Biochimica et Biophysica Acta, 298 (1973) 219–229

BBA 76239

POLYPEPTIDE COMPOSITION OF MEMBRANES DERIVED FROM NEURONAL AND GLIAL CELLS

JAN-OLOF KARLSSON, ANDERS HAMBERGER and FRITZ A. HENN*

Institute of Neurobiology, University of Göteborg, Göteborg (Sweden)

(Received September 6th, 1972)

SUMMARY

Particulate fractions from whole rabbit brain, and from preparations enriched in neuronal or in glial cell bodies, were subjected to electrophoresis in two different systems, one containing sodium dodecyl sulphate and the other Triton X-100. Most membranes seemed to have three major polypeptides in common. Their molecular weights were calculated to be 44000, 52000 and 95000. Homologous subcellular fractions derived from neuronal or glial cells showed only relatively minor differences in their polypeptide composition. Synaptosomal plasma membranes showed a more complex protein composition than the membranes surrounding neuronal or glial cell perikarya.

INTRODUCTION

In an attempt to better understand the complex functioning of the central nervous system, many descriptive studies of soluble brain proteins have been carried out. These studies have resulted in the identification of several brain-specific proteins[1–5]. This work provides a model for similar studies on the functionally important insoluble proteins localized in various membrane fractions of the brain. The characterization of the neuronal membrane proteins should reveal components particularly involved in the propagation of electrical impulses, while the characterization of synaptic membranes must involve those components involved in chemical transmission. One problem in making a descriptive survey of protein species meaningful is the localization of the sample from a histologically complex tissue such as brain. In this report we have utilized bulk prepared fractions enriched in neuronal or glial cells to serve as the starting material for preparing membrane fractions, including relatively pure fractions of neuronal and glial plasma membrane. The neuronal plasma membrane preparation recently reported by Henn *et al.*[6] provides a sample of neuronal perikaryon plasma membrane obtained from cells whose dendritic processes and axons have partially been torn away. This membrane is undoubtedly not particularly rich in those synaptic regions specialized in chemical transmission, while the preparation of synaptosomal plasma membrane[7–9] provides a preparation of predominantly neuronal plasma membrane enriched in areas of synaptic contact.

* Present address: Department of Psychiatry, Washington University, School of Medicine, St. Louis, Mo. 63110, U.S.A.

This provides what appears to be a good starting point for identification of components involved in chemical as apposed to electrical impulse transmission.

Recent studies on glial cell function have also brought the membrane properties of this cell type into prominence. Henn and Hamberger[10] have shown that glial cells contain active transport systems capable of concentrating a variety of putative neurotransmitters. In addition, the biophysical membrane characteristics of glial cells were shown to be compatible with a role as a "spacial buffer" removing excess K^+ from the synaptic region by Trachtenberg and Pollen[11]. Henn *et al.*[12] have shown that the control of the extracellular ionic environment of neurons may well involve an active as well as passive component in the glial membrane. The recent preparation of a glial cell plasma membrane reported in the previous paper by Henn *et al.*[13] provides a preparation, on which studies aimed at characterizing the molecular basis of glial cell function can be undertaken. As a preliminary step we have characterized the protein components *via* electrophoretic separation systems employing two different detergents and compared them with separations obtained on neuronal and synaptosomal membrane systems.

Finally we have also surveyed the protein components found in organelles obtained from isolated glial and neuronal cell fractions. This allows some estimation of possible differences in the proteins of neuronal organelles as opposed to glial cell organelles.

METHODS

Preparation of fractions enriched in neuronal cell bodies and in glial cells

12–15 white rabbits, weighing between 1.5–1.8 kg, were used for each preparation. The animals were anaesthetized by sodium pentobarbitone and killed by intracardiac perfusion of Ringer's solution. The procedure used for preparing the neuronal and glial fractions was according to Blomstrand and Hamberger[14,15], as described by Henn *et al.*[6]. After centrifugation of the cell suspension on a discontinuous Ficoll density gradient, the neuronal fraction was obtained of the interface below the 30% Ficoll layer, and the glial fraction of the interface below the 12% Ficoll layer. The neuronal fraction routinely contained at least 90% neuronal cells, and the glial fraction approximately 80% glial cells, as judged by light microscopy. Endothelial cells and free nuclei were the main contaminants.

