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THE POLYPEPTIDE AND THE PHOSPHOLIPID COMPONENTS OF AXON PLASMA MEMBRANES

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SUMMARY

The axon plasma membrane fraction isolated from garfish olfactory nerve was analyzed for its polypeptide composition by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. There were present over 20 well-resolved polypeptide components in this membrane, and eleven of them, with an apparent molecular weight range of 22 000–130 000, accounted for most of the membrane proteins. None of the major polypeptide species present in the membrane appeared to be glycoprotein. Based on electrophoretic mobility on sodium dodecyl sulfate-polyacrylamide gel, eight of the major polypeptides found in garfish nerve membrane appeared to be also present in the axon plasma membrane isolated from lobster walking leg nerve. Both garfish and lobster nerve membranes contained high concentration of lipids (66–76 %) which were essentially cholesterol and phospholipids. The classes of phospholipids present were phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol and sphingomyelin. Lobster nerve membrane also contained about 3 % phosphatidic acid. Assays for acetylcholinesterase in axon plasma membrane fractions isolated from different nerve sources showed a wide variation, ranging from a specific activity of 2.4 for garfish nerve to 312.5 for lobster nerve membrane.

INTRODUCTION

Since the identification of garfish olfactory (*Lepisosteus osseus*) nerve by Easton [1] as an excellent source of axon plasma membrane several reports have appeared on the biochemical [2–8] and physiological [9–14] characterization of axon membrane using this nerve. We have recently reported [15] the isolation of an axon plasma membrane fraction from this nerve. This membrane fraction was identified as axon plasma membrane from its yield, its $(Na^+ + K^+)$-activated ATPase activity and its specific interaction with ^3H-labelled tetrodotoxin [8]. The membrane was obtained in high yield and in sufficient amounts to be useful for detailed biochemical studies of excitable membrane and its components. The axon plasma membrane is unusually

rich in lipids, and a detailed study on the composition and characterization of the lipids of this membrane has been made [4, 15]. Here we report the results on the polypeptide and glycoprotein components of this membrane, obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [16]. The polypeptide components of another axon excitable membrane preparation isolated from lobster walking leg nerve [17] have also been analyzed and are compared with those of garfish nerve membrane. Several major polypeptide components of similar apparent molecular weights are present in both membrane preparations. We also report the phospholipid compositions and acetylcholinesterase activities of membrane preparations isolated from different nerve sources.

MATERIALS AND METHODS

Preparation of axon plasma membranes

The procedures used for the isolation of axon plasma membrane fractions from garfish [15] and lobster [17] nerves have been reported previously. In the case of garfish nerve it consisted of homogenization of the nerve in 0.25 M sucrose, 5 mM Tris buffer (pH 7.4), followed by centrifugation of the homogenate twice over 1.195 M sucrose in a SW 25.1 rotor for 90 min at 25 000 rev./min, and finally separation of the total plasma membrane fraction, obtained as a sharp white band at the interface over a discontinuous sucrose gradient system. The axon plasma membrane accumulated at the interface between 20 and 30 % sucrose. In the case of lobster nerve, the total plasma membrane fraction obtained on centrifugation of nerve homogenate in 0.33 M sucrose over 1.195 M sucrose was separated into two membrane fractions by centrifugation over a linear gradient between 0.66 and 1.195 M sucrose. The light membrane fraction, which collected at a region corresponding to an apparent density of 1.072 g/cm^3, was identified as the axon plasma membrane. The membranes used in most of these studies were stored frozen at −30 °C for up to 3 months in 0.25 M sucrose, 5 mM Tris, pH 7.4, until used. No differences in $(Na^+ + K^+)$-activated ATPase activity, tetrodotoxin specific binding or in polypeptide pattern on sodium dodecyl sulfate-polyacrylamide gel electrophoresis were observed when the frozen samples were compared with those of non-frozen samples analyzed immediately.

