IMMUNOCHEMICAL ANALYSIS OF GLIAL AND NEURONAL FRACTIONS OF RAT BRAIN

A. B. Poletaev, V. V. Dergachev,
V. N. Shelikhov, and T. S. Naumova

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A method of obtaining antineuronal and antiglial immune sera is described. The results of a quantitative immunochemical analysis of antigens of neuronal and glial fractions of rat brain are given. Neurons were shown to contain four, and glia three brain-specific proteins; in addition, one or two proteins are common to these cellular fractions.

KEY WORDS: *neurons*; *glia*; *brain-specific proteins*.

Despite advances in the study of the biochemical, electrophysiological, and morphological features distinguishing neurons (N) and glial cells (GC), the differential contribution of each cell type to brain activity has not yet been adequately studied [6].

The object of this investigation was to develop a method of obtaining antineuronal and antiglial immune sera (IS) and to use these sera for a comparative analysis of the properties of N and GC.

EXPERIMENTAL METHOD

Immune sera were prepared as follows. The brains of 50 rats killed by decapitation were placed in the original medium (1000 ml water, 150 g glucose, 10 g Ficoll, 8.5 g NaCl, 1.2 g KH_2PO_4, 0.2 g NaOH; pH 7.0) and broken up with a stiff brush in a mortar. The suspension was trypsinized (0.25% trypsin) for 45 min at 37°C. The trypsin was removed by centrifugation three times, at 1000g for 5 min each time, and the cell residue was suspended after each centrifugation in cold original medium. The suspension was then filtered twice under a low vacuum through steel gauze with a mesh of 140 and 70 μ.

Solutions of sucrose for use as density gradients were made up in the original medium, to contain in 100 ml of medium: a) 20 g, b) 23 g, c) 25 g, d) 18.5 g, and e) 23.5 g sucrose. The cell filtrate was layered above gradients a, b, and c and centrifuged for 20 min at 5500g. The resulting layers contained: I) an unpurified fraction of GC, II) a mixture of GC and N, III) an unpurified fraction of N, IV) a little N, fragments of blood vessels, blood cells. Layer I was collected, resuspended in medium, and recentrifuged for 30 min at 5500g on gradients d and e. The middle layer thus obtained contained the purified GC fraction. Layer III was recentrifuged for 30 min at 5500g on gradient e. The resulting cell residue contained a purified fraction of N bodies. The purity of both fractions, as phase-contrast microscopy showed, exceeded 95%. About 5% consisted of unidentified cell fragments.

The fractions of N and GC were homogenized and used to immunize 8 rabbits (four rabbits were immunized with each fraction). Two other rabbits received an injection of whole brain homogenate.

In the course of immunization the rabbits received subcutaneous injection of 2 ml homogenate with an equal volume of Freund's adjuvant once a week for one month. A month later the fifth injection was given, and a month later still the sixth injection, without adjuvant in both cases. Blood was collected after 7-10 days. The γ-globulin fraction was then salted out from IS, dialyzed, and used in the experiment.

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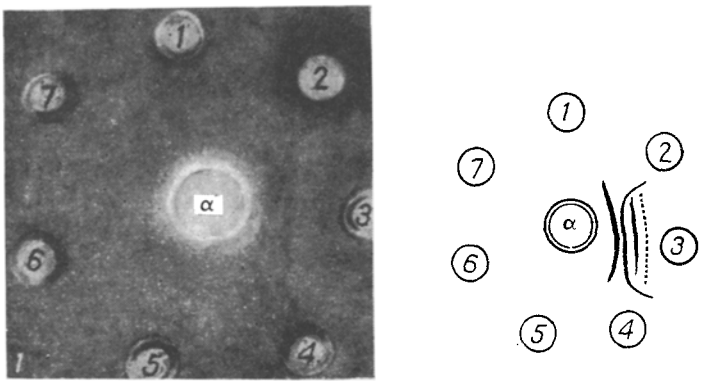

Fig. 1. Precipitation test with antineuronal γ-globulin and extract of neuronal fraction. Here and in Fig. 2: α) antineuronal γ-globulin; β) antiglial γ-globulin; 1) extract of glial fraction, 2) of liver, 3) of neuronal fraction, 4) of kidneys, 5) of intestine, 6) rat blood serum, 7) extract of spleen.