Preparation of subcellular fractions from neuronal and glial cells

The cell fractionation was essentially carried out according to Henn *et al.*[6,13]. The crude nuclear pellet obtained in the preparation of plasma membrane was resuspended, mixed with 7 vol. of 2.39 M sucrose and centrifuged for 60 min at $50000 \times g$ to sediment the purified nuclear fractions. The plasma membrane was obtained as previously described[6,13], and mitochondria were obtained from the sucrose gradients used to purify the plasma membrane in the interface below 35.5% sucrose. The microsomal fraction was obtained from the supernatant of the $3000 \times g$ spin used to prepare plasma membrane, this was spun at $10000 \times g$ for 15 min and the pellet discarded. The microsomes were obtained as a pellet after centrifugation of the resulting supernatant at $100000 \times g$ for 60 min.

Preparation of subcellular fractions from whole brain

Subcellular fractionation was carried out as described previously by Yanagihara and Hamberger[16]. The tissue was chopped with scissors and homogenized in 0.32 M sucrose 10 mM Tris–HCl (pH 7.4) with 10 up and down strokes in a glass–teflon homogenizer, driven at 1000 rev./min. The nuclear fraction was purified according to Løvtrup-Rein and McEwen[17] and separation of synaptosomes and mitochondria according to Gray and Whittaker[18] or Kornguth *et al.*[19]. Membrane fractions were prepared from the isolated synaptosomal fraction after osmotic disruption of the synaptosomes by centrifugation on a discontinuous sucrose gradient[16].

Electrophoresis; Triton X-100 system

The plasma membrane fractions were initially run on polyacrylamide gels in the presence of Triton X-100 according to the method of Lim and Tadayyon[20]. Two modifications of the techniques reported by these authors were used. First it was found that total solubilization of the membrane sample was achieved by vigorous homogenization of the sample in 8 M urea followed by the addition of an equal volume of sample solvent consisting of 100 mM K_2CO_3, 8 M urea, 20% (v/v) β-mercaptoethanol and 10% Triton X-100. We also found it advantageous to use an upper buffer, containing 0.25% Triton X-100, and run the 2-mm gels at 10 V/gel at constant voltage. The gels were stained in 1% amido black in 7% (v/v) acetic acid overnight and destained either electrophoretically or by prolonged soaking in 1% (v/v) acetic acid.

Treatment of subcellular fractions for sodium dodecyl sulphate electrophoresis

The fractions were collected as pellets and either prepared freshly for electrophoresis or stored at −30 °C during a few weeks before treatment. All fractions were dissolved in a solution of 5% sodium dodecyl sulphate, 1% β-mercaptoethanol, 1 mM EDTA in 10 mM phosphate buffer (pH 7.0). After homogenization the samples were heated at 100 °C for 3 min[21], and then centrifuged at 100000 × *g* for 20 min. This treatment solubilized almost all of the protein.

Electrophoresis; sodium dodecyl sulphate system

Approximately 100 μg of solubilized proteins were immediately applied to polyacrylamide gels containing sodium dodecyl sulphate, and electrophoresized in buffers containing sodium dodecyl sulphate. Three different electrophoresis systems were used, all based on the work of Shapiro *et al.*[22], Weber and Osborn[23], Lenard[21], and Fairbanks *et al.*[24].

System I consisted of gels, highly cross-linked with ethylenediacrylate, and gave the best separation and the most reliable results (Karlsson, J.-O., unpublished). This system was mostly used, and all results presented here are based on that system, unless otherwise stated. The gel composition was 10% (w/v) acrylamide (Eastman Co.) 0.5% (w/v) ethylenediacrylate (Borden Chem. Co), 1% (w/v) sodium dodecyl sulphate, 1 mM EDTA, 0.05% (v/v) *N,N,N′,N′*-tetramethylethylenediamine, 0.25% (w/v) ammonium persulfate, and 0.1 M sodium phosphate buffer (pH 7.0). The electrophoresis buffer was 1% sodium dodecyl sulphate, 1 mM EDTA in 0.1 M sodium phosphate buffer (pH 7.0).

System II was identical to System I, except that the acrylamide concentration was 7.5% (w/v).

System III was the system of Weber and Osborn[23], with 0.1% sodium dodecyl sulphate as previously described by Karlsson and Sjöstrand[25].

Sample solutions, containing 10% sucrose and bromophenol blue, were applied to the gels, and electrophoresis was performed at room temperature, first at a constant current of 0.5 mA/gel for 30 min, and then at 5 mA/gel for approximately 3 h. Gels were fixed and stained in 0.03% Coomassie brilliant blue (ICI Ltd), 10% acetic acid and 25% isopropanol over night. The gels were then kept in 0.003% Coomassie blue, 10% acetic acid, and 10% isopropanol for 6–9 h, and then stored in 10% acetic acid. For comparison some gels were stained with amido black.

