A STUDY OF THE ORGAN-SPECIFIC ANTIGENIC PROPERTIES OF THE HUMAN BRAIN WITH THE AID OF SERA CONTAINING ISOIMMUNE ANTIBODIES AGAINST THE BRAIN

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In experimental damage of nerve tissue and in different cases of psychic illness, it is possible to demonstrate isoantibodies against the specific brain antigen [8, 10-14, 16, 17, 18, etc.]. This has been confirmed in our earlier investigations [5,6]. As specific antigen we had used extracts of different areas of the cerebral cortex, but did not test the activity of sera against antigens from others parts of the brain. However, there is some information in the literature that antibodies, which appear in certain brain diseases, react differently with antigens from different parts of the brain [11].

We considered it of interest to study the activity of sera against antibodies from different parts of the brain, as well as against those from brains of other species of animals. This investigation was expected to show the antigenic properties of morphologically different brain structures and to demonstrate the antigenic peculiarities of brains of animals of different species.

METHODS

Blood was taken from a vein on an empty stomach. Sera were inactivated at 56°C for 30 min. In preliminary experiments sera were adsorbed on sheep erythrocytes in order to remove heterophilic Forssman's antibodies. Later adsorption was not made, because we did not note any relationship between the presence or absence of antibrain antibodies and the presence of heterophilic antibodies.

Antigens, as saline extracts, (methods of preparation and standardization were described earlier [6]), were obtained from different areas of the brain and liver of man, the same individual (street accident victims or cases of myocardial infraction), as well as the brain and liver of rats.

We did not attempt to have the individuals from whom antigens and sera were obtained matched as to blood groups, because we have established that the finding of antibodies against the brain was not related to the group antigenic differences. To demonstrate the latter, as it is known, other methods are necessary [2].

Sera were tested for the presence of antibrain antibodies by slow complement fixation reaction in the cold. The dose of the complement constituted 150% of its titer, determined by warm complement-fixation reaction.

RESULTS

The results obtained with 16 samples of sera from different persons are presented in this communication. These sera were selected, as presenting the greatest interest for the study of antigenic properties of the brain. The persons from whom the sera were obtained suffered from varying degrees of pathology of the nervous system, but we consider it unimportant to dwell on their clinical characterization.

Table 1 shows the results of experiments with these sera, as separate protocols of repeated runs. This table shows only the results of tests of the sera with brain antigens; data on tests with control antigens from the liver, which gave negative results, are not included.

As seen in Table 1, sera showed significant differences in their activity when they were tested against antigens from the frontal lobe, thalamus opticus, cerebellum and cerebral white matter of the human brain, as well as against rat brain.

TABLE 1. Reaction of Isoimmune Antibrain Sera with Brain Antigens

Serum sample number	Antigens from tissues of																			
	frontal lobe				thalamus opticus				cerebellum				white matter				rat brain			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
1	++++	++++	+++	++(+)	++++	++++	+++	++	++++	+++	+++	++	++++	++++	--(+)	+(+)	+++	++++	++	+(+)
2	++++	++(+)	+	−	++++	++++	++	±	++	-(+)	−	−	++(+)	++	+	±	++++	+++	4	−
3	++(+)	++	+(+)	±	+(+)	++	+++	±	+(+)	++	±	−	++	-+	+(+)	+(+)	++++	++++	++	+(+)
4	++	+++	+(+)	±	++	++	+++	±	+(+)	+	+	±	++	++	++	+	++	++	±	±
5	++++	+++	++	−	+++++	++++	++	−	++				++++	++++	++(+)	−	+++	++++	++	±
6	++++	++	+	−	+++	+++	+	−									++(+)	+(+)	+	−
7	−	±	−	−	−	+	±	−	−	−	−	−	++	+	−	−	++++	++(+)	++	±
8	+	±	±	−	++	+(+)	+	−	++	+	+	±	+(+)	+	±	−	++(+)	++	+(+)	−
9	+	+	±	−	±	+(+)	±	−	±	−	−	−	−	−	±	−	++(+)	+(+)	+(+)	±
10	+	+	±	−	±	±	±	−	−	−	−	−	−	−	±	−	++++	++	++	−
11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++(+)	++	+(+)	+
12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++++	+++	±	
13	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++++	++++	+(+)	+
14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++	++(+)	++	+(+)
15	+++	++(+)	++	±	++(+)	+(+)	+(+)	±	−	−	−	−	−	−	−	−	++++	+++	±	−
	40	80	160	320	40	80	160	320	40	80	160	320	40	80	160	320	40	80	160	320
16	++++	++++	++++	++++	++(+)	++	+	+	++++	++++	++++	++++	−	−	−	−	−	−	−	−

Legend: ++++, +++(+), +++, ++(+), ++, +(+), + positive reaction; ± doubtful reaction; − negative reaction; figures denote serum dilutions.

