Highly Purified Synaptosomal Membranes from Rat Brain

PREPARATION AND CHARACTERIZATION

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SUMMARY

Synaptosomal plasma membranes were isolated from whole rat brain by a method derived from several existing ones. The procedure involves the sequential centrifugation of a crude mitochondrial fraction through a discontinuous sucrose gradient and a continuous cesium chloride gradient. The material is then subjected to osmotic lysis and centrifuged through an additional discontinuous sucrose gradient. The synaptosomal membranes prepared in this way contain only very small amounts of mitochondria, endoplasmic reticulum, myelin, axonal and glial plasma membranes, and lysosomes; the level of contamination was estimated by assay for appropriate marker enzymes, and by assaying the final membrane preparation for radioactivity following the addition of previously labeled radioactive contaminants at the start of the isolation procedure. On the basis of these criteria, the contaminants examined appear to account for less than 10 to 15% of the protein in the preparation.

The isolated membranes were sequentially extracted with Triton X-100 and sodium dodecyl sulfate; as previously described, the Triton-soluble fraction contains those portions of the synaptosomal membrane not associated with the synaptic junction, whereas the sodium dodecyl sulfate dissolves the junctional complex. The fractions were subjected to electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate, and the gels were stained for protein with Coomassie brilliant blue. The results indicate that some proteins are common to both fractions, but that each fraction contains several unique protein species.

The membrane preparation also contains RNA and DNA in limited amounts. The bulk of the RNA resembles mitochondrial RNA with respect to its mobility in polyacrylamide-agarose composite gels.

Since the pioneering reports of Gray and Whittaker (1) and De Robertis et al. (2), a large number of authors have described the isolation of synaptosomes, or pinched off nerve endings from brain homogenates. Most investigators have prepared synaptosomes by centrifuging a crude mitochondrial pellet through a discontinuous gradient of sucrose or ficoll (1-7); the resulting preparation is often contaminated with free mitochondria, endoplasmic reticulum, myelin, and other membranes of unknown origin. An apparently more satisfactory method of isolating synaptosomes or synaptosomal plasma membranes is by centrifugation through a continuous gradient of sucrose (8) or cesium chloride (9, 10). Cotman et al. (8) carried out zonal centrifugation, in sucrose, of an osmotically shocked crude mitochondrial fraction, and estimated by assay for marker enzymes that the contamination of the resulting synaptosomal membranes by mitochondria and endoplasmic reticulum was in the range 10 to 20%. Kornguth et al. (9, 10), by sequential centrifugation in a discontinuous sucrose gradient and a generated cesium chloride gradient, obtained synaptosomes which by electron microscopic examination appeared to be quite pure. Unfortunately, quantitative estimates of the level of contamination were not carried out, and the method has the drawback of requiring more than 72 hours from start to finish. Accordingly, we have modified and extended this method to allow the isolation of highly purified synaptosomal membranes in approximately 24 hours. In addition, we have extensively characterized the membrane preparation with respect to its purity, and its protein and nucleic acid content.

