INVESTIGATIONS OF LIPID COMPONENTS OF NEURONE-ENRICHED MEMBRANES OF THE JELLYFISH CYANEA CAPILLATA

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Summary

The lipid components of nerve-rich tissues from the jellyfish Cyanea capillata were purified by multiple differential extractions of a single tissue pellet and detected with various chemical spray reagents following separation by thin-layer chromatography. The results indicate that gangliosides are completely absent from Cyanea capillata neurones, that the major sterol is not cholesterol, and that the five major phosphorus-containing lipids do not co-migrate with conventional phospholipid standards. The atypical lipid environment of Cyanea capillata neurones may account for some of their unusual physiological and pharmacological properties.

Introduction

Cnidarians are the lowest extant organisms to possess an identifiable nervous system. Although a considerable amount is now known about the electrical properties of neurones in these animals (Anderson, 1989; Spencer et al. 1989), essentially nothing is known about the lipid constituents of their axonal membranes. Since there is evidence that lipids can influence the electrical (Coronado et al. 1984) and pharmacological (Feller et al. 1985) properties of neuronal ion channels, it is important that we know the lipid composition of the membranes of cnidarian neurones if we are fully to understand and appreciate the electrical properties of these cells. In the case of one of the best studied cnidarians, the scyphozoan jellyfish Cyanea capillata (Anderson, 1989), there are three lines of evidence that the lipid components of its neuronal membranes may differ from those of higher animals. First, nystatin, a drug that requires cholesterol or ergosterol for insertion into membranes, does not functionally insert into jellyfish neurones (P. A. V. Anderson, unpublished observations). This result is further supported by studies showing that cnidarians cannot synthesize cholesterol de novo (Von Aarem et al. 1964). Second, the physiological action on vertebrate sodium channels of the major toxin in the venom of the scorpion Leiurus quinquestriatus (LqV) has an absolute requirement for phosphatidylethanolamine (PE) and to some extent phosphatidylycerine (PS) (Feller et al. 1985). Sodium currents in Cyanea capillata are not affected by LqV (Anderson, 1987\textsuperscript{a,b}). Third, the membranes of another cnidarian, the sea anemone Metridium senile, contain no sialic acid (Hurley et al. 1977) which, by definition, means they cannot contain gangliosides.

Key words: jellyfish, gangliosides, phospholipids, sterols, sodium current, Cyanea capillata.
Here we show that membranes from the nerve-rich perirhopalial tissue of *Cyanea capillata* have a markedly different lipid composition from the membranes of most animals; they contain no detectable levels of ganglioside, cholesterol is not the major sterol, and they contain no conventional phospholipids. These membranes, however, do contain an isomer of cholesterol, minor amounts of fucosterol, and five distinct but as yet unknown phosphorus-containing lipids.

**Materials and methods**

*Purification of a total lipid extract (TLE)*

Specimens of the scyphozoan jellyfish *Cyanea capillata*, were collected in the vicinity of Marineland, Florida. Lipid analysis was carried out on perirhopalial tissue (Anderson and Schwab, 1981), a triangular region free of swimming muscle that extends centrally from each rhopalium. Although portions of the adjacent radial and circular muscle bands were inevitably included during the dissections, the bulk of the dissected tissue consisted of the ectodermal myoepithelium, which contains two ectodermal nerve nets, and the underlying endoderm, with a single nerve net (Anderson and Schwab, 1981). The predominant ectodermal nerve net, the MNN, is composed of large (15 μm diameter cell bodies) bipolar neurones and, based on measurements from micrographs of preparations of exposed nerve net (Anderson and Schwab, 1984), occupies at least 34% of the two-dimensional area of the perirhopalial tissue. This is not, however, an accurate measure of the percentage of membrane contributed by neuronal and non-neuronal cells. Individual ectodermal myoepithelial cells are considerably thicker (100 μm) and denser than the neurones, but the surfaces of MNN neurones are elaborated into large membranous whorls (Anderson and Schwab, 1981). Thus, although we cannot reliably estimate the percentage of membrane contributed to our membrane preparations by neurones, the perirhopalial tissue is one of the most nerve-rich of all tissues in the animal and should provide sufficient neuronal membrane to permit a qualitative determination of its lipid composition.

Pieces of perirhopalial tissue (Anderson and Schwab, 1981) were removed from whole jellyfish, rinsed in clean sea water, then immediately frozen in liquid nitrogen in 2–3ml samples (approximately 10 jellyfish) and stored at −80°C. When required, tubes were thawed at room temperature, homogenized at full speed on a Polytron tissue homogenizer, then refrozen in liquid nitrogen and lyophilized overnight. The volumes of all samples and their wet and dry masses were recorded. Wet tissue density was typically 0.93 g ml⁻¹, yielding a dry mass of 3.8% (0.7% salt-corrected dry mass) of the initial wet mass.