TABLE 2. Results of Experiments with an Isoimmune Serum, Containing Antibodies Against Organospecific Antigens, Common to All Parts of the Brain (Patient P-v)

Date of bleeding	Antigen series	Organs and tissues used in the preparation of antigens	Serum dilution 1:10	1:20	1:40	1:80
Nov. 28, 1960	I	Frontal lobe	++++	++++	+++	++(+)
		Thalamus opticus	++++	++++	+++	++
		Caudate lobe	++++	+++	++	+
		Cerebellum	++++	+++	+++	++
		Midbrain	++++	+++(+)	++	+
		Cerebral white matter	++++	++++	++(+)	+(+)
		Rat brain	++++	++++	++	+(+)
		Human liver	–	–	–	–
		Rat liver	–	–	–	–
Dec. 19, 1960	II	Frontal lobe	++++	++++	++	+
		Occipital lobe	++++	++++	++	+(+)
		Thalamus opticus	++++	++++	+++	+
		Caudate lobe	++++	++++	+++	+
		Cerebellum	++++	++++	++(+)	+
		Midbrain	++++	+++	++	+
		Cerebral white matter	++++	++++	+++	++
Jan. 20, 1961	III	Frontal lobe	++++	++++	+++	++
		Occipital lobe	++++	++++	++(+)	±
		Caudate lobe	++++	++++	++++	++
		Cerebellum	++++	++++	++	–
		Cerebral white matter	++++	++++	+++	±
		Brain stem (white and grey matter)	++++	++++	+++	±
		Corpora quadrigemina	++++	++++	++(+)	±
		Corpus callosum	++++	++++	++++	++
		Rat brain	++++	++++	++(+)	±

Legend: As for Table 1.

Thus, sera of 6 persons (samples 1-6) gave sharp reactions with all the antigens tested. No selective activity of sera toward any specific antigen was noted.

Of special interest are the results obtained with the serum of one of the patients (sample 1, Table 1). The serum of this patient was tested many times during the 3 months that he had been hospitalized. Table 2 shows the separate protocols of experiments with this serum with antigens obtained from organs of several individuals, denoted as series I, II, and III of antigens. As seen in the table, the serum of this patient reacted identically with antigens from different parts of the brain: frontal lobe, occipital lobe, thalamus opticus, caudate lobe, cerebellum, cerebral white matter, midbrain (used whole), and antigens from the rat brain.

Thus, with this serum it was impossible to determine significant differences in the activity of the antigens tested; this indicates the presence within the tissues of different parts of the human brain and of animal (rat)brain, of common organospecific antigens.

It may be supposed that at a certain stage of brain damage in certain diseases, there arise antibodies against products of destruction which contain antigens common to all brain tissues of man and of other species of animals.

Different results were obtained with sera from 8 other individuals (Table 1, samples 7-14). These sera were characterized by a lack of reaction against human brain antigens, or by a weak reaction, while these sera reacted well against antigens from the rat brain.

What are the reasons for the appearance of antibodies against brain tissues of other species of animals? The answer to this question can be only hypothetical. It may be supposed that at a certain stage of disease due to damage

TABLE 3. Results of Experiments with an Isoimmune Serum, Showing Differences in the Antigenic Structure of Brain (Patient R-n)

Date of bleeding	Antigen series	Organs and tissues used in the preparation of antigens	Serum dilution			
			1:40	1:80	1:160	1:320
Nov. 8, 1960	I	Frontal lobe	++++	++++	++++	++
		Thalamus opticus	+	+++	+(+)	–
		Caudate lobe	–	++	±	–
		Cerebellum	++++	++++	++++	+
		Cerebral white matter	–	–	–	–
		Rat brain	+++(+)	++(+)	++(+)	+(+)
		Human liver	–	–	–	–
		Rat liver	–	–	–	–
Nov. 8, 1960	II	Frontal lobe	++++	++++	++++	++++
		Occipital lobe	++++	++++	++++	++++
		Corpus callosum	++++	++++	++++	++++
			1:10	1:20	1:40	1:80
Dec. 20, 1960	II	Frontal lobe	++	++(+)	++++	++++
		Occipital lobe	++++	++++	++++	++++
		Thalamus opticus	+	+	+(+)	+
		Caudate lobe	–	+	+(+)	+
		Cerebellum	++	+++	++++	++++
		Rat liver	–	–	–	–
			1:80	1:150	1:320	1:640
Jan. 18, 1961	III	Thalamus opticus	–	–	–	–
		Caudate lobe	–	–	–	–
		Cerebral white matter	–	–	–	–
		Corpus callosum	++++	++++	++++	++++
		Cerebral white matter	–	–	–	–
			1:40	1:80	1:160	1:320
Feb. 15, 1961	IV	Frontal lobe	++++	++++	++++	++++
		Thalamus opticus	++(+)	++	+	+
		Caudate lobe	–	–	–	–
		Cerebellum	++++	++++	++++	++++
		Cerebral white matter	–	–	–	–
		Rat brain	–	–	–	–
		Human liver	–	–	–	–
		Rat liver	–	–	–	–

