METHODS

INVESTIGATION OF THE CONSTITUTIVE PROTEINS OF BRAIN STRUCTURES BY ELECTROPHORESIS IN A CONTINUOUS POLYACRYLAMIDE GEL GRADIENT IN CAPILLARY TUBES

M. A. Danilovskii

UDC 612.398.7:612.82

A comparative investigation was made of the protein composition of structures of the rat brain (premotor cortex, area CA3 of the hippocampus, caudate nucleus, frontal lobe of the cerebellum) by electrophoresis in a continuous gradient of polyacrylamide gel (PAG) pores in capillary tubes. This method was shown to have much greater resolving power than electrophoresis in a uniform gel concentration. The method did not reveal any differences in the protein composition of the brain structures studied.
KEY WORDS: *brain proteins; microelectrophoresis; polyacrylamide gel gradient.*

Investigations of brain structures by electrophoresis on paper, in agar, starch, and polyacrylamide gel (PAG) have not yet provided an unequivocal answer to the question of whether functionally different parts of the mammalian brain are heterogeneous in their protein composition [1, 7, 8, 12]. The reason for this contradictory state of affairs may be differences in the sensitivity and resolving power of the methods used. The method of choice in such investigations must therefore be taken to be the technique of microdisc-electrophoresis in capillary tubes, which has justified itself well in investigations of single cells, small samples of cells, and weighed microsamples of tissues [4, 6, 10]. By means of a technique of protein separation in homogeneous PAG in capillary tubes the writer could find no difference in the composition of functionally different structures of the rat brain [2]. Although the micromethod has increased detecting power [9], the number of protein zones obtained in a microinvestigation does not completely reflect the heterogeneity of the protein composition of nerve tissue as revealed by methods of two-dimensional fractionation of protein components [13].

The method of separation of biopolymers in a continuous pore gradient has been shown [14] to have much greater resolving power than ordinary electrophoresis.

In the investigation described below the protein composition of homogenates of functionally different brain structures was investigated in a continuous gradient of PAG pores (1-30%) on a microscale.

EXPERIMENTAL METHOD

Experiments were carried out on ten noninbred male rats weighing about 200 each. Weighed microsamples of brain tissue (0.5-1.0 μg) were taken from the premotor cortex, area Ca3 of the hippocampus, the right caudate nucleus, and the right frontal lobe of the cerebellum at 4°C. Homogenization was carried out by the method described above [2] with the addition of 15 μl/mg of buffer A (0.5% Triton X-100, 0.25 M sucrose, 0.15 mM soy bean trypsin inhibitor, in 0.06 M Tris-HCl, pH 7.4). To detect protein fractions belonging to nerve tissue and coinciding with the trypsin inhibitor marker protein on the chromatograms, in some cases homogenization was carried out in buffer B (buffer A without trypsin inhibitor). Centrifugation was carried out for 90 min at 15,000g. The protein concentration after centrifugation was determined in the supernatant by the method described in [11]. A continuous PAG gradient was created by a modified method [15]. Quartz capillary tubes with an internal diameter of 560, 574, and 590 μ and 50 mm long, treated with water-repellant, were fixed

Laboratory of Physiological Mechanisms of Memory Control, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bekhtereva.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 11, pp. 621-623, November, 1978. Original article submitted April 3, 1978.

 0007-4888/78/8611-1544$07.50

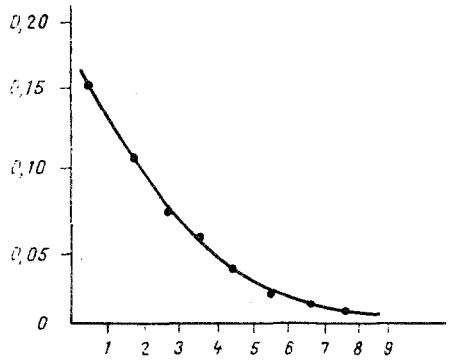

Fig. 1. Curve of distribution of polyacrylamide by weight in gradient gel. Abscissa, distance along gel (in mm); ordinate, mean weight of unit length of gel (in mg).

vertically in a slab on the stage of a Zeiss micromanipulator. Each capillary tube was filled with solution C (30% acrylamide solution [5], but instead of the solution for ammonium persulfate, the same volume of H_2O was added) to a height of 15 mm from the lower end and solution D (0.4 mg/ml ammonium persulfate in H_2O) was poured in a layer above it. Between 20 and 25 capillary tubes of the same diameter were filled simultaneously. The filling operations were carried out with a glass micropipet fixed in the holder of a micromanipulator and connected with a polyethylene adaptor to a microsyringe. The length of the micropipet was 60 mm and its external diameter about 60 μ; the micropipet was made on a Zeiss microforge. The filled capillary tubes were placed overnight in a humid chamber at 20°C. After polymerization, 10-mm segments were cut off each end of the capillary tube and the tube with the gel was placed in solution E (0.38 M Tris-HCl, pH 9.2) for 2 h at 37°C. The capillary tubes with the gel were kept in solution E at 4°C. The length of the gel thus obtained was 10-12 mm (the indeterminacy was due to the mechanically weak low-percentage part of the gel). The formation of a continuous PAG gradient was monitored by weighing (Fig. 1). The gel was cut manually into parts, each 1 mm long. Each part of the gel was placed successively in absolute ethanol and acetone for 1 h, after which it was dried at 50°C for 30 min. To increase the accuracy, corresponding parts of ten gels were pooled and weighed and the mean weight of each part calculated. Electrophoresis of the marker proteins [soy bean trypsin inhibitor (during electrophoresis of this protein in a gel gradient two protein zones were identified; according to B. I. Klement'ev's findings by means of the microgel-filtration method developed previously [3], their molecular weight was 7200 and 28,000 daltons), bovine serum albumin (molecular weight 67,000 daltons), dimer of bovine serum albumin (molecular weight 134,000 daltons), ferritin (molecular weight 450,000 daltons)] and of the solubilized protein of brain structures was carried out with stabilization of the voltage applied to the gel at 100 V for 40 min as described in [6]. Approximately 0.6 μg protein in a volume of 0.3 μl was applied to the gel. During electrophoresis of the marker proteins 0.01-0.4 μg of each marker protein in a volume of 0.1-0.4 μl was applied to the gel. In some cases combined electrophoresis of these quantities of marker proteins (trypsin inhibitor, bovine serum albumin) and of nerve tissue proteins was carried out.