EXPERIMENTAL PROCEDURE

Synaptosomal Membrane Preparation—Brains were removed from 250-g female Sprague-Dawley rats and placed on ice. The tissue was disrupted by mincing or by gentle homogenization, and was then homogenized with three strokes of a glass-to-glass hand homogenizer (pestle clearance 180 μ) in 6 volumes of 0.32 M sucrose containing 1 mM MgCl₂ and 1 mM potassium phosphate, pH 7.0 (Solution A). The homogenate was centrifuged at 800 × g for 10 min; the pellet was washed twice with Solution A, and the combined supernatants were centrifuged at 7,000 × g for 30 min. The resulting crude mitochondrial pellet was washed three times with Solution A, and was resuspended in 0.32 M sucrose containing 10 mM MgCl₂ and 1 mM potassium phosphate, pH 7.0 (Solution B). The suspension was layered over a discontinuous sucrose gradient (0.8 M-1.0 M-1.2 M sucrose containing 10 mM MgCl₂ and 1 mM potassium phosphate, pH 7.0), and was centrifuged at 75,000 × g for 2 hours in the SW 27 rotor of a Beckman model L-2
65B ultracentrifuge. The material sedimenting at the 1.0 m-1.2 m sucrose interphase was resuspended in 0.15 m NaCl containing 1 mm MgCl₂ (Solution C), and was layered carefully over an equal volume of 45.5% cesium chloride (CsCl) in Solution C. This use of an initial previously formed step in CsCl concentration dramatically reduces the time required to achieve equilibrium conditions (11). The gradient was centrifuged at 100,000 × g in the Spinco No. 50 rotor, or at 75,000 × g in the SW 27 rotor, for 12 to 14 hours. The material sedimenting at an isopycnic density of 1.16 to 1.17 was homogenized in 1 mM Tris, pH 7.0 (5 ml per g of original tissue), with a tight-fitting glass homogenizer; the suspension was allowed to stand at 4°C for 1 hour and was rehomogenized, then centrifuged at 35,000 × g for 20 min. The pellet was resuspended in Solution B, and was applied to a discontinuous sucrose gradient identical with that described above. The material sedimenting at the 1.0-1.2 m interphase in this gradient is the final synaptosomal membrane preparation. The yield is approximately 5 mg of protein per g of brain. The pellet below 1.2 m sucrose in this gradient consists of synaptosomal mitochondria.

Preparation of Radioactive Organelles and Subcellular Fractions—Fractions were prepared from rats killed 24 hours after the administration of 50 μCi of L-[4,5-3H]leucine into each lateral ventricle. Homogenization was as described above for the synaptosomal membrane preparation. The specific radioactivity of the protein in each pure fraction was determined as described previously (12).

Myelin and Mitochondria—A crude mitochondrial pellet, prepared as described above, was centrifuged through a discontinuous sucrose gradient. The myelin (0.32-0.50 m sucrose interphase) and perikaryal mitochondria (pellet below 1.2 m sucrose) were placed on separate, identical gradients, and recentrifuged to obtain the pure fractions. The material at the 1.0-1.2 m sucrose interphase was carried through the remainder of the synaptosomal membrane isolation procedure described above, in order to obtain synaptosomal mitochondria (pellet in final sucrose gradient).

Endoplasmic Reticulum—A brain homogenate was centrifuged at 7,000 × g; the pellet was washed, and the combined supernatant were centrifuged at 100,000 × g. The microsomal pellet was resuspended in Solution B and layered over a discontinuous sucrose gradient. The material sedimenting at the 1.0-1.2 m sucrose interphase was taken as endoplasmic reticulum membranes.

Axonal Plasma Membranes—Rat optic nerve and tract were carefully dissected free from the surrounding tissue. They were homogenized and subjected to the isolation procedure described above for synaptosomal membranes, except that the material sedimenting at an isopycnic density of 1.15 in CsCl was used. Since the optic nerve and tract do not contain any synaptic endings, the final preparation consists of the nonsynaptosomal portion of the axonal plasma membrane.

Glial Membranes—A glial cell-enriched fraction was prepared from whole rat brain as described in detail elsewhere (13). The cells were homogenized and subjected to the synaptosomal membrane isolation procedure, in order to obtain glial plasma membranes (isopycnic density 1.15 in CsCl). This preparation is contaminated to a slight extent by synaptosomal membranes, as the glial cell-enriched fraction is not entirely free of synaptosomes.1

1 Unpublished results.
The extent of mitochondrial and microsomal contamination of the synaptosomal membranes at various stages of the isolation procedure, as estimated by assay for appropriate marker enzymes, is shown in Tables I and II. It can be seen that, after the first discontinuous sucrose gradient, the preparation retains considerable cytochrome oxidase, monoamine oxidase, and antimycin-insensitive NADH oxidase activity (Table I, Part A), indicating the presence of significant quantities of mitochondria and endoplasmic reticulum. On the basis of the specific activities of the enzymes in the appropriate pure subcellular fractions, it can be calculated that mitochondria account for approximately 25%, and endoplasmic reticulum 20%, of the protein in the preparation. The presence of acid phosphatase activity suggests that lysosomes are also present, but a quantitative estimate of lysosomal contamination could not be made, as the specific activity of acid phosphatase in a pure lysosomal fraction was not determined. It should be noted that the preparation referred to in Part A of Table I is equivalent to the standard “synaptosome” preparations described previously (1, 2).