The total lipid extract (TLE) was obtained by extraction of the lyophilized tissue with chloroform:methanol (2:1 v/v). This was followed by multiple other extractions of a single tissue pellet using a variety of solvents to extract specific lipid components (Fig. 1). Additional sterol and phospholipid purifications were performed using only the Folch method (Folch *et al.* 1957). The volume of all extracts was reduced by rotary evaporation and further reduced to between 0.2 and 1ml by evaporation under a stream of N₂ gas. In the case of the 1-butanol:ether extract (fraction 1.2), the ether evaporated
rapidly, leaving only the 1-butanol. This, in turn, was evaporated off at low temperature by adding water to the 1-butanol to form an azeotrope of lower boiling point. All glassware was washed, acid cleaned, rinsed with deionized water, baked dry, then rinsed

Nerve-rich tissue from *Cyanea capillata* (frozen or lyophilized)

\[ \text{Extract: } 20 \text{ vols } 2:1 \text{ v/v chloroform:methanol per ml wet tissue} \]

\[ \text{Centrifuge} \]

\[ \text{Concentrate} \quad \rightarrow \quad \text{FRACTION 1} \quad \rightarrow \quad \text{Partition in } 20 \text{ vols } 6:4 \text{ diisopropyl ether:1-butanol per ml wet tissue, with} \]

\[ 10 \text{ vols } 50\text{mM}^{-1} \text{ NaCl} \]

\[ \text{FRACTION 1.1 (aqueous phase) Gangliosides?} \]

\[ \text{FRACTION 1.2 (organic phase) Sterols? Phospholipids?} \]

\[ \text{Extract: } 10 \text{ vols } 1:1 \text{ v/v chloroform:methanol per ml wet tissue} \]

\[ \text{Centrifuge} \]

\[ \text{Concentrate} \quad \rightarrow \quad \text{FRACTION 2} \quad \rightarrow \quad \text{Sterols?} \]

\[ \text{Gangliosides?} \]

\[ \text{Phospholipids?} \]

\[ \text{Extract: } 10 \text{ vols acetone per ml wet tissue} \]

\[ \text{(gangliosides do not solubilize)} \]

\[ \text{Centrifuge} \]

\[ \text{Concentrate} \quad \rightarrow \quad \text{FRACTION 3} \quad \rightarrow \quad \text{Sterols?} \]

\[ \text{Phospholipids?} \]

\[ \text{Low molecular weight contaminants?} \]

\[ \text{Extract: } 14 \text{ vols } 6:1 \text{ v/v tetrahydrofuran:0.2moll}^{-1} \text{ KH}_{2}\text{PO}_{4} \]

\[ \text{(pH 6.8) per ml wet tissue, partitioned with } 3 \text{ vols ethyl ether} \]

\[ \text{Centrifuge} \]

\[ \text{Concentrate} \quad \rightarrow \quad \text{FRACTION 4} \quad \rightarrow \quad \text{Gangliosides?} \]

Fig. 1. A flow chart illustrating the protocols used for extracting lipids from *Cyanea capillata* membranes.
with the appropriate extraction solvent immediately before use. All solvents were HPLC grade and saturated with nitrogen gas.

Separation of TLE components by thin-layer chromatography (TLC)

Samples were run on silica gel G TLC plates (Fisher Scientific and Baker Chemical Company), which were cleaned prior to spotting by allowing methanol to migrate to the top of the plate, and then baked dry at 100°C for 1h. Concentrated samples were spotted onto the TLC plates in volumes of 2–40 μl. Several solvent systems (Table 1) were employed to separate the various fractions into their components. Separated components were revealed by exposing the plates to I₂ vapour, which was then allowed to evaporate off prior to staining with specific spray reagents. When repeated or multiple solvent systems were used on a single TLC plate, the plate was thoroughly dried in an airflow hood before being developed in the next solvent.

TLC patterns were recorded as photocopies, photographs or acetate sheet tracings. In discussing the results of these TLC runs, spots are identified on the basis of their relative migration position from the origin, with spot 1 being closest to the origin. Lipid migration distances (Rᵢ) are expressed relative to that of the closest purified standards, and denoted as Rᵢ(standard), where Rᵢ is the migration distance (i.e. Rᵢ(sample)/Rᵢ(standard)) relative to the standard (std) for a given spot number (x). Rᵢ is defined as the migration distance of the sample spot divided by the migration distance of the solvent front. All fractions from each extraction (Fig. 1) were tested for various lipid components as outlined below; however, the data presented in the Results include only those spots revealed by specific detection reagents.