Subcellular fractions from whole brain, neuronal and glial fractions were always electrophoresed at the same occasion and together with one or two standard proteins. To estimate the molecular weights of the polypeptides a standard curve relating the mobility of some well defined proteins with the mobility of bromophenol blue was made (Fig. 1).

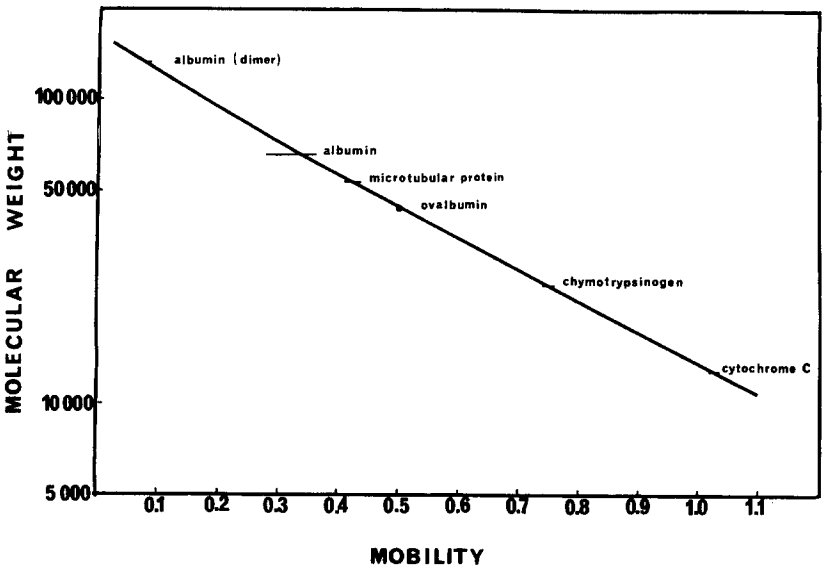


Fig. 1. Comparison of the molecular weight of some standard proteins with their electrophoretic mobilities in System I. 1.0 is the mobility of bromophenol blue. Average values from 2–5 different runs plotted together with the range (horizontal bar).

Standard proteins

Cytochrome *c* (horse heart, mol. wt 13000), chymotrypsinogen (mol. wt 25000), ovalbumin (mol. wt 45000) and serum albumin (bovine, mol. wt 67000) were obtained from Boehringer (Mannheim). Microtubular protein from rabbit brain (mol. wt approx. 55000) was isolated by precipitation with vinblastine (Velbe, E. Lilly Co.) essentially as described by Feit *et al.*[26].

RESULTS

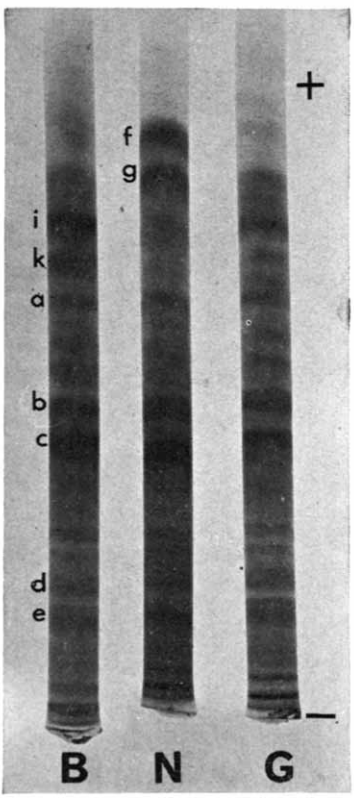
Reproducibility of electrophoretograms

The subcellular organelles were prepared from three different preparations of fractions enriched in neuronal and glial cells, all of which gave essentially the same

electrophoretic pattern. The electrophoretic separations were all reproducible in at least three different runs, and the reported molecular weights are the mean of at least three experiments. Electrophoresis with System I proved to be very reproducible (Fig. 1), and no differences were noted between freshly made up material and material stored at −30 °C for several weeks prior to solubilization. However, it was noted that storing material dissolved in the sodium dodecyl sulphate solution for more than a week tended to result in diffuse bands and poor electrophoretic separation. All samples run in sodium dodecyl sulphate System I and II showed a white band in the front, visible in incident light which did not stain with Coomassie blue, this band had a mobility of 1.25 (corresponding to a mol. wt of 7000), and apparently consists of lipids[21,27,28].

