CHANGES IN SOME PHYSICOCHEMICAL PROPERTIES OF CEREBRAL CORTICAL PROTEINS DURING CLINICAL DEATH AND RESUSCITATION AFTER MECHANICAL ASPHYXIA

L. V. Molchanova and S. I. Pylova

UDC 616-036.882-08-07:616.831-008.939.6-074

During the period of clinical death from mechanical asphyxia in dogs, changes take place in the physicochemical properties of protein of the postimitochondrial supernatant from brain gray matter homogenates. The densitogram obtained by electrophoresis of the proteins in polyacrylamide gel is modified. The total protein concentration after centrifugation and total lactate dehydrogenase (LD) activity are reduced. The combined fraction of unadsorbed β and γ globulins is reduced after adsorption on DEAE-Sephadex A-50 and the activity of LD isozymes (3 + 4 + 5) is considerably reduced. The disturbance of the behavior of proteins in sedimentation and electrical fields and changes in their adsorption properties, showing specific features at different times of clinical death, play an important role in the formation of the irreversible changes in the gray matter of the brain tissue. In the early recovery period successive restoration of the adsorption and sedimentation properties of the protein is observed, whereas the electrophoretic spectrum does not return to normal during the first day.

KEY WORDS: resuscitation; brain; mechanical asphyxia.

One of the most important problems in resuscitation is the question of restoration of brain function after revival [5]. The study of metabolic disturbances in the brain tissues themselves must evidently make a definite contribution to the solution of this problem.

The object of this investigation was to study the electrophoretic and absorption properties of proteins of the cytoplasmic fraction of brain gray matter homogenates from dogs during clinical death from mechanical asphyxia and in the postresuscitation period. Total activity of lactate dehydrogenase (LD) and its isozymes was determined at the same time in the brain tissue, as an indicator of behavior of a protein whose physicochemical properties have been studied most fully [1, 12]. Investigations of protein metabolism from this aspect may help to determine some principles governing the formation of irreversible changes in the gray matter of brain tissue.

EXPERIMENTAL METHOD

Mongrel dogs weighing 9-15 kg were used in the experiments after Pantopon (4-8 mg/kg body weight) premedication. The terminal state developed as a result of mechanical asphyxia. After clinical death lasting 5 min the dogs were resuscitated by external cardiac massage combined with intraarterial injection of physiological saline with adrenalin (0.1 mg/kg) and artificial ventilation of the lungs [4]. In all groups of experiments the skull was trephined under thiopental anesthesia (10-20 mg/kg).

In the experiments of group 1 on five animals, brain tissue was taken at the 5th minute of clinical death and in group 2 (5 animals) at the 10th minute; in group 3 (7 animals) biopsy was performed after 1 h of the postresuscitation period, and in group 4 (7 animals) after 24 h of the postresuscitation period. The control group consisted of 9 dogs. The brain gray matter homogenate was centrifuged with cooling and the postmitochondrial supernatant fraction obtained for the investigation [8]. Protein concentration was determined by

Scientific-Research 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. 87, No. 6, pp. 539-542, June, 1979. Original article submitted July 10, 1978.

