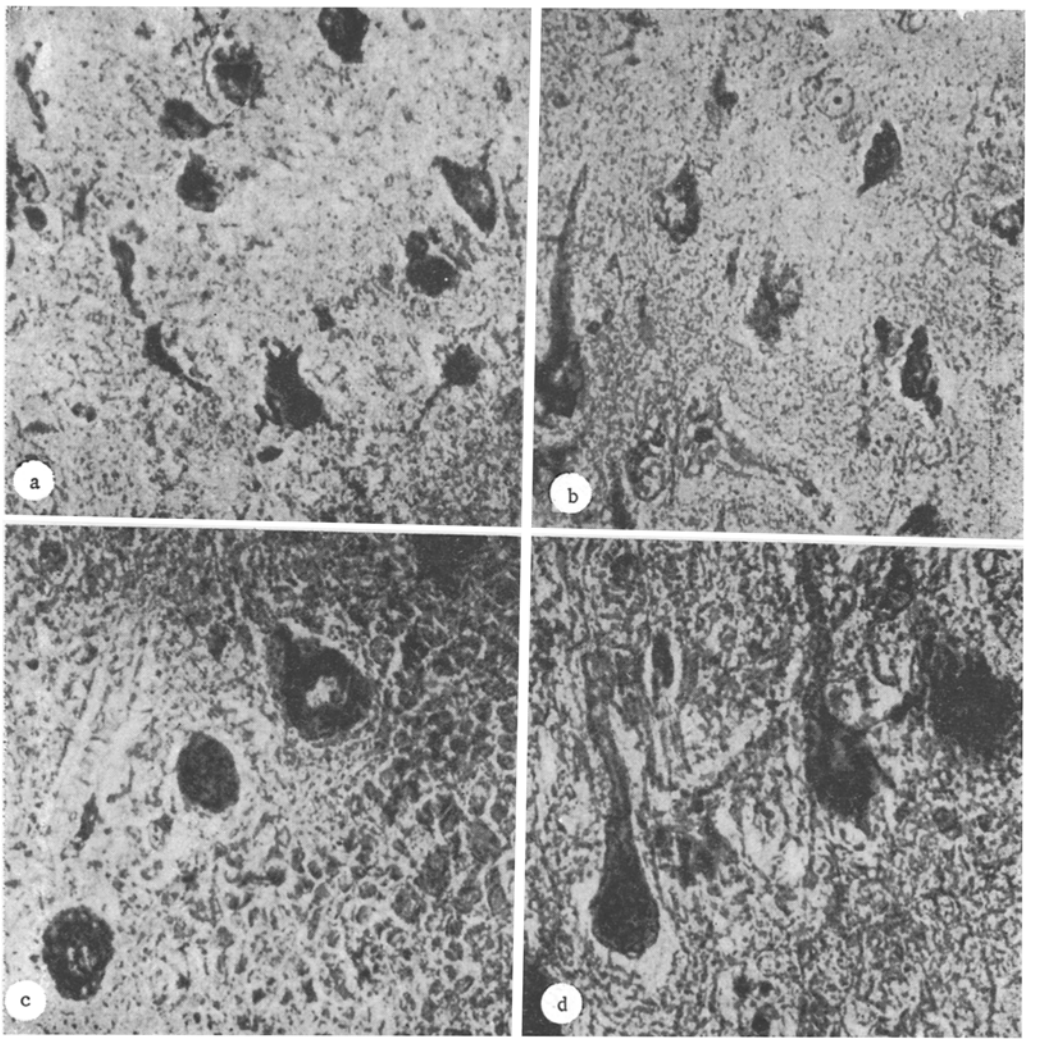

Fig. 1. Changes in acid phosphatase activity in brain neurons of dogs 1 month after resuscitation from hypovolemic hypotension lasting 4 h. a, c) Control (a – neurons in layer III of sensomotor cortex, c – Purkinje cells); b, d) experiment (same brain regions). Gomori, 400 ×.

Fig. 2. Densitograms of proteins of postmitochondrial supernatants of cortical gray matter of dogs in initial state and 1 month after resuscitation with the aid of prolonged dextran infusions. Continuous line) control; broken line) experiment.

Brain tissue for investigation was obtained by intravital biopsy through a burr-hole. The burr-hole was drilled under thiopental anesthesia (10-25 mg/kg).

The content of total RNA and DNA was determined in homogenates of the gray matter of the cerebral cortex [8, 12]. Acid phosphatase activity was studied in the postmitochondrial supernatant [10]; the protein fractions were separated by electrophoresis in polyacrylamide gel [5, 11]. Quantitative analysis of the protein fractions was carried out with a densitometer from Carl Zeiss, Jena.

Acid phosphatase activity was determined in the brain tissue by Gomori's histochemical method; the brain also was stained with cresyl violet by Nissl's method in Ritter's modification and impregnated by Cajal's method. To assess the degree of brain damage the number of Purkinje cells was counted in the cerebellar cortex [7].