**RESULTS**

The data in Table I, Part B, show that endoplasmic reticulum membranes are virtually absent from the preparation following the CsCl gradient centrifugation. The extent of contamination at this stage appears to be 2 to 3%. In addition, acid phosphatase activity could not be detected, indicating that lysosomes have also been removed. Mitochondria, on the other hand, are still present in significant quantities; the specific activities of cytochrome oxidase (an inner mitochondrial membrane marker) and monoamine oxidase (an outer membrane marker) suggest contamination levels of 14 and 18%, respectively. Many of these mitochondria may be intrasynaptic ones, as the synaptosomes are still intact at this stage of the isolation procedure.

Following osmotic lysis of the synaptosomes and centrifugation through the second discontinuous sucrose gradient, mitochondria also virtually disappear from the membrane preparation (Table II). The contamination level appears to be approximately 3%, similar to that for endoplasmic reticulum. Once again, no acid phosphatase activity could be detected. It should be noted that these mitochondrial contamination values are based on the specific activities of the enzymes in

### TABLE I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytochrome oxidase</td>
</tr>
<tr>
<td>Pure perikaryal mitochondria...</td>
<td>270 ± 40</td>
</tr>
<tr>
<td>Pure synaptosomal mitochondria...</td>
<td>305 ± 34</td>
</tr>
<tr>
<td>Pure endoplasmic reticulum...</td>
<td>N.D.</td>
</tr>
<tr>
<td>Membrane preparation after first sucrose gradient (A)...</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>Membrane preparation after CsCl gradient (B)...</td>
<td>38 ± 6</td>
</tr>
</tbody>
</table>

* N.D., activity not detectable.

### TABLE II

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Homogenate...</th>
<th>Pure perikaryal mitochondria...</th>
<th>Pure synaptosomal mitochondria...</th>
<th>Pure endoplasmic reticulum...</th>
<th>Membrane preparation after first sucrose gradient (A)...</th>
<th>Membrane preparation after CsCl gradient (B)...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
<td>Cytochrome oxidase</td>
<td>Monoamine oxidase</td>
<td>Antimycin-insensitive NADH-cytochrome c reductase</td>
<td>Acid phosphatase</td>
<td>Ouabain-sensitive Na+, K+-ATPase</td>
<td></td>
</tr>
<tr>
<td>Homogenate...</td>
<td>37 ± 6</td>
<td>1.5 ± 0.1</td>
<td>102 ± 7</td>
<td>36 ± 6</td>
<td>290 ± 15</td>
<td></td>
</tr>
<tr>
<td>Pure perikaryal mitochondria...</td>
<td>270 ± 40</td>
<td>11.5 ± 1.2</td>
<td>830 ± 100</td>
<td>1.2 ± 0.3</td>
<td>360 ± 15</td>
<td></td>
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<td>Pure synaptosomal mitochondria...</td>
<td>365 ± 34</td>
<td>12.4 ± 1.9</td>
<td>795 ± 55</td>
<td>N.D.a</td>
<td>N.D.a</td>
<td></td>
</tr>
<tr>
<td>Pure endoplasmic reticulum...</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1920 ± 170</td>
<td>N.D.</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Membrane preparation after first sucrose gradient (A)...</td>
<td>65 ± 10</td>
<td>3.1 ± 0.6</td>
<td>590 ± 90</td>
<td>4.2 ± 1.1</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Membrane preparation after CsCl gradient (B)...</td>
<td>38 ± 6</td>
<td>2.1 ± 0.2</td>
<td>170 ± 10</td>
<td>N.D.</td>
<td>N.D.</td>
<td></td>
</tr>
</tbody>
</table>

* N.D., activity not detectable.
pure perikaryal mitochondria. These specific activities are slightly higher in pure synaptosomal mitochondria (Tables I and II), and thus the contamination estimates are even lower when the latter are used as a basis for calculation. It is of interest that the activity of Na\(^+-\) and K\(^+-\)activated ouabain-sensitive ATPase is highly enriched in the final membrane preparation, relative to the homogenate; this enzyme is known to be present in high specific activity in synaptosomal membranes (17, 25).