Unfractionated material

The sucrose homogenates from the neuronal, glial and whole brain fractions showed similarities in their polypeptide pattern (Fig. 2). The major polypeptides common for all these fractions had average mol. wts of 28000, 44000, 52000, 86000 and 95000 (Bands a, b, c, d and e, respectively, in Fig. 2). Bands b, d and e seemed to



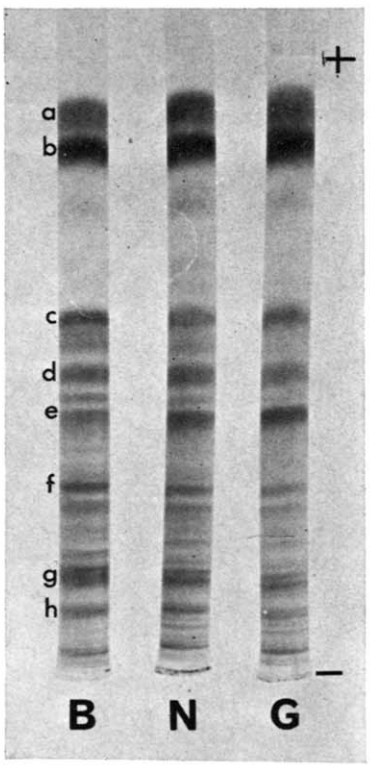


Fig. 2. Sodium dodecyl suphate electrophoresis of homogenates from whole brain (B), neuronal (N) and glial (G) fractions. For band identification see text in this and the following figures. Anode and cathode indicated.

Fig. 3. Sodium dodecyl sulphate electrophoresis of purified nuclei from whole brain (B), neuronal (N) and glial (G) fractions.

consist each of two closely spaced polypeptides. In the neuronal fraction there were two distinct, fast migrating polypeptides which were very weak in the other fractions (Bands f and g). The mol. wts for these bands were 14000 and 16000. Two polypeptides with mol. wts of 19000 and 23000 (Bands i and k in Fig. 2) were prominent in the whole brain homogenate, weak in the glial fraction and almost absent in the neuronal fraction. The other two electrophoretic systems both showed two major bands in the neuronal and glial fraction corresponding to Bands b and c of System I. These bands appeared to have mol. wts of 45000 and 52000, respectively, in these gel systems.

Nuclear fraction

The nuclear fractions from whole brain, neuronal and glial fractions showed an almost identical polypeptide pattern (Fig. 3). The major polypeptides had mol. wts of 14000, 16000, 33000, 44000, 52000, 66000, 100000 and 120000 (Fig. 3, Bands a–h respectively). The two fast-migrating polypeptides were dominating. The band with mol. wt 33000 took a reddish colour in the Coomassie stain. The two major bands in the front had mol wts of 14000 and 16000 according to System II, and 16000 and 20000 according to System III.

Mitochondrial fraction

The mitochondrial fractions were rather similar in their polypeptide patterns (Fig. 4). Most of the polypeptides had a mol. wt of more than 30000. The two dominating polypeptides in all fractions had mol. wts of 44000 and 52000 (Fig. 4a,

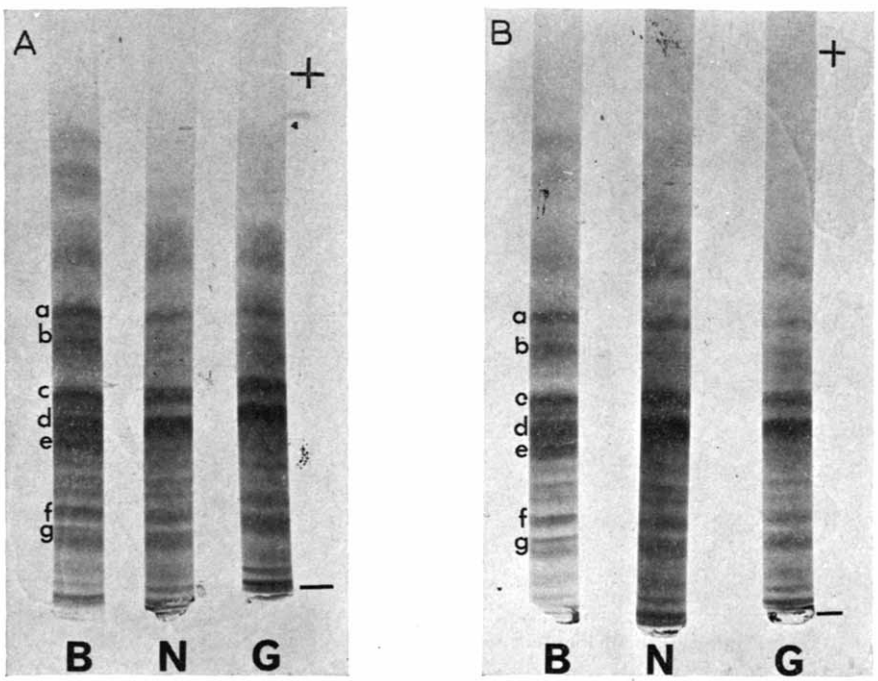
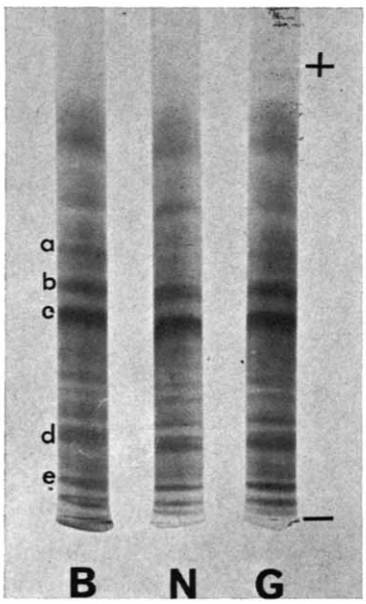