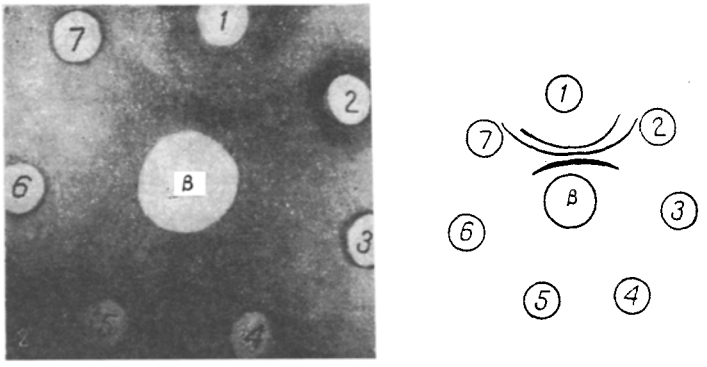

Fig. 2. Precipitation test of antiglial γ-globulin with extract of glial fraction (photograph and scheme).

EXPERIMENTAL RESULTS

Immune sera with titers of the order of 1:256-1:512 (before exhaustion) were obtained from rabbits immunized with homogenate of GC or whole brain, and with titers of not more than 1:128 from rabbits immunized with N homogenate. This suggests that GC have stronger antigenic properties than N. Immunological analysis of γ-globulin (γG), previously exhausted with empirically chosen doses of liver homogenate and serum of rats (using precipitation in agar as the control [2]), showed (Figs. 1 and 2) that antiglial γG gave four or five precipitation bands with extracts of GC and one or two bands with extracts of N. Antineuronal γG gave five or six precipitation bands with N extracts and one or two bands with GC extracts. The γG did not react with extracts of rat liver, kidney, intestine, or spleen or with rat serum.

By exhausting the antiglial and antineuronal γG with empirically chosen doses of homogenates of N or GC respectively, monospecific γG with respect to the particular cell types could be obtained, and with their aid three brain-specific proteins (BSP) were detected in the GC extract and four in the N extract. Both antiglial and antineuronal γG reacted practically equally with extracts of rat, rabbit, cat, duck, frog, and fish brain (in the precipitation test), but species-specific BSP were found only in rat brain with the aid of the antineuronal γG, in the form of two additional bands.

Brain-specific proteins were discovered more than 10 years ago [10], but as yet fewer than ten of them have been discovered [7]. This may seem paradoxical if the enormous

variety of brain proteins is taken into account (all cells of organs and tissues are known to contain several proteins specific for those cells alone [5]). These cell-specific proteins do not manifest themselves as BSP, i.e., as proteins with an organ level of specificity (contained in all or very many brain cells), because of their extremely small amounts [3, 4]. What are the possible functions of the BSP? Most BSP are known to be acid [1]. Some of them, at least at certain periods of life, are characterized by a nuclear localization [8]. In some cases they are directly connected with chromatin [9]. Evidently some BSP are those factors which, as has been suggested [9], may be directly connected with the regulation of the activity of the cell genome in the nervous system. Possibly this function of the BSP is not confined to the period of embryogenesis but plays an important role in the process of function of the mature brain, by mediating external environmental influences on the activity of the brain cell genome.

In this investigation 8 or 9 BSP were discovered, of which no fewer than 4 are neuronal and no fewer than 3 glial in origin. All the glial BSP discovered are species-nonspecific. Of the neuronal BSP about half are species-specific. The presence of different BSP in GC and N suggests a fundamental difference in the biochemical processes which determined the participation of these types of cells in specific brain activity.

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