IMMUNOELECTROPHORETIC AND ENZYMIC INVESTIGATION OF SOME BRAIN ANTIGENS OF ALBINO RATS

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The enzyme activity of precipitation arcs formed by water-soluble rat-brain antigens on immunoelectrophoresis was investigated. Enzymic activity of lactate dehydrogenase and esterase was found in precipitation arcs located in the zone of the α-, β-, and γ-globulins.

The protein composition of tissues in the course of their development is at present undergoing intensive investigation [5]. One method which can be used to detect individual proteins by differences in their antigenic properties is immunoelectrophoresis. By means of this method, 12 water-soluble antigenic components have been found in the brain of albino rats, five of them organ-specific [11], and their changes in postnatal ontogenesis have been studied.

Meanwhile, the identification of these antigens with a particular class of protein compounds is of great interest [1]. Among rat brain antigens, Kosinsky and Grabar have identified an esterase [11]. Several such investigations have been undertaken on the nerve tissue and serum of certain animals [10, 11, 18]. However, the number of these investigations is still small.

This paper describes the study of rat brain antigens obtained by a combination of biochemical, histochemical, and immunochemical methods of enzyme detection.

EXPERIMENTAL METHOD

Water-soluble antigens from the brain of Wistar rats were investigated.

Preparation of the Antigen. The brain was washed with tap water and homogenized in five volumes of 0.14 M NaCl solution, pH 7.2. The supernatant obtained from the homogenate by centrifugation at 21,500 g for 1 h was concentrated by ultrafiltration through Perlodion filters in special tubes. Protein concentration in the samples for analysis was 5-6%. This variation in the protein concentration did not affect the number or clarity of the precipitation arcs.

Preparation of the Antisera. Rabbit antisera against rats' brain were prepared by Dorfman's method [3]. The antisera were concentrated by Cohn's method in Gusev's simplified modification [2].

To detect organ-specific brain antigens, the native rabbit serum was exhausted [4] with a lyophilically dried mixture of water-soluble extracts of heterologous organs (liver, kidney, spleen, lungs, heart, thymus, serum). The exhausted sera were concentrated by the method described above. Completeness of exhaustion was verified by immunodiffusion. The antigens were identified by immunoelectrophoresis [17] in agarose gel, made up in barbiturate buffer, pH 8.6, $\mu = 0.025$. Electrophoresis was carried out in a potential gradient of 4 V/cm for 40 min. Immunodiffusion was carried out in the cold (4°C) for 48 h. After

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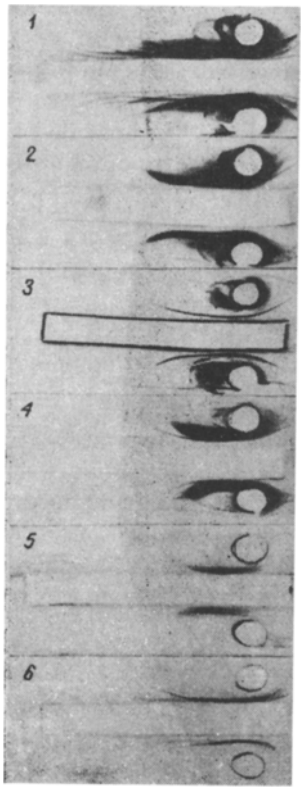

Fig. 1

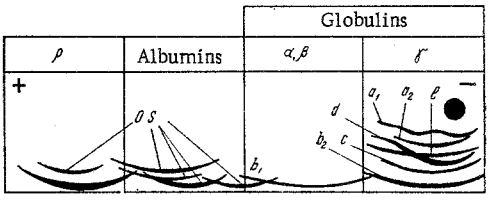

Fig. 2

Fig. 1. Immunoelectrophoresis of water-soluble brain antigens from an adult rat developed with brain antiserum. 1) Stained for protein with azocarmine; 2) Nachlas–Seligman reaction for esterase; 3) reaction for alkaline phosphatase; 4) reaction for acid phosphatase; 5) reaction for succinate dehydrogenase; 6) reaction for lactate dehydrogenase.

Fig. 2. Diagram showing mutual arrangement of precipitation arcs discovered during histochemical tests and of organ-specific water-soluble brain antigens from adult rat. a_1 and a_2 – test for esterase; b_1 and b_2 – test for lactate dehydrogenase; c – test for succinate dehydrogenase; d – test for acid phosphatase; e – test for alkaline phosphatase; OS – organ-specific antigens.

immunoelectrophoresis, the gel was washed for 3 days to remove nonreacting material in cold (4°C) 0.14 M NaCl solution, pH 7.2. The dried agarose plates were stained for protein with amide black 10B or with azocarmine.

