ACTION OF TRYPSIN ON WATER-SOLUBLE ORGAN-SPECIFIC BRAIN ANTIGENS

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The study of the action of enzymes on the antigenic properties of the tissues is important because it may be postulated that the immunogenic properties of the tissue antigens are dependent on their resistance to the enzyme systems of the body. The action of enzymic proteolysis on organ-specific brain antigens has received too little study [6, 8], partly because of the unsuitability of the gel-precipitation method used in these investigations for this purpose [6].

In the present investigation the action of trypsin on the organ-specific antigenic properties of water-soluble brain extracts was studied by means of the complement fixation reaction (CFR) in the cold and by Boyden's passive hemagglutination reaction (PHR).

EXPERIMENTAL METHOD

Saline extracts [5] from various structures of the human brain and from the whole rat's brain were diluted to give a protein concentration of 0.6-1.0 mg/ml and treated with trypsin* solution diluted 1:2-1:4 with buffered physiological saline (pH 7.4 or 8.2). The mixture of extract and trypsin (in the control series, extract and buffered solution) was incubated for 2 h at 37°, after which it was heated in a water bath at 56° for 30 min to inactivate the trypsin. The results of special investigations confirm that this heat treatment of the trypsin was adequate to ensure that, on the subsequent addition of solutions containing trypsin to the immune sera, their activity was not lowered in either the CFR or the PHR.

The investigations were carried out with rabbit and guinea pig sera agains homogenate from various morphological structures of the human brain, and also with rabbit sera against rat's brain tissues. The method of performance of the CFR and PHR was essentially the same as that described previously [2-5]. For convenience of subsequent statistical analysis of the results [1], the intensity of the CFR was assessed as a sum of conventional units [5]. For the PHR the tissue extracts were diluted so that the protein content in the control extract was 0.3 mg/ml. The trypsinized extract was diluted with the appropriate volume of buffered solution (with 1% rabbit serum), or a more concentrated extract was used in order to make the protein concentration equal to that in the control experiment, for as a result of trypsinization the protein content in the tissue extract was reduced by 2-3 times.

EXPERIMENTAL RESULTS

The results of the CFR are illustrated in the figure. They show that all the sera used possessed activity against brain tissue extracts untreated by trypsin, and reacted much more weakly with extracts from the liver (different significant), i.e., they contained organ-specific brain antibodies. As a result of treatment of the brain extract with trypsin, in most cases their activity showed a significant decrease ($P < 0.05$-0.01); the differences between the activity of the control and trypsinized extracts from the white matter of the cerebral hemispheres with sera Nos. 1276 and 10, extracts from the caudate nucleus investigated with serum No. 1270, and extracts from the gray matter of the frontal lobe in experiments with GP serum. The maximal (as a percentage of the control) lowering of activity ($P < 0.05$-0.01) was found with extracts of the cerebellum, compared with extracts from the white matter (sera Nos. 1484-1416, 1471,

*A commercial 0.25% trypsin solution prepared by the Moscow Research Institute of Virus Preparations ans also dried trypsin (Difco) diluted to the same concentration were used.

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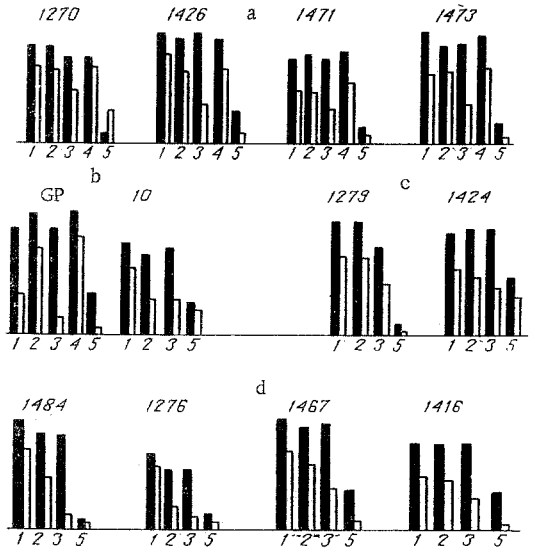

Fig. 1. Action of trypsin on organ-specific antigens of saline extracts of the human brain (from results of the CFR). The columns denote the intensity of the reaction in conventional units: black—with untrypsinized, unshaded—with trypsinized saline extracts. The numbers above the columns are the serial Nos. of the sera, the numbers below the columns denote the reaction with extracts of the white matter (1), caudate nucleus (2), cerebellum (3) and gray matter of the frontal lobe (4), and liver (5); a) antiserum to gray matter of the frontal lobe; b) antiserum to tissues of the caudate nucleus; c) antiserum to tissues of the cerebellum; d) antiserum to the white matter of the cerebral hemispheres.

1473, 1424), the caudate nucleus (Nos. 1484, 1467, 1416, 1473-1426), and the gray matter of the frontal lobe (results with sera Nos. 1270-1426 and GP). With some sera the depression of activity of extracts from the white matter was greater than with extracts from the caudate nucleus (GP) and frontal lobe (1473 and GP). Hence, by the use of trypsinized brain extracts, in some sera antibodies with relative specificity toward individual brain tissue structures could be detected: to the white matter (in sera Nos. 1484 and 1276) and the gray matter of the frontal lobe and of the caudate nucleus (GP).

In the experiments with the PHR, and also with inhibition of the PHR, it was found that the brain extracts after treatment with trypsin had completely lost their ability to react in the test.

Saline brain extracts, treated with trypsin, thus partly lose their organ-specific antigenic properties as revealed by the CFR. At the same time, specific brain antigens relatively resistant to enzymic proteolysis could be detected by this method. These antigens were not lipid substances, for they were detected by means of immune sera heated at 63° for 30 min, and as a result of this treatment they had lost their complement-fixing activity relative to extracts (in alcohol and ether) of the brain lipids.

The organ-specific brain antigens detected by the PHR were not resistant to enzymic proteolysis. This may be the reason for the relatively low immunogenic activity of the brain precipitinogens, as indicated by results showing the absence of precipitating brain antibodies (although complement-fixing antibodies were present) in the sera of patients with neuropsychiatric diseases [3], and also in some brain antisera.

Trypsin differs in its inactivating action on organ-specific antigens in saline extracts of different brain structures, and this may be used for the detection of complement-fixing antibodies with relative specificity to certain morphological structures of the brain.

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