Fig. 4. Sodium dodecyl sulphate electrophoresis of mitochondria from whole brain (B), neuronal (N) and glial (G) fractions. A, Coomassie blue-stained gels. B, amido black-stained gels.

Bands c and d, respectively). In System II and III these bands had mol. wts of 45000 and 52000, respectively. Other polypeptides common for all fractions had mol. wts of 28000, 86000 and 95000 (Bands a, f and g, respectively). The only difference that could be detected was the presence of two polypeptides in the whole brain mitochondria (Bands b and e, mol. wts 34000 and 58000, respectively) which were not prominent in the mitochondrial fractions from the glial or neuronal enriched fractions. As can be seen from the figure, there was no marked difference between amido black (Fig. 4b) and Coomassie blue (Fig. 4a) stained gels.

Microsomal fraction

The microsomal fractions from whole brain, the neuronal and the glial fractions were rather similar in their polypeptide pattern. Most of the polypeptides in all the fractions had a mol. wt higher than 20000. The dominating polypeptides in both fractions had mol. wts of 44000 and 52000 (Fig. 5, Bands B and c, respectively). Other bands common for all fractions had mol. wts of 95000 and 135000 (Bands d and e, respectively). The whole brain and glial microsomes had a polypeptide with a mol. wt of 37000 (Band a) which was difficult to detect in the neuronal fraction.



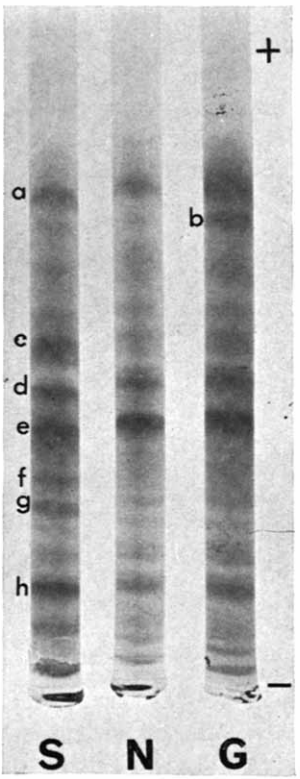


Fig. 5. Sodium dodecyl sulphate electrophoresis of microsomes from whole brain (B), neuronal (N) and glial (G) fractions.

Fig. 6. Sodium dodecyl sulphate electrophoresis of synaptosomal membranes (S), and neuronal (N) and glial (G) cell membranes.

Plasma membranes

The results of our studies using sodium dodecyl sulphate gels are shown in Fig. 6. All plasma membrane samples showed prominent bands corresponding to mol. wts of 19000, 44000, 52000 and 95000 (Fig. 6, Bands a, d, e, h, respectively). Interesting differences were also noted. First the glial plasma membranes showed a prominent band corresponding to a mol. wt of 23000 (Band b) not a major component of either synaptic or neuronal perikaryon plasma membrane. Secondly, in comparing the neuronal and synaptic plasma membrane electrophoretograms it is clear that the synaptic membrane has a more complex pattern. Polypeptides with mol. wts of 35000, 60000 and 68000 (Bands c, f, g, respectively) were prominent in synaptic plasma membrane samples as opposed to those derived from neuronal perikaryon.