The extent of contamination of the final synaptosomal membrane preparation by various subcellular fractions, as estimated by a radioassay, is presented in Table III. In these experiments, a pure fraction (e.g. myelin, mitochondria, etc.) of known protein-specific radioactivity was added to the preparation prior to the initial homogenization. The specific radioactivity of the protein in the final preparation was then measured, and the degree of contamination by the added fraction was calculated. The radioactive pure fraction was added in large excess, relative to the amount of the same (nonlabeled) fraction in the preparation, in order to avoid appreciable dilution of the radioactivity. Because of this large excess of contaminants, the data obtained probably represent overestimates of the true levels of contamination. The results in Table III show that myelin accounts for 1 to 2% of the protein in the preparation, glial and axonal plasma membranes for approximately 2 to 3% each, and endoplasmic reticulum and mitochondria (based on either perikaryal or synaptosomal mitochondria) for 3 to 5% each. It should be noted that these values for the latter two fractions are in excellent agreement with those obtained by assay for marker enzymes (Table II). Taken together, the data in Tables II and III indicate that those contaminants examined account for 10 to 15% of the protein in the synaptosomal membrane preparation. These biochemical results are confirmed by electron microscopic examination of the preparation (not shown). Virtually no myelin fragments or mitochondria were observed in the electron micrographs, and most of the membranes present could be identified as being of synaptosomal origin.

The isolated membranes were fractionated into Triton- and SDS-soluble portions, as described under "Experimental Procedure." It has been shown (26, 27) that, under appropriate conditions, Triton X-100 dissolves only those portions of the synaptosomal membrane not associated with the synaptic junction. The data in Table IV show that, under the two solubilization conditions employed by us, the Triton solubilizes 35 to 40% of the synaptosomal membrane protein, approximately 80% of the membrane acetylcholinesterase activity, and virtually none of the membrane alkaline phosphatase and 5'-nucleotidase activities. These results are identical with those presented by Cotman et al. (26) in their detailed study on Triton solubilization; thus they confirm that, in our hands, the synaptic junctional complex remains insoluble and intact following Triton treatment. Since the same enzyme activity results were obtained when solubilization was performed in the presence or absence of Ca\(^{2+}\) (Table IV), in general the latter conditions were used for the fractionation of membranes for electrophoretic studies. The electrophoretic patterns were no different when solubilization was performed in the presence of Ca\(^{2+}\).

The Triton- and SDS-soluble portions of the membranes were subjected to electrophoresis in polyacrylamide gels containing SDS, as described under "Experimental Procedure." The electrophoretic patterns following staining of protein with Coomassie blue are shown in Fig. 1. It can be seen that the two fractions have a number of protein components in common; however each fraction has, in addition, several protein species which do not occur in the other fraction. Although there do not appear to be any dominant protein bands in the Triton-soluble portion of the membranes, there are two bands in the SDS-soluble portion which account for a large part of the protein in the gel. These bands have molecular weights of 42,000 and 53,000, respectively, as estimated from the mobility of marker proteins of known molecular weight on identical gels. At least 20 to 30 additional protein bands, with molecular weights between 40,000 and 100,000, are seen in each fraction.

### Table III

Radioassay of final synaptosomal membrane preparation for various contaminants

Radioactive subcellular fractions were prepared as described under "Experimental Procedure," and their protein-specific radioactivity was determined. Each radioactive pure fraction was then added (in large excess, see text) to a separate unlabeled brain mince immediately prior to the initial homogenization step. Synaptosomal membranes were isolated as usual, and the specific radioactivity of the protein in the final preparation was measured.

