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Characterization of the Lipid and Polypeptide Components of a Tetrodotoxin Binding Membrane Fraction from *Electrophorus electricus*

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Summary. This paper reports an analysis of the lipid and polypeptide composition of a tetrodotoxin (TTX)-binding plasma membrane fraction of the eel electroplaque. Phospholipids comprise 73% of the total lipid with cholesterol and neutral glycerides constituting about 21 and 6%, respectively. The major phospholipids are phosphatidylcholine (47.3%), phosphatidylethanolamine (32.6%), phosphatidylserine (13.1%), and sphingomyelin (4.5%). Phosphatidylinositol and phosphatidic acid are minor components. Plasmalogens comprise approximately 19% of the total phosphatidylethanolamine. Each major phospholipid class was analyzed for fatty acyl composition. The results indicate a unique distribution profile for each class with respect to chain length and unsaturation. PE and PS both contain high percentages of polyunsaturated fatty acids particularly docosahexaenoic acid with constitutes 35 and 39% of the total fatty acids, respectively. However, PC and PS contain significantly lower levels of polyunsaturated fatty acids. The lipid profile observed in this preparation is compared to those previously reported for membranes from other excitable tissues. Polyacrylamide gel electrophoresis of the membranes indicates a complex distribution of peptides with several major species and at least 30 minor components. Two of the major species have molecular weights corresponding to those of the two subunits of the $(Na^+ + K^+)$-ATPase.

Propagated action potentials in electrically excitable cells are known to result from transient changes in the permeability of the cell surface membrane to cations. The resulting ion movements presumably occur through membrane pores or channels which have such unique properties as ion selectivity and gating [*see* reviews 2, 16]. There is now considerable interest in elucidating the chemical and physical properties of these channel components since such characterization will undoubtedly provide invaluable insights into the molecular basis for electrical excitability in biomembranes.

Until relatively recently, much of our understanding about the properties of these channels had been obtained from electrophysiological studies on intact nerve and muscle fibers. However, the availability of radioactively labeled channel-specific neurotoxins has now provided the potential for more detailed chemical studies and ultimate isolation of purified channel components. Using [^{3}H] tetrodotoxin (TTX), a Na^+-channel specific probe, it is now possible to identify channel sites in intact cells and in membrane preparations from a variety of electrically excitable tissues [29] including the electroplaque of *Electrophorus electricus* [28]. The latter is a particularly useful system since the electric organ which constitutes a large percentage of the total body weight is quite homogeneous and relatively large quantities of membranes can be conveniently prepared. Recently, a [^{3}H]TTX-binding protein fraction has been purified from detergent-solubilized electroplaque membranes, presumably representing at least part of the total Na^+-channel structure [1]. The electric organ therefore provides an ideal source of membranes for channel isolation and also for studies on the dynamic properties of the channel in an intact native environment where alterations in lipid-protein interactions are minimized.

In this paper, we report on some of the properties of a [^{3}H]TTX-binding plasma membrane fraction isolated from the electric organ of *Electrophorus electricus*, focusing mainly on the overall lipid composition. These studies include an analysis of not only the relative amounts of the major phospholipids but

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also the fatty acyl chain distribution within individual phospholipid classes. The results obtained are compared to those reported for other excitable tissues.

Materials and Methods

Preparation of Membrane Fragments

Electrophorus electricus (World Wide Aquarium, New York) were killed and the electric organs excised as previously described [28]. The tissue was cut into small sections, frozen in liquid nitrogen, and stored at −70 °C for later use. Portions of the tissue were thawed and used immediately for the preparation of membranes. The isolation procedure was essentially as described previously in detail [28], although minor modifications were made. The electric organ was finely minced, homogenized with a glass Teflon homogenizer in 7 volumes of ice cold 0.25 M sucrose and centrifuged at $600 \times g$ for 5 min: The cloudy supernatant (*S1*) was discarded and the particulate material resuspended in 3 volumes sucrose, and filtered several times through cheesecloth and through a fine metal mesh. After sonication for 1 min (Braunsonic, 50 watts), the suspension was filtered again (*P1*) and the particulate fraction collected by centrifugation ($40{,}000 \times g$, 45 min). Pellets were resuspended in 30% sucrose with homogenization. Following centrifugation at $10{,}000 \times g$ for 20 min, the cloudy supernatant was collected. The density of the suspension was reduced by dilution with H_2O to 15% and centrifuged for 40 min at $150{,}000 \times g$. The white loosely packed membraneous material (*P2*) was resuspended in 0.25 M sucrose taking care not to dislodge the small tightly packed tan-colored pellet (*P3*) in the bottom of the tube, which contained substantial intracellular membrane contaminants.