0007-4888/79/8706-0562$07.50

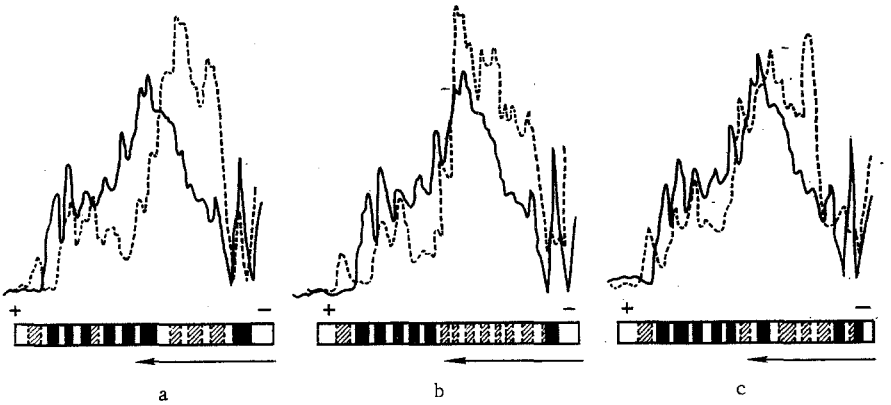

Fig. 1. Densitograms from electrophoresis of postmitochondrial supernatant proteins. Continuous line, control group; broken line: experimental group. A) After clinical death for 5 min; B) after clinical death for 10 min; C) after 24 h of postresuscitation period following clinical death for 5 min.

TABLE 1. Changes in Protein Concentration and LD Activity in Postmitochondrial Supernatant before and after Absorption on DEAE-Sephadex A-50 (M ± m)

Animals	Total protein, mg/ml	Total LD activity, IU/ml	After adsorption on DEAE-Sephadex A-50		
			LD isozymes (3+4+5), IU/ml	unadsorbed globulin fraction after electrophoresis in PAG, mm²	
				α globulins	β and γ globulins
Control	14,57±0,47	64 675±2 564	15 953±1 230	707±77	983±107
Experimental					
5 min of clinical death	10,20±0,35*	50 600±4 806*	8 315±2 961*	520±43*	735±59*
10 min of clinical death	11,60±0,18*	54 900±5 559	4 213±2 611*	623±44	775±35*
1 h after resuscitation	9,80±0,24*	50 643±4 109*	10 791±3 055	480±51*	765±31*
24 h after resuscitation	12,15±0,54*	54 560±3 315*	16 418±3 177	712±78	953±152

* $P < 0.05$ compared with control.

Lowry's method and the activity of LD and its isozyme [6] was determined on the "Era-1" instrument (from the firm "Photovolt") at a wavelength of 340 nm. Absorption of DEAE-Sephadex A-50 was carried out in bulk. Electrophoresis of protein in 7% polyacrylamide gel (PAG) was carried out with equalized protein concentration.

EXPERIMENTAL RESULTS

Electrophoresis of proteins of the supernatant fraction in PAG gave a characteristic densitogram, which is regarded as specific for brain tissue [8]. During clinical death from mechanical asphyxia the behavior of the proteins in an electric field changed, as reflected in a shift of the densitogram profile to the right, with a considerable increase in the β globulin fraction (from 10.6 ± 1.9 to $31.7 \pm 3.7\%$; $P < 0.01$) and with a decrease in the fractions of prealbumins and γ globulins (from 5.3 ± 0.4 to 3.8 ± 0.8 and from 9.9 ± 2.6 to $4.7 \pm 0.8\%$ respectively; $P < 0.01$). Lengthening the duration of clinical death increased the changes observed (Fig. 1a, b). For instance, the β globulin fraction increased to $35.9 \pm 1.8\%$, the prealbumins decreased to $2.0 \pm 0.3\%$, and the γ globulins fell to $3.6 \pm 0.6\%$ ($P < 0.01$).

The electrophoretic behavior of the proteins still remained disturbed during the first day of the recovery period (Fig. 1c), when the β globulin fraction was $32.4 \pm 1.1\%$, the prealbumin fraction $3.2 \pm 0.5\%$, and the γ globulin fraction $4.8 \pm 1.0\%$ ($P < 0.01$).

Changes in the behavior of proteins in an electric field were due not only to disturbance of the charge on the molecules, but also to a decrease in the protein concentration in the postmitochondrial supernatant. The results given in Table 1 show that during clinical death and in the first hour of the recovery period the total protein concentration in the supernatant fell significantly (from 14.6 ± 0.5 to 9.8 ± 0.2 mg/ml). Similar changes in the behavior of protein in a sedimentation field after tissue ischemia of varied etiology have been reported in the literature [2, 11]. The results showing changes in LD activity, present as five isozymes in brain tissue, are also given in Table 1. During clinical death total LD activity fell; in the recovery period this fall continued and became absolutely statistically significant.