Legend: As for Table 1.

of certain brain structures, antibodies are produced against the most "foreign" antigenic brain components; consequently, these antibodies are best revealed when the sera are tested against heterologous brains, in which such "foreign" components are, apparently, represented more fully.

This fact may be also explained in another way. Possibly, the antibodies which are formed as a result of brain tissue destruction are blocked by antigenic components which are formed as a result of a continuing destructive process and, so, cannot be demonstrated in reactions with homologous antigens, while with inadequate, but with related antigens, such as the brain of a heterologous species of animal, they can be clearly demonstrated.

Of course, only a correlation with clinical observations can answer the question at what stage of the disease process, and with damage to which brain structures, do antibodies, mainly directed against a heterologous brain, appear.

From the immunological point of view this fact is of interest also, because it confirms data on the existence of interspecific similarities and differences of organospecific brain antigens [4].

In addition to the above two types of sera, we found sera which selectively reacted with antigens from certain parts of the brain, and either did not react, or reacted weakly with antigens from other parts of the brain.

As seen in Table 1, the serum of one patient (sample 15) fixed the complement with antigens from the frontal lobe, thalamus and the rat brain, but did not react with antigens from the human cerebellum and cerebral white matter. Of special interest are the findings obtained with the serum of another patient. As seen in Table 1, this serum (sample 16) fixed the complement at high dilutions (1 : 320 ++++) with antigens from the frontal lobe and the cerebellum, but reacted much more weakly with antigens from the thalamus. Negative results were obtained with antigens from the cerebral white matter and from the rat brain.

We were able to study the blood of this patient during 3 months. The results obtained are presented in Table 3.

As seen in Table 3, this serum was tested against antigens from different parts of the brain of several individuals. In spite of this, the basic reaction type was constant, characterized by the fact that the serum in high titers reacted with antigens from the frontal and the occipital lobes, cerebellum and corpus callosum, while with antigens from the thalamus and the caudate lobe the reaction was much weaker. Also, there was a definite zone of retardation of the reaction in low titers, a phenomenon which was discovered with other sera as well (Table 1). The testing of the serum of this patient against antigens from the white matter of the cerebrum and the white matter of the brain stem gave negative results in the complement fixation reaction. Serum of Nov. 8, 1960 reacted sufficiently well with antigens from the rat brain, although weaker than with antigens from the human frontal lobe and cerebellum. A subsequent sample taken 3 months later gave a negative reaction with this antigen.

Thus, our experiments have shown that with the aid of isoimmune sera it is possible to reveal antigenic differences between different morphological structures of the human brain, as well as to demonstrate the existence of intraspecific differentiation of the organospecific brain antigens.

These data furnish still another proof for the previously established fact of the presence of specific antigenic substances in morphologically differentiated brain structures [1, 3, 7, 9, 15, 19, etc.].

Further investigations of isoimmune sera apparently will have more than only a theoretical significance; it is to be hoped that they will produce results which could help the clinical evaluation of the stage and depth of brain damage by disease processes.

SUMMARY

Sera of the 16 patients suffering from neuropsychic afflictions containing isoantibodies against the brain were used for studying the antigenic properties of the brain. Investigations were carried out by the method of prolonged complement fixation in cold conditions with water-salt extract of the human and rat brain. Some sera (6) reacted with the same intensity with antigens from various portions of the human brain: frontal lobe, optic thalamus, cerebellum, white matter of the hemispheres, as well as with the antigens from the rate brain. Sera of 8 other persons reacted mainly with the rat brain. Sera of two persons exhibited a selective activity with respect to some portions of the human brain with the absence of any reaction with other antigens. It is suggested that in affection of the brain by a morbid process there appear antibodies to various antigenic components at definite stages of the disease. Brain tissues contain organ-specific antigens, common to all of its portions as well as antigens mainly represented in the individual nerve tissue formations. Antigenic similarities and differences between the human brain and that of the heterologous species were established.

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