NEUROSPECIFIC D ANTIGEN IN POSTNATAL DEVELOPMENT OF RAT BRAIN STRUCTURES

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Much attention is currently being paid to the study of the role of neurospecific proteins in function of the nervous system under normal and pathological conditions [9]. One such protein is the D antigen of bovine brain which, in some of its physicochemical and immunologic properties, resembles acid brain-specific protein 14-3-2 and α-antigen [4, 11], a particular feature of which is its nonhomogeneity on electrophoresis, where it moves as two immunochemically identical fractions — a fast migrating and a slowly migrating component (FMC and SMC, respectively). The functional role of this heterogeneity of D antigen has not yet been adequately studied.

One approach to the discovery of the role of nervous system proteins and, in particular, of neurospecific antigens is to study biochemical processes during the animal's ontogenetic development, when correlation between the formation of the specific functions of the neuron and their molecular basis is investigated.

Accordingly, the aim of the investigation described below was to analyze the dynamics of the content of the components of D antigen in certain parts of the brain which differ in their neuronal organization and morphogenesis in postnatal development.

EXPERIMENTAL METHOD

A monospecific antiserum obtained by hypoimmunization of rabbits with a pure preparation of D antigen from bovine brain was used for the immunochemical analysis. Isolation of the antigen and preparation of the monospecific antiserum against it were done as described previously [2, 4].

Male Wistar rats aged 7, 14, 21, and 30 days and about 1 year, weighing 350-400 g were used. The animals were decapitated and the visual cortex, right and left hippocampus, and both caudate nuclei were removed. Brain structures taken from one animal were homogenized in barbital buffer, pH 8.6, with ionic strength 0.035, in the ratio of 1:3 (w/v). The homogenate was centrifuged at 8000 rpm for 55 min and 20 μl of the supernatant was taken for immunoelectrophoresis.

The method of electrophoresis used was a modification of the crossed immunoelectrophoresis technique developed by Lourell [13]. Antigens were separated electrophoretically in 1% agarose gel made up in barbital buffer, pH 8.6, with ionic strength of 0.035, using a field voltage of 12 V/cm for 22 min at 12°C. The direction of the field was then changed through 90% and electrophoresis continued in the same gel, containing 28 μl/cm^2 of antiserum and in a potential gradient of 2 V/cm for 16 h at 12°C.

After immunoelectrophoresis, the plates containing gel were washed for 3 days in 0.15 M NaCl solution, dried under filter paper at 50°C, and stained for protein with Coomassie Brilliant Blue P-250 (from Terak, East Germany). The content of components of D antigen in the brain structures was estimated from the area of the precipitation peaks in the brain structures, which was directly proportional to the concentration of the corresponding protein in the test mixture [13]. The ratio of the areas of the precipitation peaks of FMC and SMC of D antigen

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TABLE 1. Content of Components of D Antigen in Rat Brain Structures Depending on Animals' Age (M ± m, n = 5)

Age of animals, days	SMC of antigen			SMC of antigen		
	visual cortex	hippocampus	caudate nucleus	visual cortex	hippocampus	caudate nucleus
7	3,1±0,5	5,5±0,4	5,4±0,5	1,33±0,05	2,06±0,06	2,21±0,07
14	7,1±1,1	9,8±1,4	10,2±1,6	2,34±0,27	4,06±0,42	5,01±0,50
21	27,9±3,4	24,8±1,5	23,5±2,1	14,2±1,4	15,4±1,1	16,5±1,5
30	35,2±1,5	33,5±1,4	32,2±1,6	23,5±2,2	27,6±1,4	27,7±2,0
300—350	35,1±3,6	40,2±2,1	38,4±1,6	40,7±3,0	47,2±0,9	42,3±1,8

TABLE 2. Ratio of Areas of Precipitates of FMC and SMC of D Antigen as a Parameter of the FMC/SMC in Brain Structures Depending on Rats' Age (M ± m, n = 5)