The following enzyme marker activities were measured according to the indicated methods: succinate dehydrogenase (succinate-INT oxidoreductase) [25], NADPH-cytochrome *c* reductase [37], acetylcholinesterase [12]. Oubain-sensitive ($Na^+ + K^+$)-ATPase activity and [^{3}H]TTX binding were determined as described previously [28]. Protein was measured by the Lowry method [22] using bovine serum albumin as a standard.

Isolation of Membrane Lipids

Lipids were extracted by the addition of 20 volumes of chloroform/methanol (2:1) to an aqueous suspension of membranes (2–3 mg per ml). The samples were flushed with N_2, and left overnight at 4 °C. The extracts were washed once with 0.2 volumes of chloroform/methanol/H_2O (3:48:47) and twice with 0.2 vol of chloroform/methanol/0.75% KCl (3:48:47). The washed extracts were pooled and stored under N_2 at −70 °C for phospholipid, cholesterol, and triglyceride analyses or used immediately for analysis of phospholipid fatty acids. In preliminary studies samples were serially extracted as described by Rouser et al. [33]. Subsequent lipid analysis showed no differences between this exhaustive extraction and the procedure described above and the latter was routinely used.

Phospholipid phosphorus was determined by the method of Eng and Noble [13] following digestion of lipids for 1 hr at 190 °C in 70% perchloric acid. Values for phosphorus were converted to mg phospholipid by assuming a mol wt of 800 for phospholipids.

Cholesterol and triglyceride analyses were carried out using a Technicon Auto Analyzer II (Technicon Instruments Corporation, Chauncey, N.Y.) [23]. Aliquots of the lipid were dried down and redissolved in isopropanol, extracted with zeolite mixture, and analyzed for triglycerol and cholesterol.

Phosphatidylethanolamine plasmalogens were determined by the method of Schmid and Takahashi [35]. The phosphatidylethanolamine was separated from the other phospholipids by thin layer chromatography as described below. All organic solvents used for lipid analysis were BDH Glass Distilled (BDH Chemicals, Can.). High purity N_2 was routinely used.

Phospholipid Analysis

Phospholipids were separated by two-dimensional thin-layer chromatography on silica gel H plates prewashed in chloroform/methanol/H_2O (65:25:4) and activated at 120 °C. The plates containing about 45 to 55 μg lipid phosphorus were developed first in chloroform/methanol/28% ammonia (65:35:5) and in the second dimension in chloroform/methanol/acetone/acetic acid/H_2O (50:10:20:10:5) [32]. The lipids were visualized with iodine vapor and the corresponding areas removed by aspiration and analyzed for phosphorus. Several random areas of silica gel were taken off the plate to serve as blanks. In addition, an aliquot of original extract was dried down for total phosphorus analysis.

Fatty Acid Analysis

For analysis of the fatty acids the individual phospholipid classes were separated as described above, taking precautions to avoid oxidation of the lipids. The phospholipids were visualized under ultraviolet light following 2,7 dichlorofluorescein spray (0.2% in 95% methanol) and rapidly transferred to ampoules. The fatty acids were converted to the methyl esters by the addition of 4 ml of 4% H_2SO_4 in methanol. In addition, an aliquot of the original washed lipid extract was dried down and treated in a similar manner for total fatty acid determination. The ampoules were sealed under N_2 and heated at 90 °C for $1^1/_2$ hr. The methyl esters were subsequently extracted with hexane and separated by thin layer chromatography (silica gel G) in benzene. The methyl esters were visualized with 2,7 dichlorofluorescein and extracted with chloroform/methanol (2:1). The extracts were dried down under N_2 and dissolved in isooctane (G.C.-spectrophotometric grade). Fatty acid analysis by gas-liquid chromatography was carried out using a Hewlett-Packard Gas Chromatograph Model 7620A (Hewlett-Packard, Canada). Glass columns (6 feet by 2 mm ID) were packed with either 15% EGS on chromosorb W, 80/100 mesh, NAW (Chromatographic Specialities, Brockville, Canada) or 15% HI-Eff-2BP on Gas-Chrom P 80/100 mesh (Applied Science Laboratories, State College, Pa.). Operating conditions were: injector 230 °C, detector 230 °C, column 180 °C, and carrier gas (HE) 30–40 ml/min. The instrument was calibrated with standard fatty acid mixtures supplied by Nu-Prep Chek (Elysian, Minn), Sulpeco Inc. (Bellefonte, P.A.) and Serdary Research Laboratories, Inc. (London, Canada). The area under each of the fatty acid peaks was determined by a Hewlett-Packard Integrator Model 3380A (Hewlett-Packard, Canada) interfaced with the gas chromatograph. The amounts of each fatty acid present are expressed as a percentage of the total fatty acid present.