Using the Triton X-100 system we were able to reproduce identical mobilities over the concentration range employed 20–60 μg protein per gel. The synaptic pattern contains 15 distinct bands, of which three are prominent, the neuronal plasma membrane consists of 13 distinct bands, 9 of which correspond to bands found in the synaptic membrane (Fig. 7). Of these, however, there is a large quantitative difference in one band, the second band behind the front, this appears as a major component in the neuronal membranes and is seen as a minor band in the synaptic preparation.

The glial pattern in Triton X-100 gels consists of a somewhat simpler pattern than the other two membrane fractions. Nine distinct bands are noted, two of which are especially prominent. The large band noted behind the front was characteristic of the glial pattern in Triton gels, and this band was absent from the membrane fractions obtained from various parts of the neuronal plasma membrane. This band appeared to account for 20% of the protein on the gel *via* densitometer tracings.

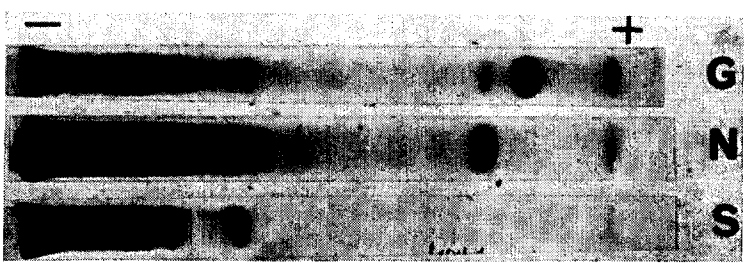


Fig. 7. Triton X-100 electrophoresis of glial (G) and neuronal (N) cell membranes and synaptosomal (S) membranes.

DISCUSSION

Comments on methodology

The molecular weights calculated for polypeptides in the present work should be considered as tentative. Some polypeptides may bind abnormally high or low amounts of sodium dodecyl sulphate per weight, resulting in a changed migration in the electrophoretic system. It is known that glycoproteins bind less sodium dodecyl sulphate per weight compared to polypeptides without covalently bound carbohydrate[29–31]. This will result in an overestimation of the molecular weights for polypeptides with a substantial amount of covalently bound carbohydrate. However,

this potential error will be less serious in the sodium dodecyl sulphate electrophoresis system used, as a relatively high degree of cross-linking and acrylamide concentration was used. It may also be mentioned that our standard curve (Fig. 1) only gives approximate molecular weights. Another source of error in interpretations of the polypeptide composition in the stained gels is that Coomassie blue may stain different polypeptides with different intensity. However, the amido black-stained gels always showed a close similarity with the Coomassie blue-stained gels.

Subcellular organelle fractions

When total protein from neuronal and glial cells is compared with that from whole brain, it is apparent that the brain is built up by cells possessing essentially similar polypeptide composition. Furthermore, homologous subcellular fractions from neurons and glia showed only relatively small differences in their polypeptide patterns. Most of the different subcellular fractions contained dominating polypeptides with mol. wts of 44000, 52000 and 95000, respectively. Although the results suggest that these polypeptides are common for a number of subfractions, we have no experimental evidence to state that the polypeptides of the same molecular weight found in the different fractions represent one common protein. The origin and function of these major polypeptides are not known. The polypeptide with a mol. wt of 52000 may be the subunit of microtubular (neurotubular) protein which is abundant in brain[32–34]. A considerable portion of this protein is found in particular fractions from brain[33,35,36]. Microtubular protein has been reported to consist of two non-identical subunits with mol. wts of 53000 and 56000[37]. Uyeda *et al.*[38] have recently isolated a glial fibrillary acidic protein with a mol. wt of 43000 which appeared to consist of two closely spaced bands on sodium dodecyl sulphate electrophoresis. The major polypeptide with a mol. wt of 44000, described in the present study, also migrated as two close bands. It is, today, impossible to identify all of the polypeptides which seem to be characteristic for different subcellular fractions. The polypeptide with a mol. wt of 19000 found only in the homogenate from whole brain may be derived from the basic myelin protein[39,40].

A pertinent question is to what degree a cross-contamination between different subcellular fractions is responsible for the similarities in polypeptide composition. However, this factor does not invalidate our proposition that the presented data reflect the properties of the different cell types, since the evaluation of the cross-contamination in the cell-enriched fractions (10–20%)[41] must also largely be valid for subcellular fractions. The purity of the subcellular fractions obtained from neuronal and glial cells has been checked with morphological and enzymatic techniques[6,13,42]. The observation that the nucleus in neuronal perikarya makes up a larger proportion of the cell when compared to glial cells is consistent with the clear peaks 14000 and 16000 of mol. wt characteristic of neuronal homogenate.

