NEUROSPECIFIC S-100 PROTEIN IN SYNAPTOSOMES OF THE RAT CEREBRAL CORTEX

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A nonspecific S-100 protein was found in the composition of low-molecular-weight acid proteins from synaptosomes of the rat cerebral cortex by capillary microdisc electrophoresis in 15% polyacrylamide gel with 0.1% sodium dodecysulfate and with the aid of a highly purified marker protein. The S-100 protein accounted for 15-20% of the low-molecular-weight acid synaptosomal proteins.

KEY WORDS: rat brain synaptosomes; microelectrophoresis of proteins; nonspecific acid S-100 protein.

Considerable interest has recently been shown in the study of acid neurospecific proteins which, in the general view, maintain brain functional activity at the highest level [1]. However, no convincing evidence has yet been obtained of the biochemical functions of these proteins in CNS neurons. Special attention has been paid to the study of the S-100 protein discovered by Moore [13]. S-100 protein has been shown to exist mainly in glial cells [8]. It has been suggested that this protein may be a modulator of synaptic conduction in nerve cells, for it can selectively bind Ca^{++} ions [5, 9]. It has also been stated that Ca^{++} and Mg^{++} facilitate the binding of S-100 protein with synaptic membranes and with membranes of synaptic vesicles (SVs) [7].

The problem of where this protein is synthesized and chiefly located in the nerve cells is thus very important. The presence of S-100 protein in trunks of the hypoglossal and vagus nerves has been demonstrated by electron autoradiography and by Miani's preparative technique [12]. Miani considered that S-100 protein is synthesized in neurons and transported by the slow axon current into synapses. Immunological studies have shown a relatively high concentration of S-100 protein in the synaptosomes of the guinea pig cerebral cortex [7]. The molecular weight of native S-100 protein is about 21,000 and the molecule consists of three identical subunits, each with a molecular weight of 7,000 [6]. It is composed chiefly of dicarboxylic amino acids, namely glutamic and aspartic acids, which account for one third of the total number of its component amino acids [6]. In the investigation described below another method was used to detect S-100 protein in rat cortical synaptosomes. The protein was identified by the use of a homologous marker protein and the sensitive technique of capillary microelectrophoresis.

EXPERIMENTAL METHOD

Male rats weighing 180-200 g were used. The original fraction of unpurified mitochondria was obtained from a 10% homogenate of the cerebral cortex by centrifugation in 0.32 M sucrose at 10,000 g for 20 min. Subsequent fractionation was carried out by centrifugation (VAC-601 ultracentrifuge, East Germany, SW-65 rotor) in a Ficoll–sucrose density gradient [4].

Fractions of myelin fragments, light and heavy synaptosomes, and mitochondria respectively were obtained.

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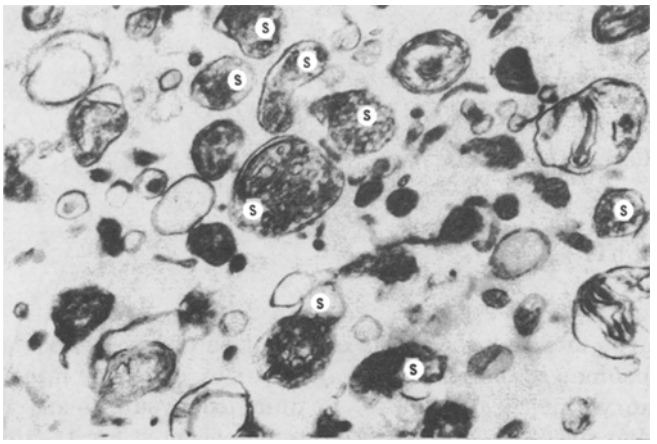

Fig. 1. Fraction of light synaptosomes. S) synaptosomes (33,750 ×).

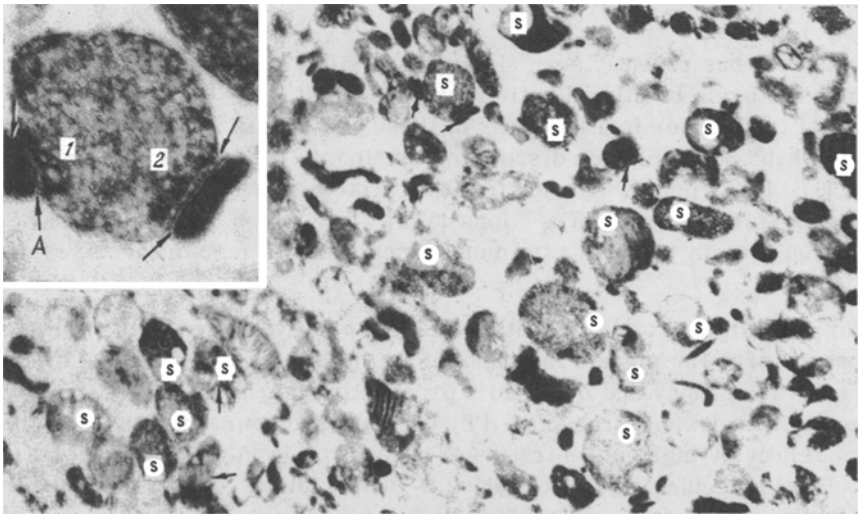

Fig. 2. Electron micrograph of fraction of heavy synaptosomes. Stained with phosphotungstic acid. S) synaptosomes (60,000 ×). Arrows indicate site of contact of synaptosomes with fragment of postsynaptic membrane and synaptic space. A) a single synaptosome with two synaptosomal complexes 139,500 ×). Structures of the vesicular lattice, consisting of electron-dense projections, cut in different planes, visible in region of presynaptic membrane: 1) section along projections, 2) plane of section at level of base of projections; 60,000 ×).

