ON THE ANTIGENIC PROPERTIES OF DIFFERENT PARTS OF THE CENTRAL NERVOUS SYSTEM

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Research on the antigenic properties of nervous tissue was initially carried out in a plan of investigations on cytotoxins. The works of I. I. Mechnikov [4], Pirone [6], V. K. Khoroshko [7], and S. Mikhailova were mainly devoted to a study of the organ- and species-specificity of brain antigens and the clarification of problems of specificity of the anti-brain sera obtained. Delesenn [9] was the first to study the antigenic structure of the nervous tissue itself. By immunizing ducks separately with cerebrum and spinal cord, cerebellum and medulla oblongata of a dog, he obtained corresponding immune sera, but could not detect any difference between them in their action on dogs. Later, Schmidt [8] succeeded in obtaining specific serum against the sciatic nerve of frogs.

Witebsky and Bherens [11] differentiated serologically the fore and hind lobes of the hypophysis and recorded some difference between the antigens of the cerebrum and spinal cord.

Reichner and Witebsky [10] gave some information on the difference between the antigens of white and grey matter. Yet they did not succeed in differentiating the antigens of the cerebrum, cerebellum, medulla oblongata and spinal cord.

A. F. Makarchenko [3] could not distinguish serologically the antigens of white and grey matter of the cerebrum, subcortical nodes and sympathetic ganglia of the dog.

Thus, information on the antigenic structure of the central nervous system is sparse and contradictory. In the meantime, a precise knowledge of the antigenic structure of nervous tissue is necessary in the immunological study of tumors of the central nervous system, particularly in the investigation of specific antigens of brain tumors by the method of anaphylaxis with desensitization. In this method guinea pigs sensitized with tumor material are injected with normal tissue antigens in order to desensitize them towards tissue proteins. Since the tumors under investigation may be derived from different parts of the brain matter, it is very important to ensure that the normal brain tissue used for desensitization is adequate, fully corresponding in antigenic composition to the tumor under investigation.

The aim of this work was to reveal the nature of the antigenic structure of different parts of the nervous tissue in man.

METHODS OF INVESTIGATION

For this work we used the method of anaphylaxis with desensitization, introduced by L. A. Zil'ber [1] for the detection of specific tumor antigens and which has been effectively used for the immunological study of tissues and organs [2, 12]. We examined protein fractions of different parts of the cerebrum, cerebellum and spinal cord. The specimens were prepared from the brains of persons who had died in street accidents or from other chance causes.

The particular parts of the brain were purified from membrane, washed with physiological saline, treated

with antibiotics (penicillin and streptomycin) for several hours, washed again with sterile physiological saline, and minced; the proteins were extracted with an alkaline medium and then precipitated with acid by the method used in the laboratory of L. A. Zil'ber. The protein preparations obtained were tested for sterility (inoculation in bouillon and on agar) and toxicity (intravenous injection into guinea pigs). For the experiments we used only sterile and non-toxic preparations. We determined the protein content in 1 ml in each preparation by the micro-Kjeldahl method.

The tested protein antigens were studied in cross reactions of anaphylaxis with desensitization. We investigated protein fractions prepared from whole cerebrum, cerebellum and spinal cord, as well as from cortical grey matter of the cerebral hemispheres, white matter from the semi-oval center of the cerebral hemispheres, cortical grey matter from the frontal, temporal and occipital lobes, and from the pia mater.

The essence of the experiments was the following. Guinea pigs were sensitized with a subcutaneous injection of antigen isolated from any one of the two portions of nervous tissue being compared. Desensitization was begun after 30-40 days. The second of the antigens being compared was injected intravenously into the guinea pigs, and the result was invariably anaphylactic shock. After 2 hours the animals again received the same antigen to test for the completeness of desensitization. If this again produced signs of shock, the injection was repeated. When a state of complete desensitization to the injected preparation ensued as a result of the repeated injections, a shocking dose of the first antigen was injected. The absence of response in the guinea pigs indicated that the state of increased sensitivity to the first antigen had been completely destroyed by the introduction of the second. If the same result was obtained with the reverse arrangement of the experiment, it meant that the antigens compared were identical. A positive response by the guinea pigs to the shocking dose indicated that the compared antigens differed from one another. Antigens used in the same experiment were, as a rule, prepared from tissue taken from the same person. The dosage of the preparations was estimated from the number of milligrams of protein contained in 1 ml. The shocking dose of protein was always slightly smaller or equal to that used in the last desensitization.

24 experiments of this kind were conducted on 101 guinea pigs.

In the first group of experiments (9) we compared tissue antigens from whole cerebrum, cerebellum and spinal cord. The results are given in Table 1.