Chemical and enzymatic analyses

Protein concentrations were determined by the method of Lowry et al. [18] after solubilization with deoxycholate, using bovine serum albumin as the standard. Lipids were extracted by the procedure of Folch et al. [19] and also by the technique of Bligh and Dyer [20]. Since the lobster nerve was shown not to contain any gangliosides [21], the extraction procedure of Bligh and Dyer, when applied to membrane isolated from this nerve, should not result in any loss of lipid in the water layer. Lipid phosphorus was determined by the micro method of Rouser et al. [22] and cholesterol by the procedure of Zlatkis et al. [23]. Phospholipid composition was determined by two-dimensional thin-layer chromatography followed by phosphorus analysis [22]. For carbohydrate determination, the membrane was purified and delipidated according to the procedure of Glossmann and Neville [24]. The dried protein residue was used for the determination of total hexoses, aminohexoses and sialic acid. Total hexose concentration was determined by the anthrone method described by Spiro [25]

using D-glucose as a standard. For hexosamine determination, the sample was hydrolysed in 2 M HCl at 100 °C for 15 h and the hexosamine determined according to the method of Gatt and Berman [26] using galactosamine hydrochloride as a standard. Sialic acid was determined with thiobarbituric acid according to the method of Aminoff [27] after hydrolysis in 0.1 M HCl at 84 °C for 30 min. *N*-Acetylneuraminic acid was used as the standard. $(Na^+ + K^+)$-activated ATPase activity was determined according to the procedure of Wallach and Kamat [28] and was estimated as the increase in the rate of ATP hydrolysis which occurred in the presence of both Na^+ and K^+ over the rate when K^+ was lacking. The $(Na^+ + K^+)$-activated ATPase was also measured under the conditions described by Grefrath and Reynolds [7]. The activity is expressed in terms of μmol of ATP hydrolysed per h per mg membrane protein. Acetylcholinesterase was assayed by the method of Ellman et al. [29] as modified by Klingman et al. [30]. The activity is expressed in terms of μmol of acetylthiocholine hydrolysed per h per mg membrane protein. The combined histochemical and electron microscopic technique for localization of acetylcholinesterase in the whole nerve is described elsewhere [31]. Live nerve samples pre-fixed in glutaraldehyde were sectioned into thin slices and these were incubated in the medium of Koelle and Friedenwald [32] containing acetylthiocholine iodide as substrate. After incubation, post-fixation, dehydration and embedding as described elsewhere [31], fine sections were observed in the electron microscope for the deposition of copper thiocholine, the end product of the enzymatic reaction. The binding of tetrodotoxin to the axon plasma membrane was determined using ^{3}H-labelled tetrodotoxin by the procedure described elsewhere [8].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis of axon plasma membrane protein was carried out according to the procedure of Fairbanks et al. [16]. The membrane fractions for electrophoresis were prepared by solubilising them in a solution (2 mg protein/ml) containing 1 % sodium dodecyl sulfate, 0.6 % dithiothreitol, 10 % sucrose, 1 mM EDTA and 0.01 % bromophenol blue in 0.01 M Tris, pH 8.0, followed by heating in a boiling water bath for 15 min. Approx. 20–40 μg protein (in 10–20 μl of solution) was used for each gel. In runs where gels were used for glycoprotein stain, samples containing up to 200 μg protein were employed. The electrophoresis was carried out at a constant current of 4 mA per gel for about 2.5 h; the tracking dye, bromophenol blue, moved to about 1 cm from the bottom of the gel. By running the electrophoresis for shorter period of time it was determined that none of the polypeptide components of the samples migrated ahead of the bromophenol blue dye. Gels were stained for protein with Coomassie blue essentially according to Fairbanks et al. [16]. For carbohydrate staining the gels were washed first according to Fairbanks et al. [16] and then stained with Schiff reagent after periodic acid oxidation according to Korn and Wright [33]. To check the technique of periodic acid-Schiff staining, ovalbumin was always used as a marker glycoprotein.