Polyacrylamide Slab Gel Electrophoresis in SDS

The method routinely used was that of Laemmli [20]. The separating gel was 10% acrylamide in 0.1% SDS and the stacking gel, 5% acrylamide in 0.2% SDS. The total gel length was about 21 cm.

Membrane proteins and standards were prepared for electrophoresis by incubation at 100 °C for 2 min. Approximately 45–60 mg of membrane protein in 30 μl was applied to the gel.

Electrophoresis was carried out at 4 °C using a conventional analytical slab gel electrophoresis unit. The gel was run at a constant current of 17 mA for about $1^1/_2$ hr. The current was then raised to 28 mA and the electrophoresis continued until the tracking dye was about 19 cm from the top of the gel (about $4^1/_2$–5 hr). The gel was then removed and stained for 30 min at 40 °C in Coomassie Blue stain [39, 40]. The gels were destained in 5% methanol plus 7.5% acetic acid. The acrylamide, bis acrylamide, Tris, and Coomassie Blue were all electrochemically pure. The molecular weight markers for SDS gel calibration were γ-globulin, phosphorylase *a*, bovine serum albumin, ovalbumin, carbonic anhydrase, and egg lysozyme. All were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Electron Microscopy

Membrane samples were prepared for electron microscopy as follows. Pelleted membranes were fixed in 2% osmium tetroxide in 0.15 M bicarbonate buffer at pH 7.4 for 90 min. Samples were dehydrated in ethanol and embedded in SPURS. Thin sections were stained with uranyl acetate and lead citrate and examined under a Hitachi HU 12 microscope.

Results

The procedure used for the isolation of a [^{3}H]TTX-enriched plasma membrane fraction from the electric eel is a modification of that previously described [28]. These modifications, although minor, nevertheless result in a somewhat lower overall yield, but the final membrane fraction (*P*2) appears to be less contaminated by intramembrane components and by connective tissue fragments. Table 1 summarizes the relative specific activities of key enzyme markers for fractions which represents the original starting material (*S*1 and *P*1) and the two final membrane fractions (*P*2 and *P*3). The fraction *P*2 is used for all the subsequent studies. The sonicated crude particulate fraction (*P*1) is chosen to represent part of the starting material since, during the very initial stages of purification, detailed and reproducible analyses were not always possible because of the large amounts of connective tissue present. Furthermore, several of the marker activities were apparently masked or latent at these stages.

The specific activities of key plasma membrane markers, oubain-sensitive ($Na^+ + K^+$)-ATPase, acetylcholinesterase, and [^{3}H]TTX binding are all enhanced in the fraction *P*2 but significantly lower in *P*3. Furthermore, if one compares the exceptionally high ($Na^+ + K^+$)-ATPase activity with that of the purified enzyme [26], as much as 25–30% of this membrane fraction may be ATPase. It should also be noted that the specific activities of two intracellular marker enzymes, NADPH-cytochrome *c* reductase and succinate dehydrogenase, are not substantially reduced in *P*2 compared to starting material, although an eightfold higher specific activity for the mitochondrial marker is found in fraction *P*3. While these data may suggest significant intracellular contamination in *P*2, this is clearly not the case. Even if one assumes that the *P*3 fraction contains as much as 50% mitochondrial membranes, then the maximum possible contribution in *P*2 can be not greater than approximately 6%. No doubt it is less than that, particularly if one compares the specific activity of