Sterol TLC: fraction 1 and fraction 1.2

Fractions 1 (TLE) and 1.2 were separated by a TLC system employing silica gel G chromatography plates developed once in system A (chloroform). Sterol spots were detected by spraying the plates with ferric chloride spray (Lowry, 1968), antimony pentachloride or a sulpho-phospho-vanillin spray reagent (see Fig. 2). In some cases, samples were run in parallel lanes. Individual spots revealed by iodine vapour were

<table>
<thead>
<tr>
<th>System</th>
<th>Solvents</th>
<th>Ratio</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Chloroform</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>Chloroform:hexane:acetic acid</td>
<td>25:70:0.5</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>Chloroform:propionic acid:1-propanol:H₂O</td>
<td>30:30:30:12</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>Chloroform:methanol:ammonia</td>
<td>65:35:5</td>
<td>3</td>
</tr>
<tr>
<td>F</td>
<td>Chloroform:methanol:H₂O</td>
<td>70:30:4</td>
<td>4</td>
</tr>
<tr>
<td>G</td>
<td>Chloroform:methanol:0.25% KCl</td>
<td>60:35:8</td>
<td>4</td>
</tr>
<tr>
<td>H</td>
<td>Propanol:H₂O</td>
<td>7:3</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td>76% aqueous phenol</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

(1) Copius-Peereboom and Beekers (1965); (2) Hofmann (1963); (3) Higgins (1987); (4) Harth et al. (1978); (5) Dain et al. (1962).
scraped off one of the two parallel lanes and the spot that corresponded to that stained by sterol-sensitive reagents was solubilized in chloroform, centrifuged, and the sterol-containing extract pipetted off. This sterol fraction was then dried under a stream of N₂ gas and acetylated by incubation in a small volume of 1:1 (v/v) pyridine:anhydrous acetic acid overnight at room temperature (25°C). Before spotting, acetyling reagents were evaporated off with a stream of N₂ gas and the resulting acetate derivatives were rinsed twice with chloroform. Both the sterol-containing fraction and its acetylated derivative were separated by TLC on 10% silver-nitrate-impregnated silica gel G plates (Kates, 1972) developed unidirectionally, three times, in system B (chloroform:hexane:acetic acid). These spots were visualized by heat charring. Acetylated cholesterol and acetylated fucosterol, prepared in the same manner as the Cyanea capillata sterol, were used as standards (see TLC run 42.B lanes 1 and 2, Fig. 3). Control groups consisted of the acetyling reagents alone, the unpurified TLE and system-A-purified fraction 1.2.

Phospholipid TLC: fraction 1

Fraction 1 was separated by TLC using 8% ammonium-nitrate-impregnated silica gel G chromatography plates developed in system C (chloroform:propionic acid:1-propanol:H₂O). Fraction 1 was also separated by two-dimensional TLC on silica gel G plates. These were developed in the first dimension in system D (chloroform:methanol:ammonia), then rotated 90˚ and developed in system E (chloroform:methanol:acetone:acetic acid:H₂O). Phosphorus was detected using Molybdenum Blue spray reagent, choline was revealed with Dragendorff’s spray reagent, and primary amines were detected with 0.5% ninhydrin in acetone spray reagent, followed by heating to 100°C. Spots on a single TLC plate were revealed by all three reagents sequentially in the order: ninhydrin, Molybdenum Blue and Dragendorff’s (see TLC run 51.1, Fig. 4 and Table 4). This order of applying spray reagents was critical, since applying these sprays in any other order resulted in a masking of proper colour development. The same results were obtained on separate TLC plates sprayed with only one reagent. Fatty-acid-containing lipids were also evident as light rosy red spots after application of a sulpho-phospho-vanillin spray reagent. Phospholipid standards (bovine) were purchased from Sigma Chemical Co. (≥98% pure) and included phosphotidylcholine (PC), phosphotidylserine (PS), phosphotidylethanolamine (PE), phosphotidylinositol (PI) and their lyso-derivatives. These standards were also used to identify the colour development of the spray reagents.

