A STUDY OF THE ANTIGENIC PROPERTIES OF THE BRAIN BY BOYDEN'S PASSIVE HEMAGGLUTINATION REACTION

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It has been shown by means of complement-fixing reactions that the brain tissues of different species of animals possess considerable antigenic similarity [2, 6, 8, 9, 11, 12, 14, 16]. Yet at the same time, the existence of species-specific and organ-specific brain antigens cannot be ruled out [2, 16].

The object of the present investigation was to study the relationship between species-specific and organ-specific antigens in brain tissue by Boyden's passive hemagglutination reaction [7].

EXPERIMENTAL METHOD

Experiments were carried out on the serum of rabbits immunized with human, rat's, and cow's brain tissue (six intraperitoneal injections of a 10% suspension of brain in physiological saline), and also of rabbits treated with encephalitis antisera obtained from horses following immunization with a virus-containing suspension of mouse brain [2]. The passive hemagglutination reaction (PHR) was performed with rabbit's erythrocytes, treated with tan-

TABLE 1. Results of PHR during Testing of Immune Brain Antisera with Antigens from Brain Tissue of Various Species of Animals

Serum No.	Brain tissue used for obtaining serum	No. of corresponding dilutions of serum with which the results of the PHR was positive							
		Antigens from tissues of							
		Man		Rats		Cow		Rabbit	
		brain	liver	brain	liver	brain	liver	brain	liver
8*	Human brain tissue	6	0	0	0				
1424*	The same	4	0	0	0				
1473**	"	4	0	1	0				
11	"	5	0	0	0				
69†	Mouse brain tissue	1	1	6	1	2	1	0	0
82†	The same	2	1	6	0	2	2		
88†	"	3	1	6	1	1	0		
96†	Mouse brain tissue	4	0	6	5				
135†	The same	2	0	5	0				
447†	"	4	0	6	5				
6‡	Cow's brain tissue	0		1	3	6	0		
1440‡	Rat's brain tissue	4	0	6	0			5	0
1418‡	The same	5	0	6	0				
1294‡	"	3	0	6	0			4	0
1420‡	"	2	0	5	0			1	0

* Inhibited by extracts of human liver.

† Absorbed by rabbit erythrocytes and inhibited by extracts of rat's liver.

‡ Inhibited by extracts of rat's liver.

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TABLE 2. Investigation of Species-Specific Properties of Organ-Specific Antigens of the Brain by Inhibition of the PHR

Sample of serum No. 1440	Inhibition by brain extract	Results of PHR (No. of dilutions 1:20-1:160 of serum with positive reaction)			
		Extracts from tissues of			
		rat		man	
		brain	liver	brain	liver
1	Control (uninhibited serum)	4	0	4	0
2	Extract of rat's brain	0	0	0	0
3	Extract of human brain	3	0	0	0
4	Extract of guinea pig's brain	4	0	0	0
5	Extract of rabbit's brain	4	0	0	0

TABLE 3. Investigation of the Relationship between Species-Specific Antigen, Specific and Nonspecific for the Brain

Sample of serum No. 1440*	Inhibition by extracts of rat's brain or liver and by rat's serum	Results of PHR (No. of dilutions 1:20-1:640 of serum with positive reaction)			
		Test antigens			
		rat's brain	rat's liver	rat's serum	human brain
1	Control (uninhibited serum)	6	6	6	0
2	Extract of brain	0	2	6	0
3	Extract of liver	4	1	6	0
4	Serum	4	4	0	0
5	Extract of liver and serum	4	0	0	0

* Rabbit serum against rat's brain.

nin in a dilution of 1:20,000 for 30 min at room temperature or for 15 min at 37°, followed by a single washing with buffered physiological saline. Otherwise, the conditions of the reaction were identical with those described earlier [3]. In the experiment to investigate the inhibitory properties of the tissue extracts, these were combined with an equal volume of the test serum in different dilutions, and after 15-30 min the antigen-treated erythrocytes were added. The reaction was assessed in accordance with criteria of the intensity of agglutination [15]. A reaction assessed as + or higher was counted positive.

EXPERIMENTAL RESULTS

The results of the investigation of the immune sera with erythrocytes sensitized by extracts of the brain and liver (control of the specificity of the reaction with the brain) of several species of animals are given in Table 1. As index of the intensity of the reaction, the number of corresponding dilutions of serum, starting with 1:10-1:20, with a positive result of the PHR is given. It is clear from Table 1 that some sera reacted mainly, or even selectively, with extracts of the brain from which they were obtained, or of the brain of a closely related species of animal; in other sera (Nos. 96, 447, 1440-1294) antibodies were found against the brain tissue of several species of animals, including the species producing the serum (Nos. 1440 and 1294). These results show that species differences can be detected by means of the PHR in the organ-specific antigen of brain tissue, and specific antigens similar for the brain of different species of animals can be detected.

Further evidence of the existence of inter-species differences in the organ-specific brain antigen was obtained in experiments of inhibition of the PHR. Immune sera, inhibited by extracts of the liver (1 mg protein/ml) were combined in various dilutions with extracts of the brain of animals of different species (0.1 mg protein/ml extract) [13]. It may seem from Table 2, where the results of one such experiment are shown, that only the extract from the

rat's brain, against which the serum was obtained, completely inhibited the PHR with all the brain extracts, in contrast to the extracts from the brain of the other species.

The relationship between the various species-specific antigens and the organ-specific antigens and the antigens of other tissues of the particular species in the brain tissue is an interesting question. It has been shown that species-specific properties of the tissues are determined by the presence of serum proteins and of proteins not identical with those in the serum [1, 4, 5, 10].

To study the content of species-specific antigens in the brain tissue, brain antisera of a rat, after preliminary inhibition with extract of human brain (thereby blocking the antibodies against the inter-species organ-specific antigens), were combined in different dilutions with extracts of the brain and liver and also with the serum of a rat, containing the same quantity of protein (0.5 or 1.0 mg/ml).

It is clear from Table 3, giving the result of one experiment of this series, that the activity of a brain antiserum inhibited in this manner fell only in relation to the corresponding test antigen; a mixture of the serum and extract of the liver blocked the antibodies against the organ-nonspecific species-specific antigen but not against the species-specific brain antigen. These results demonstrate that the species-specific antigens which were compared were not identical.

Hence, it may be concluded from this study of the antigenic properties of the brain by means of the PHR that certain organ-specific antigens of brain exhibit species differentiation, and the species-specific properties of the brain are determined also by antigens nonspecific for this tissue, not identical with the antigens of the serum protein.

SUMMARY

Investigation of immune anticerebral sera by the method of passive hemagglutination after Boyden has shown the presence in brain tissues of species-specific organ-specific antigens and species-specific antigens nonspecific for the brain, but differing from antigens of serum proteins.

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