Table 1. Distribution of enzyme marker activities and [^{3}H]TTX binding in electroplaque membrane fractions

Activity	Subcellular localization	Fractions analyzed			
		Supernatant (*S*1)	Sonicated fraction (*P*1)	Membrane fraction (*P*2)	Membrane fraction (*P*3)
(Na^+K^+)-ATPase[a] (units/mg protein)	Plasma membrane	6.7	144	457	271
Succinate dehydrogenase[b] (units/mg protein)	Mitochondrial membrane	1.36	6.44	4.70	36.7
Acetylcholinesterase[c] (units/mg protein)	Plasma membrane	4.45	30.1	70.2	29.2
NADPH cytochrome *c* reductase[d] (units/mg protein)	Endoplasmic reticulum	1.36	0.86	0.99	1.64
[^{3}H]TTX binding[e] (pmol/mg protein)	Plasma membrane	0.26	2.29	6.80	3.33

[a] Ouabain-sensitive activity, 1 unit = 1 μmol ATP hydrolyzed/hr.
[b] Succinate-INT oxidoreductase, 1 unit = 1 μmol succinate oxidized/min.
[c] 1 unit = 1 μmol acetylthiocholine hydrolyzed/min.
[d] 1 unit = 1 μmol NADPH oxidized/min.
[e] [^{3}H]TTX binding was determined with 13×10^{-9} M [^{3}H]TTX and values are corrected for radiopurity (60%) of the toxin.