It was found that sensitization of guinea pigs with antigen from the cerebrum produced a state of increased sensitivity which could not be removed completely by the introduction of the cerebellum preparation. Of 13 guinea pigs (in 3 experiments) sensitized with a cerebrum preparation, 11 responded with pronounced anaphylactic shock to a shocking dose of the same antigen, after they had been completely desensitized to the cerebellum antigen. The reverse arrangement of the experiment gave different results, namely: desensitization with a cerebrum preparation of guinea pigs sensitized with cerebellum antigen destroyed the increased sensitivity to the latter in 8 out of 12 guinea pigs. These results may be interpreted to mean that the protein fractions of cerebrum and cerebellum are non-equivalent in antigenic relation. Apparently the cerebrum contains protein components which are absent from the cerebellum. In other words, the antigenic structure of the cerebrum would seem to be richer than that of cerebellum.

In cross experiments with antigens of cerebellum and spinal cord we were unable to detect any antigenic differences between them.

A shocking dose of cerebellum antigen to guinea pigs sensitized to it did not produce anaphylactic shock in them after desensitization with antigen from the spinal cord, and vice versa. Hence, the protein fractions of cerebellum and spinal cord show no antigenic differences.

In the next group of experiments (15) we compared different parts of the cerebrum — cortical grey matter of the frontal, temporal and occipital lobes, cortical grey matter of the cerebral hemispheres and white matter of the semi-oval center, grey matter of cortex and subcortex. In one experiment we compared protein fractions of pia mater and brain matter (Table 2).

As Table 2 shows, this group of experiments clearly revealed the difference between the grey and white matter of the cerebral hemispheres. Desensitization with grey matter antigen of guinea pigs sensitized with protein fractions of white matter did not remove the state of increased sensitivity to the latter. The reverse held true. Of 22 guinea pigs sensitized with white matter antigen in 4 experiments, 21 showed anaphylactic shock following a shocking dose of the same preparation, after they had been completely desensitized the grey matter antigen.

TABLE 1

Summary of Experiments with Antigens of Cerebrum, Cerebellum and Spinal Cord

No. of expts.	Number of guinea pigs	Sensitization		Desensitization								Shocking dose			
		antigen	dose of protein in mg	first injection		reaction		last injection				antigen	dose of protein in mg	reaction	
				antigen	dose of protein in mg	from	to	antigen	dose of protein in mg	reaction from	to			from	to
19	5	Cerebrum	2.9	Cerebellum	2.5	++	++++	Cerebellum	2.5	–	–	Cerebrum	2.6	+	++
16	4	Cerebrum	2.0	Cerebellum	1.5	–	+++	Cerebellum	3.0	–	–	Cerebrum	2.0	–	++
	4	Cerebrum	3.5	Cerebellum	1.5	+	+++	Cerebellum	2.4	–	–	Cerebrum	2.8	1 individual	+++
18	3	Cerebellum	2.3	Cerebrum	2.0	++	+++	Cerebrum	2.0	–	–	Cerebellum	2	1 individual	+1 individual
20	5	Cerebellum	2.8	Cerebrum	1.5	+	+	Cerebrum	3.3	–	–	Cerebellum	2.5	–	+ 1 individual
	4	Cerebellum	3.1	Cerebrum	1.8	+	++	Cerebrum	3.5	–	–	Cerebellum	3.0	–	± 2 individuals
12	6	Cerebellum	2.6	Spinal cord	1.25	+	++	Spinal cord	2.5	–	–	Cerebellum	2.1	–	+ 1 individual
1	4	Spinal cord	2.5	Cerebrum	2.5	++	++	Cerebrum	5.0	–	–	Spinal cord	4	–	–
2	5	Spinal cord	2.5	Cerebellum	2.25	+	++++	Cerebellum	5.5	–	–	Spinal cord	4	–	–

Symbols: ++++; +++; ++; +; ± different degrees of anaphylactic shock; – absence of reaction.

TABLE 2

Summary of Experiments with Antigens of Different Parts of the Cerebrum

No. of expts.	Number of guinea pigs	Sensitization		Desensitization								Shocking dose			
				first injection				last injection						reaction	
		antigen	protein dose in mg	antigen	protein dose in mg	reaction from	reaction to	antigen	protein dose in mg	reaction from	reaction to	antigen	protein dose in mg	from	to
7	5	White matter	3.12	Grey matter	0.56	++	++++	Grey matter	4.5	–	–	White matter	2.2	++	+++
13-14	8	White matter	2.1	Grey matter	1.1	+	++	Grey matter	2.2	–	–	The same	2.0	(1 individual)	+++
21	5	White matter	2.45	Grey matter	2.5	±	++	Grey matter	2.5	–	–	The same	2.45	++	+++
24	4	White matter	2.3	Grey matter	2.0	+	+++	Grey matter	3.0	–	–	The same	3.0	+	+++
13	3	Grey matter	2.6	White matter	1.25	+	+++	White matter	2.5	–	–	Grey matter	2.1	+	++
8	4	Grey matter	2.6	White matter	1.8	+	++++	White matter	3.65	–	–	The same	3.5	(1 individual)	++
10-11	6	Grey matter	–	White matter	1.0	+	++	White matter	4.0	–	–	The same	2.2	(2 individual)	+
22	5	Grey matter	3.0	White matter	2.45	–	+	White matter	2.45	–	–	The same	2.5	(1 individual)	++
23	4	Grey matter	3.0	White matter	2.15	±	+	White matter	4.3	–	–	The same	3.0	+	++
3	4	Grey matter of frontal lobe	2.6	Grey matter of occipital lobe	1.75	+	+++	Grey matter of occipital lobe	3.5	–	–	Grey matter of frontal lobe	3.0	–	–
7	4	The same	2.6	Grey matter of temporal lobe	2.5	+	+++	Grey matter of temporal lobe	5.0	–	–	The same	4.4	–	–
12	4	Grey matter of cortex	2.25	Pallidum	0.75	+	++	Pallidum	2.5	–	–	Grey matter of cortex	2.0	–	–
17	3	Pia mater	2.6	Cerebrum	1.5	–	±	Cerebrum	3.0	–	–	Pia mater	2.2	++	++++