Molecular weight estimation

The molecular weights of the polypeptide components of axon plasma membrane were estimated from the calibration curve obtained when the relative mobilities (relative to bromophenol blue) of proteins of known molecular weights, electro-

phoresed under the same conditions as those of the membrane proteins, were plotted against their logarithms of molecular weights. The protein standards used were myoglobin (17 600), chymotrypsinogen (25 700), ovalbumin (43 000), bovine serum albumin (68 000) and γ-globulin (160 000). For electrophoresis all protein standards were solubilized in the solubilizing medium of Fairbanks et al. [16], except γ-globulin which was dissolved in the solubilizing medium containing no dithiothreitol. A plot of the relative mobility vs. the logarithm of molecular weight of the standard proteins was found to be linear.

RESULTS

Preparation of axon plasma membrane. The discontinuous sucrose density gradient centrifugation procedure developed for the isolation of membrane fraction from garfish olfactory nerve gives two subfractions of axon plasma membrane [15]. The minor subfraction (about 25 %, by protein) collects just above the interface between 20 and 30 % sucrose and the major subfraction (about 75 %, by protein) collects at the interface. A similar type of separation pattern was obtained by Cancalon and Beidler [34] when the same procedure was applied on a smaller scale for the isolation of plasma membrane fractions from garfish olfactory nerve. Grefrath and Reynolds [7] also obtained two membrane subfractions when a Ficoll-sucrose discontinuous gradient system [35] was used for the isolation of plasma membrane from this nerve. These membrane subfractions show minor differences in the total lipid to protein ratio; however, they have similar phospholipid composition and similar phospholipid to cholesterol molar ratio. They also have similar polypeptide patterns on sodium dodecyl sulfate-polyacrylamide gel electrophoresis [15]. The reason for the formation of subfractions of axon plasma membrane is not clear. It is possible that they are membrane vesicles of different purity and homogeneity, although there are probably other explanations such as they being membrane vesicles formed from membranes of different regions (either with respect to location [7] or functions of the axon fibers.

In order to see whether possible differences exist along the axon fibers with respect to the ratio of the two axon plasma membrane subfractions, olfactory nerve from uniformly sized garfish snouts (about 6 inches long) were obtained and each nerve cut into three equal segments. The corresponding segments of each nerve were combined and axon plasma membrane fractions were isolated. No qualitative difference was seen in the ratio of the two axon plasma membrane subfractions isolated from the three segments of the garfish olfactory nerve.

In electron micrographs it is always found that the light subfraction is more homogeneous and free from inter- and intravesicular non-membranous material than the other membrane fraction. Furthermore, the light subfraction has a little higher $(Na^+ + K^+)$-activated ATPase and tetrodotoxin uptake than the major subfraction. The results presented in this paper are obtained on the combined axon plasma membrane fraction.

Chemical composition. Table I gives the chemical composition of axon plasma membrane fraction isolated from garfish olfactory nerve. One of the characteristics of the garfish olfactory nerve axon plasma membrane is its high concentration of lipids (66 %) which consist entirely of phospholipids (74 %) and cholesterol (26 %). Similar

TABLE I

COMPOSITIONAL AND ENZYMATIC PROPERTIES OF AXON PLASMA MEMBRANE FROM GARFISH OLFACTORY NERVE

Protein (%)	34
Total lipid (%)	66
Phospholipids (percent of total lipids)	74
Cholesterol (percent of total lipids)	26
Total hexoses (μg/mg protein)	12.6±0.9*
Hexosamine (μg/mg protein)	7.3±1.2**
Sialic acid (μg/mg protein)	0.85±0.1***
Tetrodotoxin binding (pmol/mg protein)	
Fresh sample	3.7
Frozen sample	3.7
$(Na^+ + K^+)$-ATPase (μmol ATP/mg protein per h)	6.8†
Acetylcholinesterase (μmol acetylthiocholine/mg protein per h)	2.4

* D-Glucose as standard.

* D-Galactosamine as standard.