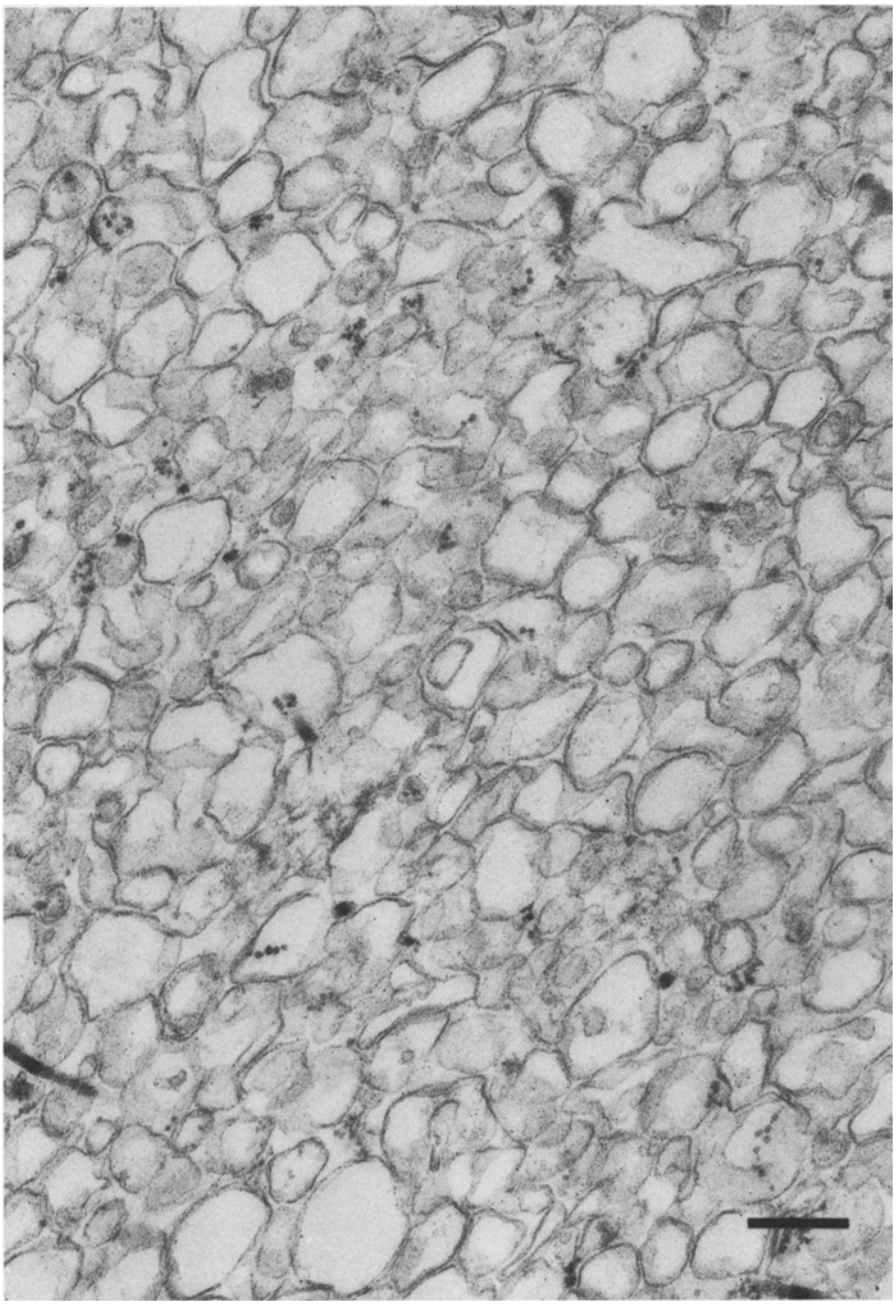

Fig. 1. Electron micrograph of the electroplaque membrane preparation. Membranes were thin sectioned and stained with uranyl acetate and lead citrate as described in the text. The bar corresponds to 300 nm

isolated muscle mitochondria [25] of approximately 100 nmoles min^{-1} mg^{-1} with 4.7 nmoles min^{-1} mg^{-1} in our preparation.

The specific activity of the endoplasmic reticulum marker is also low compared to literature values of approximately 44 nmoles min^{-1} mg^{-1} [37] for "purified" microsomal fractions from rat liver. Although tissue differences may account for any variance in relative activities, the activities in fraction *P*2 are still nearly half the values reported for a purified nerve plasma membrane preparation [3].

It should be noted that the fraction *P*2 contains plasma membranes from both the innervated and non-innervated surfaces of the electroplaque. Although additional density gradient fractionation yields a membrane fraction with higher $(Na^+ + K^+)$-ATPase activity, such manipulation invariably leads to loss of [^{3}H]TTX binding in other fractions.

The membrane fraction *P*2 routinely used in our studies appears to consist predominantly of closed vesicles ranging in diameter from about 50 to 300 nm, as shown in the electron micrograph (Fig. 1). Al-

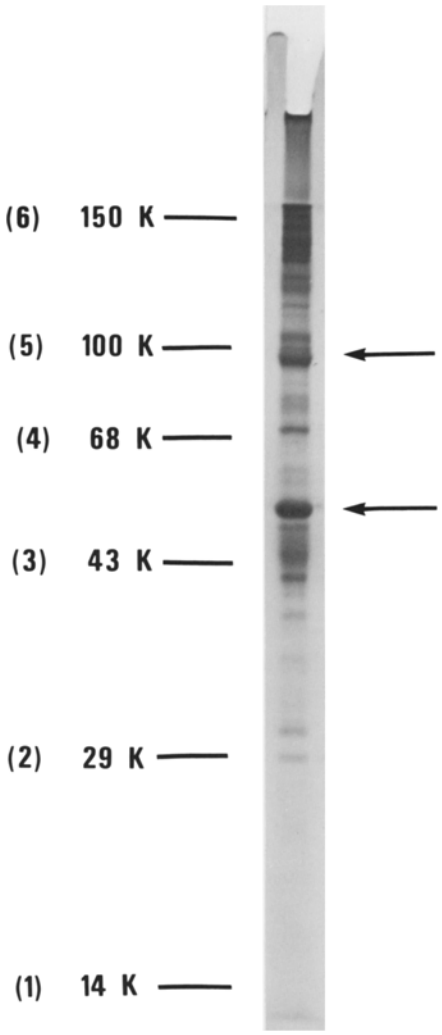

Fig. 2. Polyacrylamide sodium dodecyl sulfate gel electrophoresis of proteins from the electroplaque membranes. The molecular weight markers as indicated are (*1*) lysozyme, (*2*) carbonic anhydrase, (*3*) ovalbumin, (*4*) bovine serum albumin, (*5*) phosphorylase *a*, and (*6*) unreduced γ-globulin. The arrows correspond to proteins of mol wt 52,000 and 93,000

though the vesicles are very leaky to $^{22}Na^+$, the general permeability properties have yet to be examined.