Age of animals, days	Visual cortex	Hippocampus	Caudate nucleus
7	0,33±0,03	0,34±0,02	0,33±0,02
14	0,30±0,02	0,37±0,01	0,42±0,03
21	0,48±0,02	0,60±0,03	0,67±0,02
30	0,61±0,04	0,69±0,03	0,77±0,05
300—350	1,05±0,01	0,93±0,03	0,95±0,03

was calculated as the ratio between their respective contents (FMC/SMC) in the brain structures. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Antiserum against D antigen, reacting in crossed immunoelectrophoresis with extracts of rat brain structures, revealed two precipitation peaks corresponding to FMC and SMC. No reaction was found with rat liver extracts or blood serum. The results of a study of the dynamics of the two components of the antigen in ontogeny are given in Table 1. They show that maturation of brain structures is accompanied by a regular increase in their content of components of D antigen.

In animals aged 7 and 14 days, the content of FMC of D antigen was lower in the visual cortex than in the hippocampus and caudate nucleus ($P < 0.05$) and was closer to the FMC level of these formations at the age of 21 days, of the biochemical maturation of the neocortex and other two brain structures. The content of components of D antigen increased most rapidly in these formations between the ages of 14 and 21 days. Particularly sharp changes were observed in the visual cortex. For instance, the FMC level increased sixfold during this period, the SMC level approximately fourfold, the difference evidently being attributable to the formation of visual function in the rats, accompanied by an increase in the concentrations of cytoplasmic RNA and proteins [7]. The data show that during the first month of postnatal ontogeny, the content of SMC of D antigen in the various brain structures was similar to that in animals 1 year old. The content of SMC of D antigen at the age of 30 days was considerably lower than in the brain structures of the year-old rats, in which its level was 150-170% of that found at the age of 1 month.

Dependence of the ratio between FMC/SMC levels of D antigen in brain structures on the animals' age is shown in Table 2. This ratio increased with the animals' age; in the hippocampus and visual cortex a significant increase occurred after 1 month of postnatal development. In the animals aged 1 year, the FMC/SMC ratio was higher in the visual cortex than in the other two structures ($P < 0.05$). The changes in the ratio between the corresponding components of neurospecific protein 14-3-2 in rat brain, observed by other workers [12], were similar in character.

The increase in content of components of D antigen found in these brain structures correlates with differentiation of neurons [10] and, in particular, with the formation of synaptic connections between them [1, 5, 6]. As was shown previously, a temporary connection exists

between the appearance of neurospecific proteins in nerve tissue culture and synaptogenesis [8]. It is also important to note that a study of the subcellular distribution of D antigen showed it to be predominantly localized in the synaptosomal fraction [3].

The results of the present investigation, together with data in the literature, thus indicate the important role of neurospecific D antigen in processes taking place in synapses. The dominant role in this phenomenon is perhaps played by FMC, the content of which is persistently higher than that of SMC of D antigen in the course of ontogeny.

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INDUCTION OF INTERFERON PRODUCTION BY DEXTRAN SULFATE

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It has recently been shown that dextran sulfate is a polyclonal stimulator of B lymphocytes [2]. Its ability to stimulate peripheral blood lymphocytes has been demonstrated [3]. The marked leukocyte-mobilizing activity of dextran sulfate is attributed to its possession of polyanionic properties. Certain polyanions are known to be able to induce interferon production in vertebrates.

The investigation described below was carried out to verify the interferon-inducing activity of dextran sulfate when administered by parenteral and enteral routes, and in the latter case its resistance to the action of enzymes of the gastrointestinal tract was noted. Certain other polyanionic substances also were investigated for the same purpose.

EXPERIMENTAL METHOD

Venezuelan equine encephalomyelitis virus strain 230 was used. The virus was subjected to passage through cell cultures. A continuous line of mouse cells L-929, obtained from the Tissue Culture Laboratory of the D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, was used.

Noninbred albino mice weighing 10-12 g were used to induce interferon. To study the time

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