<table>
<thead>
<tr>
<th>Fraction added</th>
<th>Specific radioactivity of pure fraction</th>
<th>Specific radioactivity of final preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm/mg protein(^\times)</td>
<td>cpm/mg protein(^\times)</td>
</tr>
<tr>
<td>Myelin</td>
<td>2,150 ± 20</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>Glial plasma membranes</td>
<td>7,400 ± 14</td>
<td>180 ± 12</td>
</tr>
<tr>
<td>Axonal plasma membranes</td>
<td>5,600 ± 14</td>
<td>150 ± 16</td>
</tr>
<tr>
<td>Perikaryal mitochondria</td>
<td>11,600 ± 30</td>
<td>370 ± 22</td>
</tr>
<tr>
<td>Synaptosomal mitochondria</td>
<td>6,200 ± 30</td>
<td>290 ± 20</td>
</tr>
<tr>
<td>Endoplasmic reticulum</td>
<td>37,000 ± 500</td>
<td>1,200 ± 190</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean of duplicate determinations.

\(^{b}\) Mean ± S.E. for three experiments, with duplicate determinations.

### Table IV

Solubilization of synaptosomal membrane components by Triton X-100

Synaptosomal membranes were extracted with Triton X-100 (I) under the conditions described by Cotman et al. (26) or (II) in the absence of calcium, and the extent of solubilization of protein, and of acetylcholinesterase, alkaline phosphatase, and 5'-nucleotidase activities, was determined. The data represent the mean ± S.E. for three experiments, with duplicate determinations.

<table>
<thead>
<tr>
<th>Solubilization conditions</th>
<th>Extent of solubilization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
</tr>
<tr>
<td>I. 3 mm Tris-Cl, pH 8.0, 3 mm CaCl(_2)</td>
<td>0.24% Triton X-100</td>
</tr>
<tr>
<td>III. 0.25% Triton X-100</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>II. 62.5 mm Tris-Cl, pH 6.8, 10% glycerol, 0.25% Triton X-100</td>
<td>35 ± 2</td>
</tr>
</tbody>
</table>
Nucleic acid content of final synaptosomal membrane preparation

DNA and RNA were estimated in the final synaptosomal membrane preparation, and in whole synaptosomes, as described under "Experimental Procedure." The latter fraction was obtained by carrying out the usual isolation procedure, but with the osmotic lysis step omitted. The values represent the mean ± range for two samples.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>RNA content</th>
<th>DNA content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole synaptosomes</td>
<td>5.48 ± 0.28</td>
<td>3.98 ± 0.25</td>
</tr>
<tr>
<td>Synaptosomal membranes</td>
<td>5.71 ± 0.03</td>
<td>2.14 ± 0.03</td>
</tr>
</tbody>
</table>

Discussion

Although synaptosomes and synaptosomal membranes have been extensively studied with respect to their structure and metabolic activity in vitro, few authors have attempted to quantitatively estimate the degree of purity of their fractions. Electron microscopic examination of nerve endings, prepared by conventional discontinuous gradient techniques, indicates (1, 2, 28) that there may be extensive contamination by mitochondria, myelin, and other membranous elements. Our results show that as much as 45% of the protein in such "synaptosome" preparations may be attributed to mitochondria and endoplasmic reticulum (Table I, Part A). This finding is supported by that of Lemkey-Johnston and Dekirmenjian (28), who estimated that less than 50% of the membranes appearing in electron micrographs of synaptosome preparations are in fact of synaptosomal origin.

Cotman, Mahler, and Anderson (8) isolated synaptosomal membranes with continuous sucrose gradients, and estimated by assay for marker enzymes that mitochondria and endoplasmic reticulum account for 10 to 20% of the protein in their preparation. On the basis of both the enzyme assays and the radioassay, our membranes appear to contain less than one-third as much of these contaminants. Although neither of the methods of estimating contamination is foolproof, the good agreement between them (Tables II and III) provides an indication that the results are valid, and that these synaptosomal membranes are in fact purer than any that have previously been described in the literature.