*** *N*-Acetylneuraminic acid as standard. Each value is a mean value from six determinations.

† Estimated according to the procedure described in ref. 7.

high concentrations of lipids were also found in the axon plasma membrane fractions isolated from lobster nerve (76 %) and squid stellar nerve (70 %) [36]. Table I also gives data on the amount of carbohydrate in the axon plasma membrane protein. The membrane contains 12.6 and 7.3 μg of total hexose and hexosamine, respectively, per mg protein. These concentrations are low compared to the amounts of total hexose and hexosamine reported for other types of plasma membranes [24]. Sialic acid, if present, occurs only in traces in this membrane. This low concentration of carbohydrate in the membrane protein is reflected in the low level of periodic acid-Schiff staining of polypeptide bands in sodium dodecyl sulfate-gel electrophoresis which is described elsewhere.

Tetrodotoxin binding. ^{3}H-labelled tetrodotoxin binds to the membrane with a dissociation constant of $5.5 \cdot 10^{-9}$ M and a maximal binding of 3.7 pmol of tetrodotoxin per mg of membrane protein (Table I). This tetrodotoxin binding ability of the membrane is not diminished if stored frozen as a suspension in 0.25 M sucrose, 5 mM Tris, pH 7.4, up to 3 months.

$(Na^+ + K^+)$-ATPase activity. The specific activity of $(Na^+ + K^+)$-activated ATPase of the axon plasma membrane estimated as the difference in activity in the presence and absence of 0.5 mM ouabain (strophanthin-G) under the conditions described by Grefrath and Reynolds [7] is 6.8 (Table I) and it is about 70 % of the total $(Na^+ + K^+ + Mg^{2+})$-activated ATPase. This value is similar to that estimated before (4.5–6.7) [15] by the procedure of Wallach and Kamat [28]. A higher $(Na^+ + K^+)$-activated ATPase activity (25.8, 28.8) was reported for an axon plasma membrane preparation from frozen garfish olfactory nerve by Grefrath and Reynolds [7]. The reason for this difference is not known.

Acetylcholinesterase activity. The axon plasma membrane fraction isolated recently from lobster leg nerve [17, 37] has been shown to possess a high degree of acetylcholinesterase activity. The concentration of this enzyme in the axon plasma membrane of garfish olfactory nerve was found to be surprisingly low (Table I). It is

less than 1 % of that found for lobster axon plasma membrane. No specific enrichment of the enzyme activity was found in the axon plasma membrane fraction during its purification from the nerve homogenate. The nerve homogenate, when analyzed for the enzyme, showed a similar low activity, thus ruling out the possibility that acetylcholinesterase has been removed during the preparation of the membrane. Inclusion of Triton X-100 (0.1 %) in the assay medium did not increase the enzyme activity [34]. The very low activity of acetylcholinesterase in the garfish olfactory nerve was confirmed by histochemical techniques combined with electron microscopy. In agreement with the small amount of the enzyme detected by the biochemical measurements, a virtual absence of positive reaction to acetylcholinesterase activity was observed when attempts were made to locate the enzyme histochemically in the nerve according to the procedure of Koelle and Friedenwald [32] (Fig. 1). The same technique when applied to lobster nerve gave a positive reaction (Fig. 2), as evidenced by the deposition of dense granules of copper thiocholine end product, mostly at the interface between the axon and the Schwann cell plasma membranes.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and polypeptide composition. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis and staining procedures of Fairbanks et al. [16] were found to yield the best results when applied to the analysis of garfish axon plasma membrane proteins. Fig. 3 shows the polypeptide