Figure 2 shows a polyacrylamide SDS gel electrophoresis pattern of the membrane proteins. The molecular weights indicated were determined from standards of known molecular weight. There appear to be several major bands and at least 30 minor components indicating, not surprisingly, that the membrane is composed of a heterogenous population of proteins. Most of the protein subunits are in the mol wt region between about 40,000 and 150,000 with few bands observed below about 30,000. Two major bands indicated by arrows occur at apparent mol wt of approximately 93,000 and 52,000. $(Na^+ + K^+)$-ATPase is known to be present in the electroplaque plasma membranes in high concentrations and has been shown to be composed of two subunits corresponding to molecular weights of 47,000 and 93,000 [26]. Thus these two bands in our preparation could well be the two subunits of $(Na^+ + K^+)$-ATPase since, as discussed above, very high levels of this enzyme have been shown to be present here. In addition, the lower mol wt subunit of the $(Na^+ + K^+)$-ATPase is known to be a carbohydrate containing protein [26] and we have also found that the 52,000-dalton species stained positive with periodic acid-Schiff reagent as expected for glycoproteins *(unpublished observations)*.

Table 2. Lipid composition of the plasma membranes of *Electrophorus electricus*

Lipid class	mg/mg protein (mean ± SD[a])
Total lipid	0.52 ± 0.07
Phospholipid	0.38 ± 0.05
Cholesterol	0.11 ± 0.01
Other lipids[b]	0.03 ± 0.01
Ratio: phospholipid/cholesterol (mole/mole)	1.67

[a] Standard deviation. Values are for 3 separate membrane preparations.
[b] Mainly glycerides.

Table 2 shows the values obtained for the total lipid composition of the electroplaque membranes. Phospholipids comprise about 73% of the total lipids with cholesterol and glycerides making up 21 and 6%, respectively. Similar studies with rat brain synaptosome plasma membranes indicated that neutral glycerides made up only 1–2% of the total lipids [7]. The higher neutral glyceride content that we observe could be due to the fact that, unlike other animals, fish store neutral lipids, mainly triglycerides, in their muscle [38]. Since electroplaque is believed to be of myogenic origin, it may not be surprising to find higher than normal concentrations of glycerides in this tissue.

The distribution of cholesterol (0.11 mg/mg protein) found for the electroplaque membranes is similar to that reported for other excitable tissues. Schapira et al. [34] found cholesterol levels of 0.13 mg/mg protein in sarcolemma membranes from rat skeletal muscle. Also, cholesterol values of 0.15–0.20 mg/mg protein were reported for rat synaptosomal plasma membrane lipids [7].

The phospholipid content (0.38 mg/mg protein) is also comparable to values reported for other excitable tissues [3, 7, 34]. The molar ratio of phospholipid

Table 3. Distribution of phospholipid classes

Phospholipid	% of total phosphorus (mean SD[a])
Phosphatidylcholine	47.3±1.9
Phosphatidylethanolamine	32.6±2.1
Phosphatidylserine	13.1±0.8
Sphingomyelin	4.5±0.8
Phosphatidylinositol	0.8±0.7
Phosphatidic acid	0.2±0.2[b]
Lysophosphatidylcholine	Trace
Others	1.4±0.7

[a] Values are for 4 separate membrane preparations.
[b] $n=3$.

to cholesterol in the electroplaque plasma membranes is 1.67 and similar ratios have been reported for other plasma membrane preparations such as skeletal muscle sarcolemma [34].

Table 3 shows the distribution of phospholipid classes for four membrane preparations. Values are expressed as percent of total lipid phosphorus and are corrected for plate blanks. The average recovery of total lipid phosphorus from the plate was greater than 92%. The major phospholipids are phosphatidylcholine (47.3%), phosphatidylethanolamine (32.6%), and phosphatidylserine (13.1%) with sphingomyelin occurring in much lower levels (4.5%). There is little phosphatidylinositol and phosphatidic acid and virtually no detectable lysophosphatidylcholine. In addition, no cardiolipin was detected. The percent distribution of the major phospholipid classes observed here is significantly different from that reported recently by Rosenberg et al. [31] for isolated eel electroplaque membranes. In this latter analysis phosphatidylcholine was reported to comprise 62% of the total phospholipid with phosphatidylethanolamine, phosphatidylserine and sphingomyelin comprising 20, 7, and 11%, respectively. We have no explanation for these differences.

