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ENZYME IMMUNOASSAY OF SPECIFIC BRAIN ANTIGENS AS A CRITERION OF BLOOD-BRAIN BARRIER PERMEABILITY IN RATS WITH ACUTE CEREBRAL ISCHEMIA

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Cerebral ischemia remains one of the most important problems in medicine today, for the anoxia of the brain cells which results from it lies at the basis of many diseases or is one of their manifestations [1, 8]. Information on the function of the blood-brain barrier (BBB) in acute ischemia is by no means consistent [6, 9]. This difference in the results can be explained by the use of different approaches by different authors to the study of BBB permeability. The function of this morphological and functional system can be evaluated, on the one hand, by studying changes in permeability for low-molecular-weight substances of varied origin [9, 11], but on the other hand, by examining the passage of compounds specific for the brain through BBB [2, 12]. An interesting development in the study of BBB function is the use of specific brain antigens for these purposes [2, 14].

In the investigation described below changes are brought about in BBB permeability for specific acid gliofibrillary antigen (GFAP) and for brain α_2-globulin (α_2-M) in rats with acute cerebral ischemia.

EXPERIMENTAL METHOD

Altogether 50 experiments were carried out on noninbred rats of both sexes weighing 200-220 g. The animals were anesthetized by intraperitoneal injection of 1 ml of 1% hexobarbital solution/100 g body weight. A model of cerebral ischemia was produced as described previously [3]. Blood serum from control and ischemia animals served as the test object.

The barrier function of BBB was studied by an immunoenzyme method [7]. Antisera to neurospecific α_2-M and GFAP were obtained by immunizing chinchilla rabbits with purified preparations of these antigens, obtained by methods in [14] and [5] respectively. Antibodies to the above-mentioned brain antigens were isolated from monospecific antisera on immunosorbents prepared on the basis of CNBr-sepharose 4B (Sigma, USA) and purified α_2-M and GFAP preparations by the method in [15]. The concentration of immunoglobulins in the fraction of isolated antibodies was determined by Ouchterlony's double immunodiffusion

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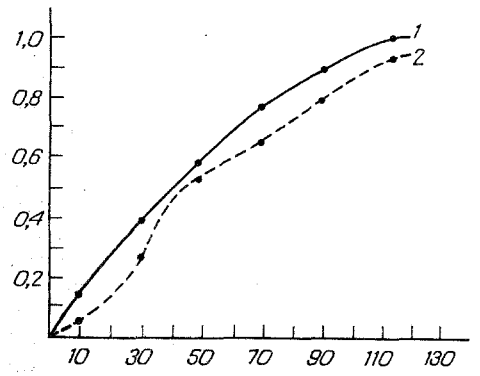

Fig. 1

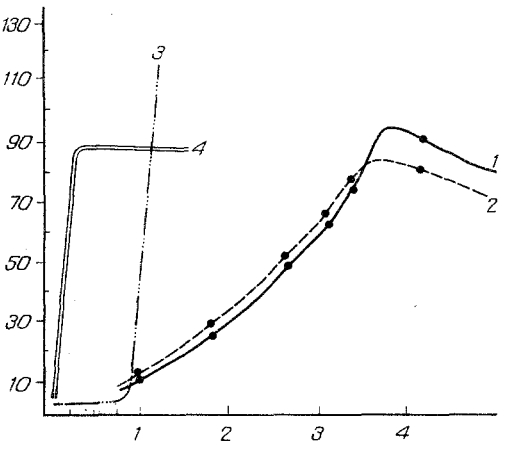

Fig. 2

Fig. 1. Dependence of concentration of specific α_2-M (1) and GFAP (2) on optical density. Abscissa, concentration of specific proteins (in ng/ml); ordinate, optical density at 450 nm (in relative units).

Fig. 2. Dependence of concentrations of specific α_2-M (1, 3) and GFAP (2, 4) in rats' blood serum on time after unilateral (1, 2) and bilateral (3, 4) ligation of carotid arteries. Abscissa, time after ligation (in days); ordinate, concentration of specific proteins (in ng/ml).

method, using donkey antiserum against rabbit immunoglobulins (Behringwerke, West Germany). The concentration of specific antibodies to α_2-M and GFAP was determined by immunodiffusion in the modification [2]. A "sandwich" version of enzyme immunoassay was used in the experiments [13].