The changes in activity of LD isozymes (3+ 4+ 5) after adsorption of the postmitochondrial supernatant on DEAE-Sephadex A-50 were different in character. The decrease in activity was already significant after clinical death for 5 min, and it continued as the duration of clinical death increased, whereas in the early postresuscitation period isozyme activity returned to its initial level. The increase in these indices could be the result of a disturbance of permeability of the vascular endothelium [10] or the cell membranes. The latter is confirmed by experimental data showing an increase in the activity of the organ-specific isozymes of acid phosphatase and LD (3+ 4+ 5) in arterial blood in the early postresuscitation period.

It will be clear from Table 1 that the fractions of unadsorbed α, β, and γ globulins decreased after electrophoresis in PAG. These results can be attributed to an increase in the absorption properties of the globulin fraction, and this is confirmed by the data showing a decrease in LD isozyme (3+ 4+ 5) activity. It can be tentatively suggested that this was the result of dissociation of protein globules into subunits. Tissue hypoxia is known to be characterized by a reduction in the energy potential and a decrease in NAD concentration, and these in turn induce rapid dissociation of complexes of protein subunits [7, 13]. Thus, during clinical death from mechanical asphyxia, the behavior of proteins of brain gray matter in sedimentation and electrical fields changes, as do their sorption properties. The changes observed in the physicochemical properties of the protein of the brain gray matter also were found by the writer in terminal states of other etiology, namely acute blood loss and compression ischemia of the brain [3, 9]. Lengthening the duration of clinical death, leading to gross neurological disturbances after resuscitation, is accompanied by quantitative changes in the activity of LD isozymes and in the electrophoretic properties of the proteins. In the postresuscitation period after clinical death lasting 5 min, successive recovery was observed, first of the adsorption and later of the sedimentation properties of the protein, whereas normal behavior of the protein in an electric field was not restored during the first day. It can be concluded from these results that cerebral hypoxia in terminal states leads to injury of the protein molecular structure.

LITERATURE CITED

1. V. L. Kretovich, Introduction to Enzymology [in Russian], Moscow (1974), pp. 295-296.
2. A. A. Krichevskaya, A. I. Lukash, and K. B. Sherstnev, Izv. Sev. Kav. Nauch. Tsent. Vyssh. Shkoly, Ser. Estestv. Nauk, No. 3, 21 (1973).
3. L. V. Molchanova and V. L. Kozura, Byull. Éksp. Biol. Med., No. 12, 17 (1975).
4. V. A. Negovskii, Direct Cardiac Massage and Expiratory Artificial Respiration [in Russian], Moscow (1966).
5. V. A. Negovskii and A. M. Gurvich, in: Fundamentals of Resuscitation [in Russian], Tashkent (1977), pp. 330-347.
6. D. Amelung, Fermentdiagnostik interner Erkrankungen, Stuttgart (1964), pp. 117-122.
7. G. S. Bailey and Soo Thye Lim, J. Biol. Chem., 252, 5708 (1977).
8. R. Caplan, C. Shelly, Y. Cheung, et al., J. Neurochem., 22, 517 (1974).
9. V. L. Kozura, L. V. Molchanovah, and S. I. Pylova, Resuscitation, 6, 29 (1978).
10. U. Rafalowska, M. Erecinska, and B. Chance, J. Neurochem., 25, 497 (1975).
11. W. Y. Reville, M. G. Harrington, and R. L. Joseph, Biochem. J., 125, 104 (1971).
12. L. Venkov, L. Rosental, and M. Manolova, Brain Res., 109, 323 (1976).
13. M. Yakata, S. Osami, and Y. Etsuku, Acta Med. Biol., 25, 53 (1977).