The percentage fatty acid composition of the individual phospholipid classes as well as for neutral and total lipid is shown in Table 4. It is particularly noteworthy that each phospholipid class appears to have a unique fatty acid distribution profile. When the individual fatty acids for the different lipid classes are compared both phosphatidylethanolamine and phosphatidylserine were found to contain large amounts of long-chain, polyunsaturated fatty acids. Both contain similar levels of docosahexaenoic acid (22:6), 34% for phosphatidylethanolamine and 39.3% for phosphatidylserine. The other major unsaturated fatty acid in phosphatidylethanolamine is arachidonic acid (20:4) (13.1%) and the major saturated species are palmitic (16:0) (10.2%) and stearic (18:0) (19.4%) acids. Phosphatidylserine, on the other hand, has only about 3% arachidonic acid (20:4) but 13.2% of (22:5) with the major saturated fatty acid being stearic acid (25.4%).

The composition of phosphatidylcholine and sphingomyelin with respect to fatty acyl chain distribution is substantially different. In these lipids, docosahexaenoic acid constitutes less than 10% of the total fatty acyl chains. In phosphalidylcholine, the major

Table 4. Percentage fatty acid composition of individual phospholipid classes[a]

Fatty acid	Phosphatidyl-ethanolamine	Phosphatidyl-serine	Phosphatidyl-choline	Sphingomyelin	Neutral lipid	Total[b]
14:0	0.2	2.3	0.6	3.2	3.9	0.5
14:1	0.4	N.D.[c]	0.8	0.1	0.6	0.6
16:0	10.2	1.5	40.0	11.6	23.3	23.6
16:1	1.2	0.8	3.4	3.2	7.3	2.5
16:2	0.7	0.1	0.3	0.1	1.1	0.3
18:0	19.4	25.4	3.3	39.8	19.1	14.9
18:1	8.0	4.8	28.4	5.3	17.4	16.1
18:2	1.4	1.2	1.0	3.4	3.6	1.8
18:3	0.4	0.7	0.7	3.8	2.6	0.9
20:3	1.7	1.0	1.3	5.1	5.2	1.0
20:4	13.1	2.7	4.2	9.4	5.0	7.1
20:5	1.9	1.4	2.0	1.5	1.9	2.0
22:4	2.5	6.1	0.4	2.3	0.8	2.1
22:5	4.0	13.2	1.2	6.6	2.2	4.3
22:6	34.0	39.3	9.7	7.3	7.4	21.2
Unknown	1.3	0.3	1.6	1.8	6.9	1.6
% Unsaturated	69.3	71.3	53.4	48.1	55.1	59.9

[a] Data expressed as the average percent by weight; three different membrane preparations were analyzed.
[b] Total lipid extract.
[c] Not detectable.

Table 5. Comparison of degree of polyunsaturation[a] of fatty acids of some major phospholipid classes of various excitable tissue preparations

Phospholipid	Preparation		
	Electrophorus electricus electroplaque[b]	*Torpedo marmorata* electric organ[c]	Rat brain synaptosomes[d]
Phosphatidylethanolamine	57.2	58.3	56.0
Phosphatidylserine	63.7	36.3	43.2
Phosphatidylcholine	18.8	16.9	7.5

[a] A 20-carbon chain with 3 or more double bonds.
[b] Plasma membrane (present analysis).
[c] Kreps et al. [19].
[d] Breckenridge et al. [7].

fatty acids are palmitic acid (40%) and the monounsaturated, oleic acid (28.4%). In sphingomyelin, the major components are both saturated (palmitic 11.6%, and stearic 39.8%).

Table 5 shows a comparison of the distribution of the long-chain polyunsaturated fatty acids of the major phospholipid classes in three excitable tissues, the electric organ of *Torpedo marmorata* [19], rat brain synaptosomes [7], and the eel electroplaque membrane (fraction *P2*). In all three tissues, the distribution of these fatty acids is particularly high in both phosphatidylethanolamine and phosphatidylserine but low in phosphatidylcholine. The exceptionally high levels in phosphatidylserine in the electroplaque membranes are particularly striking. Recently, similar fatty acid chain distribution patterns have been reported for acetylcholine receptor-enriched membranes from the *Torpedo* [27].