While this manuscript was in preparation, Morgan et al. (29) reported the preparation of synaptosomal membranes comparable in purity to the present ones. However, their yield is 50-fold less, and thus the present technique would appear to be more suitable for routine studies. The recently described method of Cotman and Matthews (18) also results in a lower yield of membrane proteins.
branes, and the cytochrome oxidase contamination is approximately 7%. Several features of the present method deserve special emphasis. We have found that the contamination of the synaptosomal membranes by endoplasmic reticulum is 2 to 5 times higher when a centrifugal force greater than 7,000 × g is used to obtain the crude mitochondrial pellet. Other workers routinely use 10,000 to 20,000 × g for this purpose (1–10). In addition, this pellet must be washed and recentrifuged a minimum of two times to avoid extensive microsomal contamination. It is also important to note that intact rather than lysed synaptosomes are subjected to centrifugation in the continuous gradient, in contrast to the method of Cotman et al. (8). This allows us to efficiently separate synaptosomes (isopycnic density 1.16 to 1.17) from mitochondria (density 1.19) and from endoplasmic reticulum, gial, and axonal membranes (density 1.14 to 1.15). When lysed synaptosomes are centrifuged in CsCl, the synaptosomal membranes (density 1.15) cannot be well separated from the latter fractions.

The synaptosomal membranes isolated in this way have a large number of polypeptide components resolvable by polyacrylamide gel electrophoresis in the presence of SDS. The pattern appears to be somewhat more complex than that described previously (3, 30, 31), possibly because of differences in solubilization of the proteins, or because our electrophoretic method combines the high resolving power of conventional disc electrophoresis with the advantages of an SDS-containing system (23). The multiplicity of bands cannot be an artifact resulting from the action of proteases, as the protease inhibitor phenylmethylsulfonyl fluoride was present throughout the extraction, dialysis, and electrophoresis of the proteins. Of particular interest is the finding that the Triton- and SDS-soluble portions of the membrane each contain several unique protein species. Since under the conditions employed in the present study Triton X-100 dissolves only those portions of the synaptosomal membrane not associated with the synaptic junction, leaving the junctional complex to be dissolved in SDS, the unique species may be important for the specialized functions of that part of the membrane in which they contain.

It is difficult to interpret the finding that the membrane preparation contains significant amounts of DNA and RNA. This may represent contaminants, from other cell organelles, which simply stick nonspecifically to the membranes during the isolation procedure. However, the excellent reproducibility of the quantity of nucleic acid found, from sample to sample, appears to preclude this explanation. Furthermore the bulk of the RNA migrates as 13 to 14 S and 16 to 18 S material, as does mitochondrial RNA at 2° (32), despite the fact that mitochondria are very unlikely to break and discharge their contents under the conditions of the isolation procedure. Thus it is possible that synaptosomal membranes in vivo contain mitochondrial-like RNA, perhaps supplied by mitochondria which are transported along the axon from the perikaryon, and break open when they reach the nerve terminal, as has been suggested by Weisz (33). The small amount of 28 S RNA may be derived from 60 S subunits (of 80 S ribosomes) present as contaminants in the membrane preparation.

Because of their high purity and good yield, synaptosomal membranes isolated in this way appear to provide an excellent preparation for the study of membrane structure and function. One membrane function of particular interest to neurobiologists, the ability to catalyze the incorporation of amino acids into membrane proteins in vitro, is examined in detail in the following paper (34).

Acknowledgments—We are grateful to Dr. Staffen Smeds for taking the electron micrographs, and to Professor Holger Hydén for his hospitality and support.

REFERENCES

1. GRAF, F. G., AND WHITAKER, V. P. (1966) J. Physiol. 103, 2
27. FISHER, S., AND DE ROBERTIS, E. (1967) Brain Res. 5, 31
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