To separate low-molecular-weight synaptosomal proteins a technique of vertical microdisc electrophoresis in capillary tubes [3] with certain modifications [2] was used. The proteins were solubilized in medium consisting of 2.5% mercaptoethanol and 2.5% sodium dodecylsulfate in 0.01 M Na-phosphate buffer, pH 7.2 [11]. In this way 100% solubilization of the proteins of the synaptosomal fractions was achieved in 30 min at 20°C.

The protein (0.15-0.20 μg in a volume of 0.3-0.5 μl) was applied to a capillary tube (9 mm long) containing the gel and electrophoresis was carried out. In control experiments the equivalent amount of S-100 marker protein was fractionated, also in 15% polyacrylamide gel (PAG). The gels were stained with Amido Black. The stained gels were scanned in a two-color densitometer.

For morphological investigation a suspension of light and heavy synaptosomes was fixed in 0.05 M cacodylate buffer, pH 7.4, with the addition of 2% paraformaldehyde and 2% glutaraldehyde and then post-fixed in 2% OsO_4 in the same buffer. Electron-dense structures of the synaptic membranes in the region

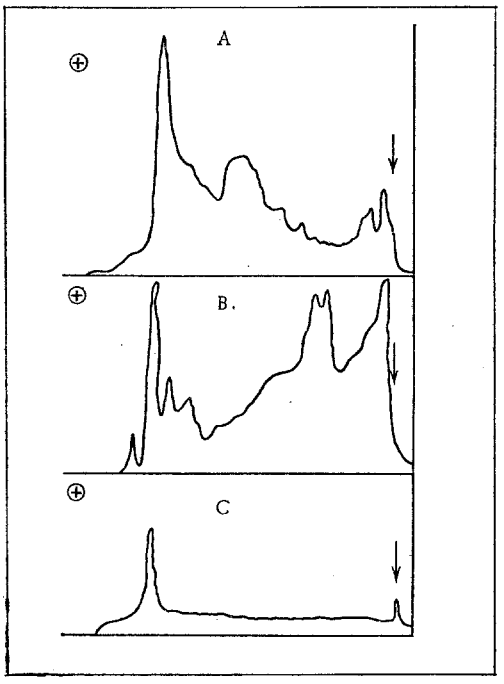

Fig. 3. Densitograms of electron micrographs of acid proteins of cerebral cortical synaptosomes fractionated in 15% PAG with 0.1% sodium dodecylsulfate. Arrows indicate start. A) Light synaptosomes, B) heavy synaptosomes, C) S-100 marker protein.

of the active zone were demonstrated by staining the fixed material with phosphotungstic acid in absolute ethanol by Jones' method [10]. The material was embedded in Epon or Araldite. Sections were cut on the LKB (Sweden) ultratome to a thickness of 400 Å, stained with uranyl acetate and lead citrate, and examined in the Hitachi electron microscope under a voltage of 75 kV.

EXPERIMENTAL RESULTS

The isolated fraction of light synaptosomes contained a high proportion of membrane-closed nerve endings measuring 0.1-0.2 μ. The preparations contained no fragments of myelinated fibers, nuclei, or structures of the perikaryon. Synaptosomes of the light fraction had a very low SV content, a short zone of contact with the postsynaptic membrane, and few intrasynaptosomal mitochondria (Fig. 1).

Mainly nerve endings with densely packed SVs and intrasynaptosomal mitochondria were found in the fraction of heavy synaptosomes (Fig. 2). Elements of the active zone of synapses were distinctly visible. The preparations contained no admixtures of myelin fragments, free mitochondria, microsomes, or lysosome-like structures. The fraction of heavy synaptosomes contained appreciable numbers of fragments from processes of neurons, mainly fragments of dendritic spines. In some cases postsynaptic elements of the zone of contact were clearly visible among the above-mentioned fragments.

The results of electron-microscopic investigation of the isolated subfractions of synaptosomes thus showed that fractions of the corresponding organelles conformed to existing requirements of purity and homogeneity.

The typical picture of fractionation of low-molecular-weight synaptosomal proteins in 15% PAG with 0.1% sodium dodecylsulfate is shown in Fig. 3. In this type of gel, a clear and homogeneous zone of protein with the relative anodic mobility of the S-100 marker protein was found in the composition of proteins of both heavy and light synaptosomes. The value obtained for the relative anodic mobility of S-100 protein in PAG with detergent agreed with data in the literature [15]. Quantitative densitometry of this protein fraction showed that it accounts for 15-20% of all low-molecular-weight synaptosomal proteins. The relative mobilities of the proteins and their molecular weights were calculated relative to the mobility of Bromphenol Blue. The molecular weight of the S-100 protein, according to these findings, was about 23,000.

The results of these experiments thus show that rat cortical synaptosomes contain a relatively high concentration of S-100 protein. This conclusion agrees with the observations of Donato and Michetti [7].

The work of Hyden [9] has shown that neurospecific S-100 protein exists not only in the free state, but also bound with membranes. According to Hyden's hypothesis, S-100 protein in the synaptic membrane binds Ca^{++} ions and interacts competitively with the contractile protein of the neurofilaments for Ca^{++} ions. Because of this competition, S-100 protein may be a modulator of the size of the synaptic space and may thus facilitate or impair conduction of the electrical impulse.

Synaptosomal S-100 protein may differ in its origins. According to one view [12], this protein is synthesized in neurons and carried by the slow axon current into nerve endings. There is as yet no convincing evidence in support of local synthesis of S-100 protein although components of a protein-synthesizing system have been found in synaptosomal membranes [14].

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