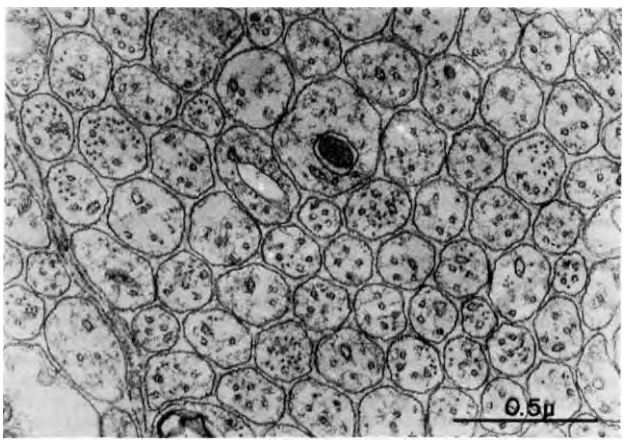

Fig. 1. Attempted localization of acetylcholinesterase in axon fibers of garfish olfactory nerve. Nerve was incubated in Koelle-Friedenwald medium containing acetylthiocholine and copper sulfate. No acetylcholinesterase activity is seen. Acetylcholinesterase if present would be indicated by the formation of dense granules of copper thiocholine end product. For positive reaction of this enzyme see Fig. 2. Magnification × 58 000.

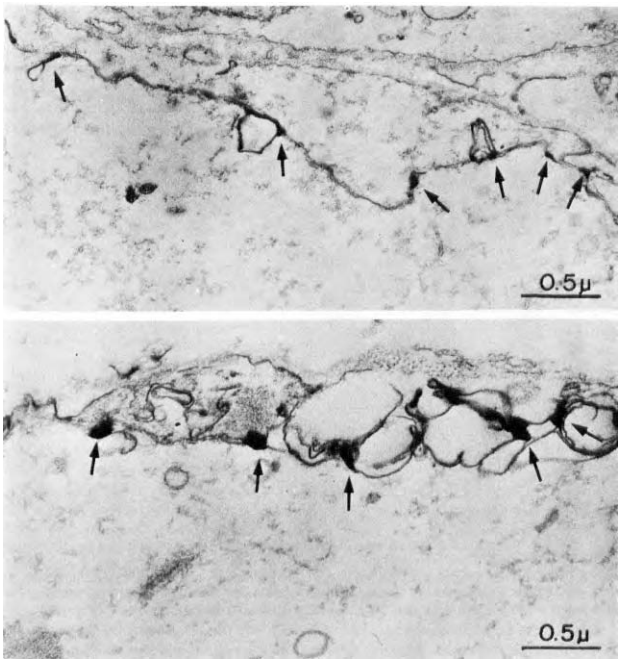

Fig. 2. Localization of acetylcholinesterase in lobster walking leg nerve. Nerves were incubated in Koelle-Friedenwald medium containing acetylthiocholine and copper sulfate. Electron dense granules of copper thiocholine end product are seen at the interface between axon and Schwann cell plasma membranes (arrows). Magnification, × 30 000.

pattern of the garfish nerve axon plasma membrane (G) separated on a 5.6 % polyacrylamide gel containing 1 % sodium dodecyl sulfate and stained with Coomassie blue. Although about 20 well-resolved polypeptide bands are present, eleven of them (arbitrarily named I–XI) account for more than 90 % of the total polypeptide components. The most predominant component is V and the other components occur in nearly equal concentration except I and II which are present only in relatively small concentrations. In addition, about ten minor polypeptide components are found in garfish axon plasma membrane, and these are consistently seen in the preparations.

Molecular weights of polypeptides. The estimated molecular weights of the

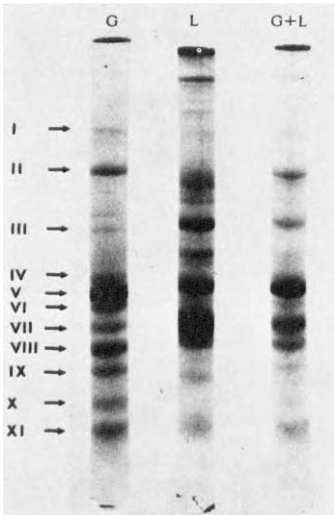

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of axon plasma membranes of garfish olfactory nerve (G), lobster walking leg nerve (L) and an equal mixture of garfish and lobster nerves (G+L). Membrane polypeptides were separated into components on a 1% sodium dodecyl sulfate gel of 5.6% polyacrylamide and stained with Coomassie blue according to the procedure of Fairbanks et al. [16]. The major bands are identified arbitrarily I–XI.