We have also examined the level of plasmalogens and find that the plasmalogens constitute as much as 18.7% of the total phosphatidylethanolamine, clearly a significant portion of this phospholipid. Plasmalogen levels about 10–15% higher than this were reported for rat brain synaptosome plasma membranes [7, 18].

Discussion

The electric organ of the electric eel is a valuable source of membranes for the study of the chemical and physical properties of the voltage-dependent Na^+-channel. The plasma membrane fraction we are studying binds [^{3}H]TTX with affinities comparable to those reported for other excitable tissues ($K_d = 6\text{–}9 \times 10^{-9}$ M). Despite the obvious usefulness of this preparation as a model system, it has not been characterized in detail with respect to chemical composition as have other preparations of excitable tissues. These general properties may be important in future functional reconstitution studies with the purified Na^+-channel components.

Recently there has been much speculation as to the structural-functional relationship of membrane components. Since excitability is known to be based on transient alterations in membrane permeability to cations, an understanding of the physical and chemical properties of the membrane may serve to provide a framework for models to explain the coordinated series of permeability changes in the membrane, possibly even cooperative mechanisms at the molecular level. Specific phospholipids in plasma membrane preparations from a wide range of excitable tissues have been found to contain high levels of long-chain polyunsaturated fatty acids, particularly docosahexaenoic acids [7, 9, 19, 21, 24]. Similar patterns have been found in the electrically-excitable electroplaque membranes, most notably in the serine and ethanolamine phospholipids.

Considerable evidence is now available to implicate polyunsaturated fatty acids in regulating many of the dynamic properties of bilayers, particularly those reflecting bilayer fluidity [10, 14]. Recently, Georgescauld and Duclohier have shown that electrical activity in intact nerve axons coincides remarkably well with changes in microviscosity of the membrane [15]. Whether these dynamic alterations are restricted to discrete lipid domains particularly enriched in phosphatidylserine and ethanolamine has yet to be determined.

Electrical excitability in intact nerve axons and in isolated electroplaques has been shown to be inhibited by phospholipase A_2 treatment [4, 30]. Furthermore, phospholipase A_2 treatment has also been shown to affect the properties of the Na^+-channel directly, as indicated by the significant reduction observed in the binding of [^{3}H]TTX in several isolated membrane preparations [5, 28, 39]. These data strongly suggest that the structural and functional organization of the ion-mediating components in the membrane is remarkably sensitive to alterations in the physical state of the phospholipid environment. In view of the striking similarities among excitable membranes with respect to the distribution of unsaturated fatty acids among specific phospholipids, it is tempting to speculate that these lipids may impart unique properties to the membrane to control the formation of active ion conducting states. Recent studies by Sklar and his colleagues [36] on key physi-

cal properties of rod outer segment membranes have provided strong evidence to suggest that the presence of highly unsaturated fatty acyl chains may modulate Ca^{++}-dependent thermal phase separations. What implication this may have on the excitation process remains unclear.

It should be pointed out that, in several cases, relatively high levels of fatty acyl chain unsaturation have been reported in inexcitable cells. For example, a comparison of periaxonal and axonal plasma membranes by Chacko et al. [9] indicated little significant differences with respect to unsaturation. On the other hand, Breckenridge et al. [8] have found substantial differences in levels of unsaturated fatty acids between the electric organ and the liver in *Torpedo marmorata*, particularly for docosahexaenoic acid. High levels of this fatty acid have been observed by others not only in the plasma membranes of excitable tissues, but also in other subcellular fractions of excitable tissues [6, 17]. It has previously been suggested that this distribution may simply reflect the fact that these cells are actively involved in the metabolism (i.e., biosynthesis) of these phospholipids [7].

It should be emphasized that in all of the studies reported here we have focused only on the bulk lipid composition of the membrane. Although our approach cannot implicate specific lipids as being directly responsible for the excitation phenomenon, studies by Cook et al. [11] suggest a role for phosphatidylserine in the propagation of action potentials in intact nerves. We are currently using the very high selectivity of certain phospholipase A_2s in modulating the structure of the Na^+-channel to explore the possibility that specific lipid domains may be associated with these ion-conducting membrane components. Nevertheless, isolation and direct characterization of individual membrane protein components such as those directly involved in ion movement or gating is fundamental in understanding the molecular events which underlie excitability in biomembranes.

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