Polystyrene plates were activated with a 0.0005% solution of antibodies to α_2-M and a 0.002% solution of antibodies to GFAP in 0.05 M carbonate buffer (pH 9.6) for 20 h. During preparation of the conjugate of antibodies to brain antigens with the enzyme, type VI peroxidase (Sigma) was used; the antibodies were conjugated with peroxidase by the periodate method [10]. A 0.08% solution of 5-aminosalicylic acid was used as the conjugant. The results were recorded on a Titerteck Multiscan multichannel spectrophotometer (Flow Laboratories, England).

EXPERIMENTAL RESULTS

The immunoenzyme system developed in the course of the experiment for the detection of α_2-M in biological fluids was capable of determinining α_2-M within the range of concentrations from 0.8 to 102 ng/ml. Optimal working of the system was obtained by the use of antibodies in a concentration of 5 µg/ml and conjugate in a dilution of 1:50 to activate the polystyrene plates. The immunoenzyme system for analysis of GFAP was able to detect it in concentrations of between 1 and 128 ng/ml. Optimal working of the system was observed when antibodies were used in a concentration of 20 µg/ml to activate the polystyrene plates and the conjugate of antibodies with enzyme was used in a dilution of 1:60. Graphs showing dependence of the concentration of α_2-M and GFAP in the test sample on optical density at 420 nm, plotted on the basis of the results of 30 experiments, are given in Fig. 1. Statistical analysis of the standard deviation of the points, which did not exceed 1.1%, demonstrated the reliability and good reproducibility of the systems developed. The immunoenzyme method of detection of α_2-M and GFAP was used to investigate permeability of BBB in rats of the control group, and also in animals with experimentally induced acute cerebral ischemia.

Blood serum from control rats (100 animals) contained α_2-M in a concentration not exceeding 7 ng/ml, and GFAP in a concentration of not exceeding 4 ng/ml. These concentrations were taken as the upper limits of the normal levels of the above-mentioned antigens in rat blood serum.

Unilateral ligation of the common carotid artery caused marked ipsilateral cerebral ischemia, accompanied by a breach of BBB and the release of the glial cell protein GFAP and the astrocyte antigen α_2-M into the blood stream. However, this change of permeability was

not a rapid response and it was not recorded until 16-20 h after occlusion. Concentration of α_2-M and GFAP rose sharply to reach a maximum after 4-5 days, and this was followed by a gradual fall (Fig. 2). This character of the curve of dependence of brain antigen concentration on the duration of experimental ischemia can be explained, on the one hand, by the rapid response of the body to disturbance of BBB function, followed by correction of permeability, and on the other hand, by activation of antibody-producers by free brain antigens and their subsequent binding of the antibodies.

Bilateral ligation of the carotid arteries caused marked bilateral cerebral ischemia, as a result of which massive penetration of GFAP into the blood was recorded 1 h after occlusion. Its level rose to reach a maximum after 4-6 h of ischemia. So far as α_2-M, the astrocyte marker, is concerned, it leaked into the blood only after cardiac arrest, which was observed in all the experimental animals between 4 and 22 h after carotid artery ligation. When these results are analyzed, it is apparent that the mechanisms of elimination of specific brain proteins into the blood stream differ depending on the degree of ischemia; moreover, they are evidently highly specific for individual brain proteins or, at least, they correlate with their function in the brain cells.

Disturbance of BBB permeability in cerebral ischemia having been discovered, the data [6, 8] showing high resistance of BBB to ischemia and anoxia cannot be disputed. However, the absence of any disturbance of BBB permeability after complete interruption of the blood supply to the brain for 3 h [8] can be explained either by imperfections of the method used to record permeability, or the low level of informativeness of the approach used in that study (the degree of disturbances of BBB function was estimated by the level of extravasation of albumin labeled with Evans' blue).

Two approaches are nowadays used to study the permeability of BBB [1]. One of them is based on investigation of permeability in the blood–brain direction [8, 11], the other on determination of specific brain antigens in the blood [2, 12, 14]. Since during the study of specific proteins as detectors of BBB permeability it is possible to investigate the functions of the cells producing them, and also to elucidate the mechanisms of elimination of antigens into the blood, the second approach would seem to be more informative.

Thus the enzyme immunoassay of specific brain antigens offers more than definite prospects for the assessment of BBB function in ischemia and anoxia. The investigation of specific brain antigens in these states provides an obvious basis for the development of analysis of certain stages of metabolism in brain cells.

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