After the end of electrophoresis the capillary tube with the gel was placed in a Petri dish with 7% acetic acid and the quartz capillary tube was accurately broken with forceps. Staining and densitometry of the protein zones were carried out by the method described previously [2]. The protein zones detected were identified relative to protein with a molecular weight of 28,000 daltons, a component of the marker protein trypsin inhibitor.

The probability of nonrandom appearance (PNRA) of protein zones on the chromatogram was calculated by the method described in [2].

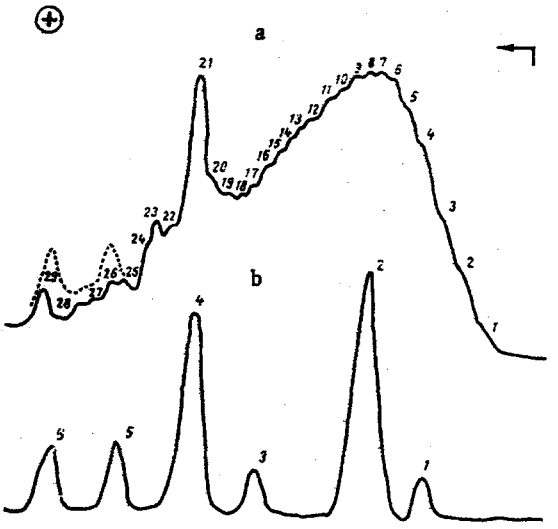

Fig. 2. Microelectrophoresis of proteins in continuous PAG: a) Densitogram of solubilized proteins of rat premotor cortex. Broken line shows position of marker proteins on chromatogram after homogenization in buffer A; b) Densitogram of marker proteins: 1) high-molecular-weight protein, a component of ferritin with unknown molecular weight, 2) ferritin, 3) dimer of bovine serum albumin, 4) bovine serum albumin, 5 and 6) protein components of soy bean trypsin inhibitor (molecular weight 7200 and 28,000 daltons respectively). Arrow indicates direction of migration of proteins during electrophoresis and position of start.

EXPERIMENTAL RESULTS

Electrophoretic spectra of solubilized proteins of brain structures and of marker proteins of different molecular weights, obtained by the method of microdisc-electrophoresis in a PAG pore gradient are illustrated in Fig. 2. The results of electrophoretic separation of marker proteins reveal high reproducibility of the method used. By means of this method 25 protein zones (from the 5th to the 29th) with a PNRA of $P \geqslant 0.95$ and four protein zones (from 1 to 4) with PNRA of $0.50 \leqslant P < 0.95$, corresponding to components of the brain structures studied were detected, many more than the number of protein zones obtained in the previous investigation by the microdisc-electrophoretic method in a homogeneous gel concentration [2]; the increase in the number of identified protein fractions was due to the greater resolving power of the method, for methods of solubilization of the proteins of the brain structures in the two investigations were for practical purposes indistinguishable from each other.

A densitogram of solubilized proteins of the rat cerebral cortex is shown in Fig. 2a; the protein spectra of the remaining brain structures differed from that shown only very slightly and only quantitatively. For instance, protein zones 16-18 of the cerebellum were slightly smaller in height than those shown in Fig. 2a, whereas protein zones 14-16, belonging to the caudate nucleus, were more clearly defined. The protein spectrum of the hippocampus also was more marked in the region of zones 14-16. It must be emphasized that no qualitative differences were found in the protein composition of the structures tested. A protein zone corresponding in position to bovine serum albumin (zone 21) was identified. On combined electrophoresis of nerve tissue proteins and marker proteins, protein zone 21 and bovine serum albumin were found to coincide exactly on the densitogram. Comparison of the protein composition of nerve tissue and of the marker proteins (Fig. 2a, b) showed that proteins of different molecular weight are represented very unequally. Proteins with a molecular weight of over 68,000 daltons (zone 21) constituted about 80% of the total protein content. Eight low-molecular-weight proteins were found. It must be pointed out that the ar-

guments on the molecular weight of the protein zones discovered are only approximate, for the place occupied by the protein during electrophoretic fractionation in a continuous pore gradient depends not only on the molecular weight, but also on the shape of the protein.

The results thus provide further evidence of the absence of any marked heterogeneity of protein composition of the brain structures despite differences in their neuronal-glial composition, their functions, and their phylogenetic gauge.

The author is grateful to V. S. Repin for advice in the course of the investigation.

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