CHANGES IN PHOSPHATASE AND ACID CATHEPSIN ACTIVITY IN THE CEREBRAL CORTEX OF DOGS IN TERMINAL STATES

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UDC 616-036.882-092.9-07:616.831-008.931:[577.152.313+577.152.344

The activity of acid and alkaline phosphatases and acid cathepsins in subcellular fractions (especially in the postmitochondrial supernatant) of the gray matter of the dog brain rises proportionally to the duration of compression ischemia. The activity of the last two enzymes also rises during clinical death from blood loss preceded by hypotension of varied duration or with different levels of the arterial pressure. Comparison of the changes observed in the specific activity of these enzymes in subcellular fractions of the gray matter indicates the existence of a general mechanism for the activation of proteolysis in nerve tissue in the terminal states investigated.

KEY WORDS: cerebral cortex; clinical death; hypovolemic hypotension; lysosomal enzymes; alkaline phosphatase.

The final outcome of resuscitation measures is known to depend on the degree of hypoxic brain damage in terminal states [3, 4]. Under these conditions the study of the biochemical mechanisms determining the development of autolytic processes in cerebral cortical cells could contribute to the further understanding of the pathogenesis of irreversibility [2, 7, 8].

The object of this investigation was to study changes in phosphatases and acid cathepsins in the gray matter of the brain in various terminal states.

EXPERIMENTAL METHOD

Terminal states were induced in acute and chronic experiments on 54 dogs of both sexes by completely arresting the circulation in the brain only (compression ischemia) or by blood loss after premedication of the animals with Pantopon (4-8 mg/kg). Compression ischemia was induced [6] in the artificially ventilated dogs by injecting physiological saline into the cisterna magna under a pressure of 360 mm Hg (group 1). The terminal states produced in the dogs with blood loss were as follows: clinical death for 2 min, preceded by hypotension for 1 h from bleeding, with an arterial blood pressure (BP) of 40 mm Hg (group 2) or 20 mm Hg (group 3). Group 4 included animals surviving hypovolemic hypotension for 4 h with a BP of 40 mm Hg without clinical death. The BP in these groups of animals was maintained at the assigned level by bleeding or by injection of 10-15 ml blood into the femoral artery as required.

Brain tissue was obtained from the animals by biopsy through a burr-hole at the 17th, 21st, and 45th min of compression ischemia, after clinical death for 2 min, and at the end of the 4th h of hypovolemic hypotension. Brain biopsy of the control animals was carried out immediately after the operation of trephining the skull (control to group 1) or after fixation to the frame for 1 or 4 h (controls to groups 2, 3, and 4). After biopsy the gray

Laboratory of Experimental Physiology of Resuscitation, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 12, pp. 17-20, December, 1975. Original article submitted March 28, 1975.

TABLE 1. Specific Enzyme Activity (in i.u./mg protein/ml) in Supernatant Fraction of Gray Matter at Different Periods of Compression Ischemia (M ± m)

Enzyme	Control	Compression ischemia (group 1)		
		17 min	21 min	45 min
AcP	5,42±0,31	7,62±0,35*	6,70±0,23 *	12,32±2,25*
AC	0,66±0,15	1,14±0,55	1,20±0,61	2,59±0,21*
AeP	1,95±0,40	3,50±0,78*	6,50±0,31*	4,99±0,26*

*Here and in Table 2, $P < 0.05$.

TABLE 2. Specific Enzyme Activity (in i.u./mg protein/ml) in Supernatant Fraction of Gray Matter During Hypovolemic Hypotension (M ± m)

Enzyme	Control	Group		
		2	3	4
ACP	6,77±1,56	6,14±0,45	9,85±1,34	4,28±0,92
AC	0,25±0,14	0,90±0,45	2,53±0,45*	1,65±0,58
AeP	1,19±0,27	2,04±0,33*	3,20±0,67*	2,86±0,79*

matter was homogenized in the cold in 0.32 M sucrose solution with EDTA, centrifuged with cooling, and the postmitochondrial supernatant fraction was obtained at 16,000 g [11]. Activity of acid (AcP) and alkaline (AlP) phosphatases was determined against sodium p-nitrophenylphosphate [5, 10], and activity of acid cathepsins (AC) was determined against hemoglobin. The protein concentration was determined by Lowry's method [9].

In chronic experiments on seven animals surviving compression brain ischemia for 21 min the course of recovery of CNS functions was observed.

EXPERIMENTAL RESULTS AND DISCUSSION

As Table 1 shows, after 17 min of ischemia of the CNS the activity of both phosphatases was increased; as the period of ischemia grew longer this activity continued to rise or remained at a high level. Acid cathepsin activity was significantly increased only after 45 min of compression ischemia.

In clinical death preceded by hypotension for 1 h to BP = 40 mm Hg (group 2) no significant changes were found in the activity of hydrolases other than AlP (Table 2). In animals surviving clinical death preceded by hypovolemic hypotension to BP = 20 mm Hg (group 3) an increase in activity of all hydrolases was found, with AC activity increased by 10 times and AlP activity increased by 2.7 times. After hypotension for 4 h to BP = 40 mm Hg (group 4) an increase in AC and AlP activity also was observed in the supernatant fraction of the brain homogenate.

Comparison of the specific activity of the enzymes in the control groups (Tables 1 and 2) showed no significant changes arising as a result of prolonged recumbency in the frame.

Changes in activity of the lysosomal enzymes and AlP in the postmitochondrial supernatant of brain tissue thus indicate activation of proteolysis both in animals surviving clinical death preceded by hypotension (group 3) and in dogs subjected to prolonged hypovolemic hypotension only (group 4). The changes depended not only on the mean BP level during the terminal state after equal periods of clinical death, but also on the duration of the hypotension without cardiac and respiratory arrest.

In chronic experiments on animals surviving 21 min of compression ischemia complete visible recovery of CNS functions took place in only one of the 7 dogs. The animals surviving 45 min of ischemia in the acute experiments were therefore regarded as a group with irreversible CNS changes. The specific activities of the enzymes investigated in the subcellular fraction of the brain homogenate from these dogs were close to those in animals surviving hypovolemic hypotension to BP = 20 mm Hg and clinical death for 2 min.

It was also found that the protein concentration in the supernatant fraction was lower than in the control animals, and the more severe the animal's terminal state the more it was lowered. Compression ischemia led to a reduction of the protein concentration by 19.8% after 17 and 21 min and by 47.2% after 45 min. In animals surviving terminal states induced by blood loss (groups 2, 3, and 4) the protein concentration in the supernatant fraction was reduced compared with the control by 11.4, 30.7, and 30.1% respectively.

Enzyme activity also was determined in the unpurified mitochondrial fraction of gray matter homogenate. In the animals of group 1 a statistically significant increase in specific AcP and AlP activity in this fraction was observed first after 17 min of cerebral ischemia and it continued to rise proportionally to the duration of ischemia. Acid cathepsin activity increased significantly only after 45 min of compression ischemia and in the animals of group 4.

The results of comparison of the data showing increased enzyme activity in the gray matter of the brain of dogs after massive blood loss or with isolated cerebral ischemia points to severe hypoxic damage to the CNS in terminal states preceded by severe hypovolemic hypotension. This is confirmed by the high mortality of the animals in these states [1]. High catheptic activity in the tissues of the gray matter of the dog brain is evidence of a common mechanism for the activation of proteolysis in nerve tissue in the terminal states studied.

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