PROTEINS AND GLYCOPROTEINS OF RAT CEREBRAL CORTEX SUBSYNAPTOSOMAL FRACTIONS: EXTRACTION WITH SODIUM DODECYLSULPHATE AND ANALYTIC ELECTROPHORESIS*

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1. Introduction

Study of nerve impulse transmission has led to the development of techniques for isolation of "synaptosomes" or nerve ending particles [1–5] which retain the morphology and transmitter content of the original terminals. Recent reports have indicated that protein [6–8], glycoprotein [9] and glycolipid [9] synthesis occurs in synaptosomes. This laboratory [10] has recently demonstrated that protein, glycoprotein, lipid, and glycolipid synthesis occurs in isolated synaptosomes *in vitro* and that the intraneural mitochondrion is one subsynaptosomal particle independently carrying out these syntheses.

Because of the occurrence of protein and glycoprotein synthesis in isolated synaptosomes it seemed of import to determine the extraction and the electrophoretic separation of the proteins of subsynaptosomal fractions and to determine which of these proteins are glycoproteins. The present communication describes such a study. Waehneldt and Mandel [11] have recently described the extraction with sodium dodecyl sulfate and electrophoresis of rat brain myelin proteins; this report gives the electrophoretic patterns of other fractions of rat brain solubilized by sodium dodecyl sulphate (SDS).

2. Materials and methods

Particle isolation. Synaptosomes and subsynaptosomal particle fractions were prepared by the method of Whittaker et al. [1–3] as previously described [10, 12] at 0–4° from rat cerebral cortex (brain stem transected between superior and inferior colliculi). Rats weighing 150 g and which had been starved 16 hr before sacrifice

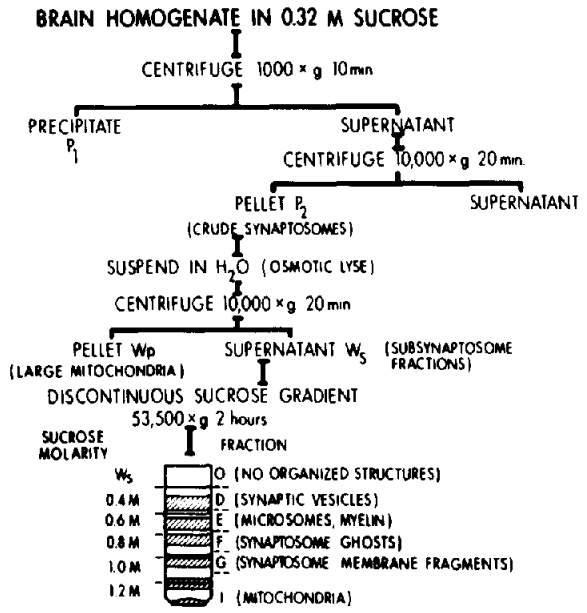

Fig. 1. Schematic representation of procedures used for the isolation of synaptosomes and subsynaptosomal fractions. Most procedures used and nomenclature are those of Whittaker et al. [1–3]. All manipulations were carried out at the temperature of melting ice.

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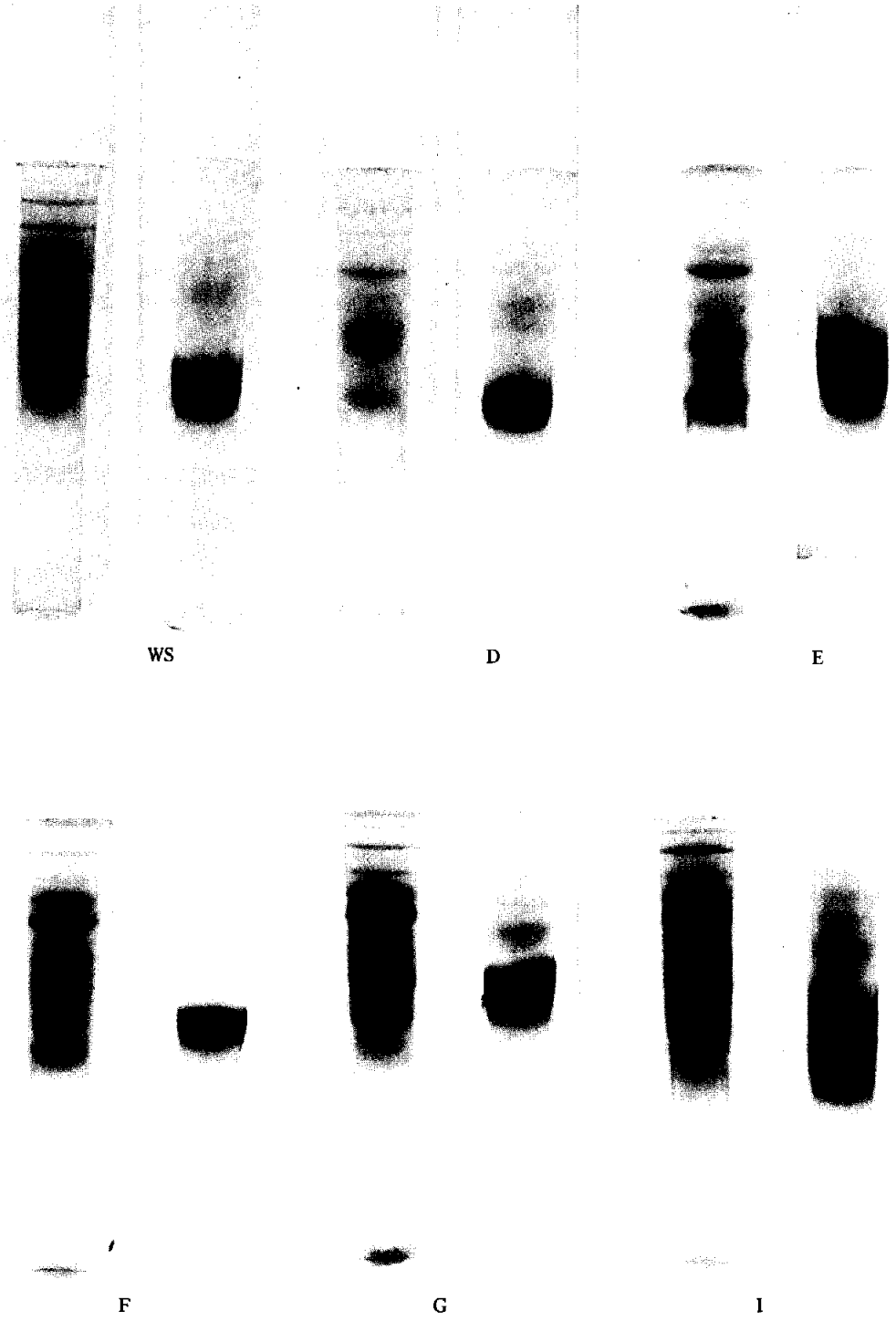

