HYDROLASE ACTIVITY IN THE GRAY MATTER OF THE DOG BRAIN DURING CLINICAL DEATH AND RECOVERY

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The state of clinical death induced by electric shock is characterized by an increase in the activity of acid phosphatase, acid cathepsins, and plasminogen activator while the alkaline phosphatase activity remains constant in subcellular fractions of the gray matter of the dogs' brain. In the recovery period after clinical death the normal brain hydrolase activity of the animals is restored, and the plasminogen activator activity falls significantly.

The state of clinical death, produced in various ways, is always associated with the development of hypoxia and a decrease in the intensity of energy metabolism [2, 3]. Even a limited supply of oxygen to the brain causes uncoupling of oxidative phosphorylation and respiration, and this disturbance modifies all biological processes taking place in the brain tissue [12].

The activity of some acid and alkaline hydrolases of the gray matter of the brain was investigated in control dogs and also in animals in a state of clinical death caused by electric shock, during the period of clinical death and after resuscitation.

EXPERIMENTAL METHOD

The control group (11 dogs) included animals not subjected to electric shock and others from which the brain was removed 1 min after electric shock. Brain tissue was also taken from the animals in clinical death 10 min (nine dogs), 1 h (six dogs), and 24 h (five dogs) after infliction of the shock. Brain tissue was also investigated from the animals 1 h (five dogs) and 24 h (eight dogs) after resuscitation. Resuscitation began after clinical death had lasted 10 min, using the method adopted in the laboratory [4].

After premedication with trimeperidine, the skulls of all the dogs were trephined under thiopental anesthesia (10-25 mg/kg body weight), and the brain tissue removed. The time occupied in processing the gray matter of the brain in the cold was strictly checked [9]. The tissue was homogenized in a solution of sucrose with EDTA and fractionated in a TsLR-1 refrigerator centrifuge [1]. The resulting fractions of mitochondria, light mitochondria, and supernatant were treated with the detergent Tween-80. Protein was determined by the method of Lowry et al. [10]. Activity of acid and alkaline phosphatase was determined relative to sodium p-nitrophenylphosphate and expressed in international units (i.u.)/mg protein/ml. Proteolytic activity was determined by Ansen's method [5] and expressed in Dilpier–Fruton units/mg protein/ml. The plasminogen activator activity was expressed in square millimeters [8].

EXPERIMENTAL RESULTS

Activity of acid phosphatase ($P < 0.05$) and acid cathepsins ($P < 0.05$) was increased in the brain tissue during clinical death, chiefly in the mitochondrial fraction (Fig. 1). Predominant among the postmortem

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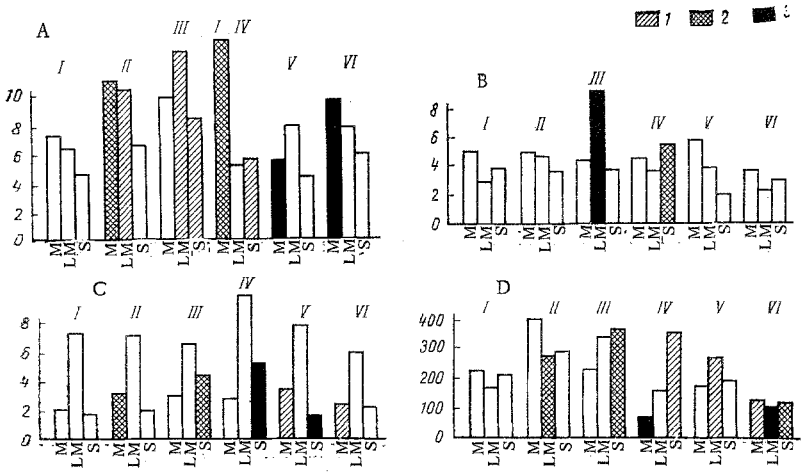

Fig. 1. Diagram showing changes in relative activity of acid phosphatase (A, in i.u.), cathepsin (B, in DF units), alkaline phosphatase (C, in i.u.) and plasminogen activator (D, in mm^2) activity in subcellular fractions of gray matter of the brain from various groups of dogs. M) Mitochondria; LM) light mitochondria; S) supernatant. 1) $P < 01$; 2) $P < 0.05$; 3) $P < 0.02$. Groups of animals: I) normal; II, III, and IV) 10 min, 1 h, and 24 h, respectively after electric shock; V, VI) recovery period 1 h and 24 h, respectively after clinical death lasting 10 min. Abscissa, subcellular fractions; ordinate, enzyme activity.

changes under these experimental conditions 60 min after electric shock was a change in the profile of enzyme activity, i.e., in the ratio between the activities of the enzymes in the fractions. Acid phosphatase activity continued to rise in the fraction of light mitochondria ($P < 0.1$), while at the same time there was an absolutely significant increase in the alkaline phosphatase activity ($P < 0.02$). Activity of the cathepsins and plasminogen activator increased in the post mitochondrial supernatant ($P < 0.05$), on the basis of which a disturbance of the permeability of the subcellular structures, which are considerably increased after autolysis for 24 h ($P < 0.02$), was postulated.

The increase in activity of the lysosomal enzymes in the supernatant fraction as a result of disturbance of membrane permeability in subcellular structures has been reported previously by several workers, both in liver tissue during acute protein deprivation [7] and also during postmortem changes in muscles [13].

It can be concluded from the results of these experiments that activity of the acid cathepsins D and B, is of great importance in the transition from clinical into biological death [2, 11]. This is confirmed indirectly by the results of determination of enzyme activity in the groups of resuscitated animals: for instance, after 1 h of the recovery period, characterized by the severest disturbances of metabolism [2, 3], the enzyme profile was not restored, but acid phosphatase activity was absolutely significantly ($P < 0.02$) lower than the level of its activity after postmortem changes for 1 h. The proteolytic activity of the resuscitated animals, however, was lower and was localized in the mitochondria and light mitochondria, but not in the supernatant ($P < 0.02$). After 24 h of the recovery period a further decrease in the activity of the cathepsins ($P < 0.05$) and plasminogen activator ($P < 0.02$) and restoration of the enzyme profile were observed. There was also a significant secondary increase in acid phosphatase activity in the mitochondrial fraction.

The experimental results show that activation of certain hydrolases of the brain takes place in clinical death; activity of the acid cathepsins continues to increase during the postmortem changes, whereas the proteolytic activity in the subcellular fractions of the animals' brain returns to normal in the first day of the recovery period.

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