Plasma membrane fractions

The studies on the plasma membrane fractions obtained from two distinct regions of the total neuronal plasma membrane are of special interest. In these studies, using two distinct detergent systems, non-ionic Triton X-100 and ionic sodium dodecyl sulphate, the increasing structural complexity of the synaptic regions was apparent. The separation obtained with both Triton X-100 and sodium dodecyl sulphate

compare favorably with other electrophoretic studies of synaptic membranes. Bosmann *et al.*[43] have reported 14 distinct bands for a synaptic membrane preparation, solubilized and subjected to electrophoresis in the presence of sodium dodecyl sulphate. Mahler and Cotman[44] and Cotman *et al.*[45] using a phenol–acetic acid–urea system, reported 10 distinct bands when they examined a synaptic membrane preparation. In the present investigation the plasma membrane fractions were found to consist of three major polypeptides with mol. wts of 44000, 52000 and 95000 which was common to all membrane preparations. The study of the polypeptide composition of synaptic plasma membranes from rat brain by Banker *et al.*[46] demonstrated that the dominating polypeptides in their preparations had mol. wts of 41500, 52400 and 99000, i.e. in close agreement with our results. Similarly, in a recent study[47] on highly purified synaptosomal membranes from rat brain it was found that the two dominating polypeptides had mol. wts of 42000 and 53000.

The differences noted in the polypeptide components of the nerve-ending portion of the neural plasma membrane as compared to the membrane surrounding the neural perikaryon probably reflects the functional heterogeneity of the neuronal membrane. It may be that regional differentiation, related to specialized function, is common throughout the limiting membrane systems found in nature.

The glial membrane was found to contain a major band not present in synaptic or neuronal membrane preparations in both detergent systems. This band accounted for approximately 20% of the protein present in the gel, using the Triton X-100 system, and it is interesting to speculate if this might represent a structural component of the glial membrane, absent in the more complex neuronal membrane. It is of interest that the problem of glial contamination of synaptosomal preparations, which has been raised but never quantitated, can at least be qualitatively answered by comparing the gel patterns from glial and synaptosomal membranes. There are distinct differences between these two membranes in both detergent systems, tending to support the view that synaptosomal preparations predominantly are of neural origin, and glial vesicles are, in all probability, not of major quantitative concern.

Summarizing the present investigation, all brain membrane fractions, irrespective of cellular origin, contain a few major common polypeptides, although minor cell and organelle characteristic components were observed. The present methodological approach limits the possibility to do a correlative evaluation between cell function and the present finding which are of descriptive character.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Medical Research Council (No. B 72-13X-2226-06A, No. B 72-12X-2543-04A and No B 72-12X-164-08C) and Ollie and Elof Ericssons Stiftelse.

We are indebted to Mrs Britta Nyström for her skilful techical assistance.

F. A. H. was a fellow of the Life Insurance Medical Research Fund, and gratefully acknowledges support from the Smith, Kline and French Foundation.

REFERENCES

1 Moore, B. W. and McGregor, D. (1975) *J. Biol. Chem.* 240, 1647–1653
2 Wolfgram, F. (1965) *Ann. N.Y. Acad. Sci.* 122, 104–115
3 Tomasi, L. G. and Kornguth, S. E. (1968) *J. Biol. Chem.* 243, 2507–2513