For the bio-histochemical tests, the washed agarose plates after immunoelectrophoresis were placed in the corresponding media and incubated as described in [6-9, 14-16]. The following tests were carried out: for esterase using α-naphthyl acetate as substrate and fast blue RR, for acetylcholinesterase by the method of Gerebtzoff and Uriel (determined after immunoelectrophoresis of both total and organ-specific antigen), for acid and alkaline phosphatases by the azo-coupling method (α-naphthyl phosphate, fast blue BB), for lactate, alcohol, malate, glutamate, and succinate dehydrogenases and monoamine oxidase. In the case of the reaction for esterase, the control plates were treated with solutions of $5 \cdot 10^{-5}$ M eserine, 10^{-3} M $CuSO_4$, and 10^{-5} M E-600 by the usual methods [16]. For the determination of lactate dehydrogenase, media containing urea and sodium lactate in high concentrations (3M and 1-2 M, respectively), as recommended by Brody [7] also were used. In the other cases, the controls were set up in accordance with the usual histochemical recommendations [6, 16].

EXPERIMENTAL RESULTS

By the use of rabbit antibrain serum, 12 antigenic components were detected in the water-soluble extract from rat brain. Five of these antigens were organ-specific. These results are in agreement with data in the literature [11]. Comparison of the electrophoretic mobility of the separated fractions with the electrophoretic mobility of the components of native rat serum led to the distinction of five zones corresponding to prealbumins, albumins, and α-, β-, and γ-globulins. Identification of the enzymes in the precipitation arcs showed that they were all in the α-, β-, and γ-zones.

The clearest histochemical results were obtained by the tests for esterase and lactate dehydrogenase. A positive test for esterase was found in two precipitation arcs lying principally in the zone of the γ-globulins and partly in the zone of the α- and β-globulins (Figs. 1 and 2). The enzymic activity of one of them was reduced by the action of a $5 \cdot 10^{-5}$ M solution of eserine, although it was not completely abolished. In some specimens this arc was detected by the methods of Gerebtzoff and Uriel for acetylcholinesterase.

The antigen described can be assumed to contain acetylcholinesterase activity. This was confirmed indirectly by the results of investigation of the changes in esterase activity of this arc during postnatal ontogenesis of rats. The degree of its identifiability by the histochemical methods used was found to be parallel to the increase in acetylcholinesterase activity in the brain. The nature of the second arc is less clear, for its esterase activity fell slightly as the result of treatment with 10^{-3} M $CuSO_4$ solution, and also to a lesser degree under the influence of 10^{-5} M E-600 solution. Leone and Anthoni found a similar arc with evidence of an aromatic esterase in the serum of dogs [12].

Lactate dehydrogenase activity was found in two arcs (Figs. 1 and 2), both in the zones of the α-, β-, and γ-globulin; one lay mostly in the zone of the α- and β-globulins, the other entirely in the zone of γ-globulins. On incubation of the agarose blocks in medium containing 3 M urea solution, the lactate dehydrogenase activity of both arcs was abolished, while if an excess of sodium lactate (1.4 M) was present in the medium, its activity remained. In rats at birth and at the age of four days, both arcs gave a very weak reaction for lactate dehydrogenase (in the newborn animals it was hardly visible), while at the ages of 6 and 8 days the intensity of their stain began to increase, and at 11 days it was clearly defined.

Tests for acid and alkaline phosphatases by the method of double azo-coupling each stained one arc (mainly in the zone of γ-globulins; Figs. 1 and 2). However, after heating and treatment with inhibitors (0.002 M $CuSO_4$ and 10^{-3} M cysteine solution respectively), staining was not completely abolished, so that the results in this case were less clear than in the previous case, and were most probably due to a nonspecific reaction.

Traces of a reaction for succinate dehydrogenase were found in one arc in the zone of γ-globulins (Figs. 1 and 2).

No precipitation arcs giving positive reactions for alcohol dehydrogenase, malate dehydrogenase, glutamate dehydrogenase, or monoamine oxidase were found. It may be, however, that the activity of these enzymes was inhibited by antibodies. Evidence has been found that the activity of lactate dehydrogenase and other enzymes is inhibited by antibodies against them [13]. Under the present conditions, the antigen–antibody reaction evidently took place in such a way that at least some of the active centers of the enzymes remained free, thus allowing the corresponding enzymes to be identified by histochemical tests. None of the precipitation arcs of the five organ-specific antigens showed any definite enzyme activity, in agreement with the findings of Kosinsky and Grabar [11].

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