major polypeptide species of garfish axon plasma membrane are shown in Table II. They fall within the range of the molecular weights of the standards (17 500 for myoglobin to 160 000 for γ-globulin). The predominant species, V, has a molecular weight of 53 000, and the other major species range in molecular weights from 130 000 (I) to 22 000 (XI). When considering all the polypeptide species, the molecular weights range from 22 000 to over 200 000. Although the range of the molecular weights of polypeptide components is comparable to those reported for erythrocytes [38] and other plasma membranes [39], the axon plasma membrane contains very little of high molecular weight species.

Comparison of garfish axon plasma membrane polypeptides with those of lobster axon plasma membrane. Comparative studies on the polypeptide components of axon plasma membranes of different species could provide information on polypeptides involved in the functioning of excitable membrane. The axon plasma membrane fraction isolated from lobster nerve recently by Barnola et al. [17] was therefore analyzed for its polypeptide pattern by sodium dodecyl sulfate-polyacrylamide gel

TABLE II

MOLECULAR WEIGHTS OF MAJOR POLYPEPTIDES OF GARFISH AXON PLASMA MEMBRANE, ESTIMATED BY SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS

The polypeptides are labelled in Fig. 3.

Polypeptide	Molecular weight
I	130 000*
II	110 000*
III	78 000*
IV	57 000
V	53 000*
VI	47 000
VII	42 000*
VIII	38 000*
IX	32 000*
X	26 000
XI	22 000*

* Polypeptides of similar apparent molecular weights are also present in lobster nerve axon plasma membrane.

electrophoresis and compared with those of the garfish nerve membrane. Fig. 3 shows the electrophoretic patterns of the polypeptides of the lobster nerve membrane (L) and of a mixture of garfish and lobster nerve membranes (G+L). A remarkable similarity appears to exist between the polypeptide components of the two axon plasma membranes. Both have nearly the same number of polypeptide subunits. Eight major polypeptides with similar apparent molecular weights are found in both membrane preparations, although the relative concentrations of these components in the two membranes are different. In addition, several minor components with similar electrophoretic mobilities are also present in both membranes. A polypeptide component in one membrane is considered similar to a component of another one when these polypeptide components do not split into two bands when a mixture of the membranes is electrophoresed, although it should be emphasized that similar electrophoretic mobilities in the gel are not convincing proof for the identity of the polypeptides. The IV, VI and X polypeptide components found in garfish nerve membrane appear to be missing in lobster nerve membrane, but it contains a major component with a molecular weight of about 66 000 not found in gar nerve membrane.

It is found that in the case of lobster nerve membrane, a considerable amount of material does not enter the gel and remains at the origin of the gel as Coomassie blue staining material, although under the same conditions all the garfish nerve membrane material enters the gel. It should also be noted that whereas component II of gar nerve membrane always appears as a sharp discrete band on sodium dodecyl sulfate gel, the band corresponding to II in the lobster nerve membrane appears broad suggesting not only the possible difference between these two components but also the presence of more than one molecular species in component II of lobster nerve membrane.

Glycoprotein of axon plasma membrane. The periodic acid-Schiff staining procedure of Korn and Wright [33] gave the best results for detection of glycoprotein

components after sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the membrane proteins. No background coloration is formed if the gels are first washed according to the procedure of Fairbanks et al. [16] before the periodic acid-Schiff staining. As little as 0.2 μg total hexose containing glycoprotein (10 μg ovalbumin) could be detected. For identification of the polypeptide species, the gels after periodic acid-Schiff stainings were stained lightly with Coomassie blue and compared. None of the major polypeptide species present in gar nerve membrane were found to show any noticeable periodic acid-Schiff staining when as much as 200 μg membrane protein was used for the electrophoresis and staining. However, two broad, weak periodic acid-Schiff staining areas were seen in the high molecular weight region of the gel where no prominent Coomassie blue staining component was present. In the case of lobster nerve membrane a sample containing 50 μg protein showed the presence of a periodic acid-Schiff staining band, which appears to correspond to component VII in the Coomassie blue stain (molecular weight, 42 000). This component is the most prominent component in lobster nerve membrane but is present only in relatively small concentration in gar nerve membrane.