EXPERIMENTAL RESULTS

As the results given in Table 1 show, the content of total RNA and DNA in homogenates of cortical gray matter on the 14th-28th day of the postresuscitation period after hypovolemic hypotension lasting 4 h, was close to the control values.

RNA synthesis in the nuclei of the neurons and glia was evidently activated, as shown by the less compact structure of the nucleoli and swelling of the nuclei.

In the postresuscitation period disappearance of neurons occurs in large foci in the cerebral cortex of animals recovering from prolonged hypovolemic hypotension [3, 7]. However, as the present investigation demonstrated, when prolonged infusions of dextran were given only single tiny foci of death of nerve cells were observed, and not in every field of vision. Further evidence of the better preservation of the nerve cells in animals treated intensively with dextran was given by the absence of any significant decrease in the number of Purkinje cells in the cerebellar cortex: In the experimental group they numbered 1870 ± 244, in the control group 2265 ± 145. In animals receiving dextran during the early postresuscitation period only, they numbered not more than 1188 ± 38 [3].

Histochemical investigations of the cerebral cortex of the experimental animals revealed an increase in acid phosphatase activity (Fig. 1); reaction products in the form of numerous large granules filled the bodies and processes of the neurons as a result of migration of lysosomes [9]. The enzyme level also rose in the bodies and processes of the glial cells and in the endothelium of the blood vessels.

The results of biochemical determination of acid phosphatase activity in the postmitochondrial supernatant correlated with the results of the histochemical investigation. For instance, relative acid phosphatase activity in the experimental group was 7.3 ± 0.8 and in the control 4.76 ± 1.0 i.u./mg protein · ml ($P < 0.05$). The increase in acid phosphatase activity can probably be regarded as a specific reflection of the intensive repair processes taking place in the brain tissue ("metabolic stress" [6]). Evidence of activation of repair processes in the brain tissue also was given by swelling and proliferation of the astrocytes and oligodendroglia.

Comparison of densitograms of proteins obtained by electrophoresis of the postmitochondrial supernatant of the experimental and control animals showed that prolonged dextran infusions produced normalization of that part of the protein densitogram which reflects the behavior of fast-moving fractions (Fig. 2). No significant difference from the control was found in fractions corresponding to prealbumins, albumins, postalbumins, ceruloplasmin, transferrin, and α-globulins. Meanwhile the high-molecular-weight protein fractions (β- and γ-globulins) still showed considerable changes compared with the control: The β-globulin content was doubled but the α-globulin content was reduced by 41% (Table 1).

Comparison of the results of this investigation with the control and with results obtained previously [3] thus indicates that prolonged administration of dextran infusions in accordance with the scheme suggested above gives a large measure of protection to brain cells against destruction and activates plastic repair processes. Although biochemically inert, dextran helps to ensure fuller adaptation by increasing the effective circulating blood volume [1]. The more prolonged the massive blood loss, the more marked the pathological retention of blood in the depots, requiring not only replacement of the lost blood but also administration of an extra volume of transfusion media [4]. Dextran infusion, by improving the state of the hemodynamics and the rheologic characteristics of the blood can, it is tentatively suggested, maintain the cerebral blood flow at a level which corresponds to the metabolic demands of the nerve cells which have survived prolonged circulatory hypoxia. The results described above demonstrate the importance of the use of prolonged dextran infusions in conjunction with other resuscitation measures for the treatment of patients in hypovolemic states.

LITERATURE CITED

1. V. B. Koziner and N. A. Fedorov, The Mechanism of Action of Dextran [in Russian], Moscow (1974).
2. V. L. Kozhura, Byull. Éksp. Biol. Med., No. 10, 39 (1975).
3. V. L. Kozhura, L. V. Molchanova, and S. I. Pylova, Byull. Éksp. Biol. Med., No. 8, 933 (1976).
4. V. A. Negovskii, T. P. Bel'skaya, Z. A. Topchiashvili, et al., in: Proceedings of the 24th Congress of the International Surgical Society [in Russian], Vol. 1, Moscow (1972), p. 217.
5. S. I. Pylova, L. V. Molchanova, and V. L. Kozhura, in: Scientific Research in Resuscitation [in Russian], Saransk (1976), p. 40.
6. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1975).
7. N. P. Romanova, in: Fundamentals of Resuscitation [in Russian], Moscow (1975).
8. A. S. Spirin, Biokhimiya, 23, 656 (1958).
9. G. Benetato and E. Gabrielescu, Rev. Roum. Physiol., 5, 257 (1968).
10. D. L. Di Pietro and F. S. Lengerle, J. Biol. Chem., 242, 3391 (1967).
11. A. H. Greenhouse and L. B. Speck, Am. J. Med. Sci., 115, 333 (1971).
12. G. Schmidt and A. Thannhauser, J. Biol. Chem., 161, 83 (1945).