Symbols: ++++; +++; ++; +; ± different degrees of anaphylactic shock; – absence of reaction.

In the reverse arrangement (5 experiments) 18 guinea pigs out of 22 gave a positive reaction.

It should be noted that the degree of shock in the guinea pigs was considerably different even within the same experiment, varying in individual cases from complete absence to fatal results, which is probably due to individual differences in the reactive powers of the organisms. This circumstance may also explain the negative reactions in 5 out of the 44 ginea pigs used in these experiments. The difference between the antigenic properties of protein fractions of white and grey matter, discovered in these experiments, is in good correspondence with the results of biochemical investigations, which have revealed the different protein content of grey and white matter.

A comparison of protein fractions obtained from grey matter of different lobes of the brain did not reveal any differences between the frontal lobe, on the one hand, and the temporal and occipital lobes, on the other. The injection of temporal lobe antigen into guinea pigs sensitized with a frontal lobe preparation completely removed the state of anaphylaxis to the latter. The same result was obtained in a comparison of the frontal and occipital lobes.

The protein fractions of cortical and subcortical grey matter showed no differences. Guinea pigs sensitized with cortical antigen of the cerebral hemispheres did not react to a shocking dose of the same preparation when they had been previously desensitized with antigen from the subcortex (pallidum).

The pia mater and brain matter, on the other hand, differ markedly in the antigenic properties of their protein fractions. Guinea pigs sensitized with piamatral antigen and desensitized with brain matter preserved increased sensitivity to the first. Cross reactions were not tested in the last three cases.

Summing up the results of this work, we may say that in the experiments described we have succeeded in revealing the difference between the antigenic properties of protein fractions of nervous tissue of different origin. We have found that cerebral tissue possesses a greater set of antigens than cerebellar tissue. Grey and white matter of the cerebrum contain common antigens, as well as antigens peculiar to each. Cerebral tissue is very different in antigenic relation from pia mater. These features can be linked with the different histological structure, function and metabolism of these parts of the central nervous system. Metabolic processes of a different nature bring about changes in the chemical composition and structure of proteins which are the main carriers of the antigenic functions of cells. Research of the immunological peculiarities of cells or tissues, of course, must not be confined solely to the study of protein components; it is necessary to consider also the lipoids and polysaccharides, which also possess antigenic functions. Yet since our investigations here are only a preliminary stage in the study of specific antigens of tumors of the central nervous system, and the latter were first discovered in the protein fractions, it was convenient to begin the study of the problem with the protein antigens.

The inhomogeneity of the antigenic structure of nervous tissue, shown by our results, must be taken into account in the study of specific antigens of tumors of the central nervous system by the method of anaphylaxis with desensitization.

As already mentioned, in studying brain tumors of different localization, it is very important to choose for desensitization an adequate part of the normal brain tissue. Thus, considering the results of our experiments we must not, for the case of a tumor localized in the cerebrum for example, use for desensitization cerebellar tissue, since it is poorer in antigenic relation than cerebral. The opposite case — desensitization with cerebral tissue when the tumor is located in the cerebellum — would appear to be quite in order, since in the cerebellum there are no antigens which are absent from the cerebrum. In experiments with tumors of piamatral origin cerebral tissue should not be taken for desensitization. For intracerebral tumors localized in different lobes of the cerebrum, it is possible to use cerebral tissue of any lobe. Since all neuro-ectodermal tumors contain some elements of grey and white matter, either white or grey matter may be used for desensitization in these cases.

SUMMARY

Characteristic features of the antigenic structure of different areas of the nerve tissue were studied by the method of reaction of anaphylaxis with desensitization. It was established that grey and white matter of the brain have different antigens. There were no differences in the antigens found in various portions of the grey matter. The concentration of the antigen in the cerebral hemispheres is higher than in the cerebellum. There is a pronounced difference in the antigens of the brain and the pia mater.

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