Phospholipid composition. Table III compares the phospholipid composition of axon plasma membranes from different sources. Major phospholipid classes are phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine and sphingomyelin. In the lobster nerve membrane, phosphatidylethanolamine is the component present in the highest concentration whereas phosphatidylcholine is present in the highest concentration in the membranes of garfish [15], squid stellar nerve [36] and squid retinal nerve [40]. A relatively high concentration of phosphatidic acid (3.3 %) is found in the lobster nerve membrane. A similar phosphatidic acid concentration was reported by Sheltawy and Dawson [21] in the total lipids of lobster nerve. Anal-

TABLE III

PHOSPHOLIPID COMPOSITION OF AXON PLASMA MEMBRANES ISOLATED FROM DIFFERENT SOURCES

The values are represented as percentage of total lipid phosphorus.

Phospholipid	Lobster leg nerve	Squid stellar nerve*	Garfish olfactory nerve**	Squid retinal nerve***
Phosphatidyl-ethanolamine	43.3	34.4	33.6	37.3
Phosphatidyl-choline	25.4	45.9	42.2	39.7
Phosphatidylserine	12.0	10.4	12.0	9.7
Phosphatidylinositol	3.1	–	4.0	–
Sphingomyelin	12.8	10.0	7.5	3.9
Phosphatidic acid	3.3	–	tr	–
Diphosphatidyl-glycerol	tr	–	tr	–
Lysophospholipids	–	–	–	6.6

* Values taken from ref. 36.
** Values taken from ref. 15.
*** Values taken from ref. 40.
tr, trace.

yses of membrane fractions isolated from lobsters collected at different times of the year, as well as from lobsters of two different sizes (358 g vs. 1225 g average weight) showed no difference in their phospholipid compositions.

DISCUSSIONS

The finding of an insignificant amount of acetylcholinesterase activity in the garfish axon plasma membrane preparation and in the whole nerve was unexpected because of the ubiquitous presence of this enzyme in nerve tissues in general, the detection and preferential location of the enzyme in high concentration in axon plasma membrane fraction of lobster nerve [17, 37] and because of the possible role of acetylcholinesterase and acetylcholine in the mechanism of nerve impulse conduction [41]. However, assays for the enzyme in axon plasma membrane preparations isolated from different sources of nerve have shown a wide variation. Thus the specific activities of acetylcholinesterase of axon plasma membranes from garfish olfactory, squid optic, squid stellar and fin, and lobster leg nerves are 2.4, 8.9, 44.9, and 312.5, respectively. Such a large variation of enzyme activity does not indicate that acetylcholinesterase has a specific role in the mechanism of nerve impulse conduction. The amount of enzyme may, however, be related to the extent of axon-glial cell membrane interaction that may exist in these nerves. At least in squid stellar nerves there is enough evidence to suggest the existence of axon-Schwann cell interaction, mediated by a cholinergic system [42]. In the garfish olfactory and the squid optic nerves, which have the lowest concentration of acetylcholinesterase, the majority of axons do not contact the glial cell membrane and the axons occur in bundles of several hundreds, each bundle surrounded only by a single glial cell.