Fig. 2. Polyacrylamide gel electrophoresis of SDS extracts of rat brain subsynaptosomal fractions. Letter corresponds to the fraction as given in fig. 1. In each instance the gel on the left is stained with Coomasie Blue and the gel on the right is stained with the PAS technique. 5% polyacrylamide; pH 7.1, 0.1% SDS in samples, gels and buffers. Samples were loaded on the top of the gel and migrated toward the bottom, which is the anode (+).

were the source of cerebral cortex. Bands of sucrose gradients were diluted 3:1 with 0.1 M tris buffer, pH 7.6, and centrifuged at 100,000 *g* for 30 min to remove sucrose. Routinely, 6 rats were used per experiment, giving a yield of between 5 and 8 g wet weight of cortex. The procedures and the fraction identification of predominant component are outlined in schematic form in fig. 1.

Extraction and electrophoresis were carried out by the method described by Mainzel et al. [13, 14]. Fifty micrograms (as protein [13]) of each fraction were extracted at pH 7.1 in 1% SDS and 1% 2-mercaptoethanol (2-Me) for 3 hr at 37°. The fractions were dialyzed for 16 hr against 0.01 M phosphate buffer pH 7.1 which was 0.1% SDS and 0.1% 2-Me. The samples were subjected to electophoresis at 8 v/cm for 2 hr in 125 mm 5% polyacrylamide gels which were 0.1 M phosphate and 0.1% SDS. The electrophoresis buffer was 0.1 M phosphate buffer pH 7.1 which was 0.1% SDS. All gels were run toward the anode.

Following electrophoresis the gels were stained for either protein or glycoprotein. For protein, the gels were fixed in 20% sulfosalicylic acid for 16 hr, stained with 0.25% Coomasie Blue for 3 hr, and destained with several washes of 7% acetic acid. For glycoprotein, the gels were stained with a modified periodic acid–Schiff (PAS) technique exactly as described by Zacharius et al. [16].

3. Results and discussion

SDS extracts of the various rat cerebral cortex subsynaptosomal fractions were analyzed for proteins and glycoproteins by electrophoresis on 5% SDS polyacrylamide gels (fig. 2). The total subsynaptosomal fraction WS was characterized by 21 bands of protein, four of which were predominant. Of the 21 distinct bands in WS, 9 were identified as glycoproteins, one of which was predominant. The synaptic vesicle, fraction D, had 11 protein bands, 4 of which were predominant; of these 11 proteins, 5 stained as glycoproteins, one of which was highly stained. Fraction E, composed of microsomes and myelin, had 10 protein bands, 3 of which were predominant, and 5 glycoprotein bands, 1 of which was very diffuse and very predominant. It is of interest that Waehneldt and Mandel [11] found rat brain myelin to be composed of 3 bands. Fraction F, the synaptosome ghost or outer membrane fraction, was fractionated into 14 bands, of which 3 were predominant. Of the 14 protein bands, 5 were identified as glycoproteins, of which one band was prominent. The synaptosome membrane fragment, fraction G, was similar to fraction F; there were 14 bands, 6 of which were of glycoprotein nature. The intraneural mitochondria fraction was separated into 10 fractions, 5 of which were heavily stained for protein. Of the 10 fractions present, 7 stained for glycoproteins (fig. 2).

The subsynaptosomal fractions are thus composed of a variety of proteins, many of which are glycoproteins. One rather common feature of the fractions is the fast migrating, heavily stained glycoprotein fraction. Since in the SDS–polyacrylamide gels the molecular weight of the proteins is reflected by the rate of migration [13], this component of these fractions seems to be a low molecular weight glycoprotein. None of the fractions was characterized by high molecular weight glycoproteins; the fraction I, intraneural mitochondria, had the highest molecular weight glycoproteins present. The present results indicate that glycoproteins are present in the rat cerebral cortex subsynaptosomal fractions and the SDS solubilization is a suitable procedure for their study.

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