4 Bennett, G. S. and Edelman, G. M. (1968) *J. Biol. Chem.* 243, 6234–6241
5 Grasso, A., Cicero, T. and Moore, B. W. (1969) *Abstr. 2nd Int. Meet. Int. Soc. Neurochem., Milan*, 201
6 Henn, F. A., Hansson, H. A. and Hamberger, A. (1972) *J. Cell Biol.* 53, 654–661
7 Kornguth, S. E., Anderson, J. W. and Scott, G. (1969) *J. Neurochem.* 16, 1017–1024
8 Cotman, C. W. and Matthews, D. A. (1971) *Biochim. Biophys. Acta* 249, 380–394
9 Morgan, I. G., Wolfe, L. S., Mandel, P. and Gombos, G. (1971) *Biochim. Biophys. Acta* 241, 737–751
10 Henn, F. A. and Hamberger, A. (1971) *Proc. Natl. Acad. Sci. U.S.* 68, 2686–2690
11 Trachtenberg, M. C. and Pollen, P. A. (1970) *Science* 167, 1248–1252
12 Henn, F. A., Haljamäe, H. and Hamberger, A. (1972) *Brain Res.* 43, 437–443
13 Henn, F. A., Hansson, H. A., Hamberger, A. *Biochim. Biophys. Acta*, submitted
14 Blomstrand, C. and Hamberger, A. (1969) *J. Neurochem.* 16, 1401–1407
15 Blomstrand, C. and Hamberger, A. (1970) *J. Neurochem.* 17, 1187–1195
16 Yanagihara, T. and Hamberger, A. (1971) *Exp. Neurol.* 31, 87–99
17 Løvtrup-Rein, H. and McEwen, B. S. (1966) *J. Cell Biol.* 30, 405–415
18 Gray, E. G. and Whittaker, V. P. (1962) *J. Anat.* 96, 79–88
19 Kornguth, S. E., Flangas, A. L., Siegel, F. L. Gerson, R. L. O'Brien, J. F., Lamar, Sr, C. and Scott, G. (1971) *J. Biol. Chem.* 246, 1177–1184
20 Lim, R. and Tadayyon, E. (1970) *Anal. Biochem.* 34, 9–15
21 Lenard, J. (1970) *Biochemistry* 9, 1129–1132
22 Shapiro, A. L., Viñuela, E. and Maizel, J. V. (1967) *Biochem. Biophys. Res. Commun.* 28, 815–820
23 Weber, K. and Osborn, M. (1969) *J. Biol. Chem.* 244, 4406–4412
24 Fairbanks, G., Steck, T. L. and Wallach, D. F. H. (1971) *Biochemistry* 10, 2606–2617
25 Karlsson, J.-O. and Sjöstrand, J. (1971) *J. Neurochem.* 18, 2209–2216
26 Feit, H., Dutton, G. R., Barondes, S. H. and Shelansk, M. L. (1971) *J. Cell Biol.* 51, 138–147
27 Dutton, G. R. and Barondes, S. H. (1972) *J. Neurochem.* 19, 559–562
28 Ramirez, G., Levitan, I. B. and Mushynski, W E. (1972) *Brain Res.* 43, 309–313
29 Pitt-Rivers, R. and Impiombato, F. S. A. (1968) *Biochem. J.* 109, 825–830
30 Segrest, J. P., Jackson, R. L., Andrews, E. P. and Marchesi, V. T. (1971) *Biochem. Biophys. Res. Commun.* 44, 390–395
31 Bretscher, M. S. (1971) *Nature New Biol.* 231, 229–232
32 Wisniewski, H., Shelanski, M. L. and Terry, R. D. (1968) *J. Cell Biol.* 38, 224–229
33 Weisenberg, R. C., Borisy, G. G. and Taylor, E. W. (1968) *Biochemistry* 7, 4466–4479
34 Dutton, G. R. and Barondes, S. H. (1969) *Science* 166, 1637–1638
35 Dahl, D. R., Redburn, D. A. and Samson, F. E. (1970) *J. Neurochem.* 17, 1215–1219
36 Feit, H. and Barondes, S. H. (1972) *J. Neurochem.* 17, 1355–1364
37 Feit, H., Slusarek, L. and Shelanski, M. L. (1971) *Proc. Natl. Acad. Sci. U.S.* 68, 2028–2031
38 Uyeda, C.T., Eng, L. F. and Bignami, A. (1972) *Brain Res.* 37, 81–89
39 Carnegie, P. R., Bencina, B. and Lamoureux, G. (1967) *Biochem. J.* 405, 559–565
40 Martenson, R. E., Deibler, G. E., Kies, M. W., McKneally, S. S., Shapira, R. and Kibler, R. F. (1972) *Biochim. Biophys. Acta* 263, 193–201
41 Blomstrand, C. (1971), Thesis, University of Göteborg, Sweden
42 Hamberger, A., Blomstrand, C. and Lehninger, A. L. (1970) *J. Cell Biol.* 45, 221–234
43 Bosmann, H. B., Case, K. R. and Shea, M. B. (1970) *FEBS Lett.* 11, 261–264
44 Mahler, H. R. and Cotman, C. W. (1970) in *Protein Metabolism of the Nervous System* (Lajtha, A., ed.), pp. 151–183, Plenum Press, New York
45 Cotman, C. W., Herschman, H. and Taylor, D. (1971) *J. Neurobiol.* 2, 169–180
46 Banker, G., Crain, B. and Cotman, C. W. (1972) *Brain Res.* 42, 508–513
47 Levitan, I. B., Mushynski, W. E. and Ramirez, G. (1972) *J. Biol. Chem.* 247, 5376–5389