Compositional studies on axon plasma membranes from several sources now indicate that these membranes have a high content of lipids and that the lipids consist essentially of cholesterol and phospholipids. The phospholipids are phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol and sphingomyelin. The axon membrane from lobster nerve also contains about 3 % phosphatidic acid. The fatty acids associated with these phospholipids are unusually rich in polyunsaturated fatty acids [4, 15, 40].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of garfish axon plasma membrane protein has yielded eleven major and ten minor polypeptide species. This polypeptide pattern is somewhat different from the pattern reported by Grefrath and Reynolds [7] on the axon plasma membrane isolated from frozen garfish olfactory nerve. Differences are found in the number, the relative concentration and also in the apparent molecular weights of the polypeptide species. This variation may be due to the differences in the techniques of sodium dodecyl sulfate electrophoresis, although the differences in the method of isolation of the membrane fractions from the nerve or the condition of the starting nerve tissue (fresh or frozen) may also have contributed to the variation in the results. The concentration of sodium dodecyl sulfate used to solubilize and electrophorese the membrane protein in the procedure of Fairbanks et al. [16], which we have used, is 1 % whereas a sodium dodecyl sulfate concentration of 0.1 % was used by Grefrath and Reynolds [7] in their studies. It may be that under these conditions the polypeptide chains were not completely disaggregated and the electrophoresis resulted in fewer of polypeptide species. The conclusion,

based on the presence of periodic acid-Schiff-positive material at the dye front in the sodium dodecyl sulfate gels that the axon plasma membrane contains the glycolipid, reported earlier [4] in the lipids of the whole nerve, is also at variance with our results. This glycolipid is not found in any appreciable concentration in the isolated axon plasma membrane fraction, although it is found in the whole nerve lipids. The strong periodic acid-Schiff positiveness found at the dye front of the gel seems due not to the glycolipid but to the plasmalogens (which liberate aldehydes under acid conditions) which are present in high concentration in the phosphatidylethanolamine fraction of the lipids of this membrane [4].

The concentration of glycoproteins present in the garfish axon plasma membrane is low, as indicated by estimation of carbohydrates in membrane protein, as well as by periodic acid-Schiff staining of the polypeptide species separated on sodium dodecyl sulfate gel. It is significant that only traces of sialic acid could be detected. On sodium dodecyl sulfate gel two faint broad periodic acid-Schiff-positive areas in the high molecular weight region of the gel could be seen when as much as 200 μg protein-containing membrane was used for electrophoresis. However, these bands do not correspond to any major polypeptide species stained by Coomassie blue. Glycoproteins are widely distributed, even though as minor components, in biological membranes and have been considered to play several important biological roles [43] such as in regulation of cell growth, antigenicity, cellular recognition, and cell adhesion. A relative low concentration of glycoproteins in the axon plasma membrane of garfish olfactory nerve is compatible with these postulated roles of glycoproteins, since only a limited cell-to-cell or membrane-to-membrane interaction is likely to exist in these nerves between axons and glial cells.

The apparent similarity in polypeptide components between the garfish and lobster axon plasma membranes is remarkable. Based on electrophoretic mobility on sodium dodecyl sulfate-polyacrylamide gel, eight of the eleven major plus several minor polypeptides found in garfish membrane appear to be present also in the lobster membrane. There are, however, differences in relative concentrations among the individual polypeptides of the two preparations. It may be significant that the predominant polypeptide component V of garfish membrane is also present in relatively equal concentration in lobster. The quantitative differences among the other polypeptide components may be due to the functional differences of the membranes in the two species, or may be due to the differences in the technique of isolation of the two axon membrane preparations. In any case, the close similarity between the polypeptide components of axon plasma membrane of distantly related species suggests that each of these apparently similar polypeptides may play an essential role in the structure and function of excitable membrane. This type of similarity in membrane polypeptide components has been reported for erythrocyte membranes of different species [44].

Because of the specialized function of axon plasma membrane, a relatively small number of polypeptide components might be expected in this membrane. It is likely that several of these polypeptide species found on sodium dodecyl sulfate gel electrophoresis exist together as a functionally viable unit in the membrane. The identification and characterization of these polypeptide components with respect to their function would require the isolation of functionally viable entities such as tetrodotoxin binding components, and their subsequent analysis. Specific modification of

the membrane components followed by chemical and functional analyses of the membrane could also be expected to provide information on the functional role of not only the polypeptide components but also phospholipids. Experiments along these lines are in progress in our laboratory.

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