Ganglioside TLC: fractions 1, 1.2 and 4

Fraction 1 was separated by TLC according to Harth et al. (1978). Briefly, samples and standards were spotted on silica gel G plates and run once in system A, then once to about four-fifths of the way to the top in system F (chloroform:methanol:H₂O), and finally twice in system G (chloroform:methanol:0.25%KCl). This plate was dried before being sprayed with resorcinol spray reagent (aged 4h to 1 week) (Higgins, 1987), covered with a clean glass plate and heated to 135°C for 6–20min (Yates and Thompson, 1977). Resorcinol-positive spots were dark blue to violet and were compared to purified ganglioside standards (Sigma G-7641 and Sigma G-9886) and crude bovine brain
extracts containing various gangliosides (Sigma B-1014 and B-1877). The ganglioside composition of fractions 4 and 1.2 was further examined by TLC on silica gel G plates with system H (propanol:H$_2$O) or system I (76% aqueous phenol). Gangliosides were verified by detection of both sialic acid with resorcinol spray reagent and fatty acid with sulpho-phospho-vanillin reagent (Saifer and Feldman, 1971). The sulpho-phospho-vanillin reagent was modified to a spray by mixing 60ml of 85% phosphoric acid, 25ml of concentrated sulphuric acid, 15ml of 0.6% vanillin and 10ml of deionized distilled water.

**Results**

**Sterols**

Samples of fraction 1.2 were run using system A in two parallel lanes (lanes 1 and 2) and the resulting spots revealed with I$_2$ vapour (Fig. 2). Subsequent application of antimony pentachloride to one of the two lanes (lane 2) revealed a single antimony-pentachloride-positive spot. This spot initially appeared red then, after 1–2min, it assumed a bluish hue, interspersed with a few red speckles (resulting in a purplish-blue colour from a distance). Under the same conditions, fucosterol also turned red initially, but within a few minutes became dark purple. Antimony pentachloride immediately turned the cholesterol, stigmasterol, campesterol and B-sitosterol standards blue, while it turned lathosterol brown.

Further work showed that the antimony-pentachloride-positive spot in fraction 1.2 did
not correspond to the bile acids taurocholate, cholic acid, deoxycholic acid or 5-cholenic acid-3\beta-ol, or to the steroids testosterone, 5α-androstan-17β-ol-3-one or ergosterol, as demonstrated by the much lower migration rates and/or differently coloured antimony pentachloride reaction products of these structurally cholesterol-like molecules.

Individual \( I_2 \)-positive spots in the parallel lane (lane 1) had been scraped off the plate prior to antimony pentachloride staining. Approximately two-thirds of the material corresponding to the antimony-pentachloride-positive spot (lane 2) was acetylated and

Table 2. Comparison of the properties of the major sterol in *Cyanea capillata* neurones with other cholesterol-like sterols

<table>
<thead>
<tr>
<th>Sterol</th>
<th>( \text{FeCl}_3 ) reaction colour</th>
<th>( \text{SbCl}_5 ) reaction colour</th>
<th>( R_t ) normal system A</th>
<th>( R_t ) acetylated system B</th>
<th>Characteristic absorption spectrum</th>
<th>( GC_r ) (cholesterol) migration rate</th>
<th>Relative molecular mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanea sterol</td>
<td>Purple</td>
<td>Red */ purple</td>
<td>100 %</td>
<td>118 %</td>
<td>No</td>
<td>110 %</td>
<td>386</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Purple</td>
<td>Blue</td>
<td>100 %</td>
<td>100 %</td>
<td>No</td>
<td>100 %</td>
<td>386</td>
</tr>
<tr>
<td>Lathosterol</td>
<td>Purple</td>
<td>Brown</td>
<td>100 %</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>386</td>
</tr>
<tr>
<td>Fucosterol</td>
<td>Purple</td>
<td>Red */ purple</td>
<td>100 %</td>
<td>97 %</td>
<td>NT</td>
<td>NT</td>
<td>413</td>
</tr>
</tbody>
</table>

*See text for description of \( \text{SbCl}_5 \) colour reaction; NT, not tested.

\( R_t \), lipid migration distance (see Materials and methods); \( GC_r \), migration rate compared with that of cholesterol.
run on TLC using system B (Fig. 3, lane 3). The remaining third of this material was run in parallel (Fig. 3, lane 7), without prior acetylation, together with a non-acetylated sample from fraction 1.2 that had not been previously subjected to any TLC (lane 6). Heat charring of the plate revealed that the acetylated antimony-pentachloride-positive spot (lane 2) had separated into three distinct spots. The lower two minor spots had migration rates similar to those of acetylated fucosterol and acetylated cholesterol while the major spot had a migration rate greater than either of these acetylated standards (Table 2 and Fig. 3).

Like cholesterol (Heilbron et al. 1928), system-A-purified *Cyanea capillata* sterol has no characteristic absorption spectrum. Gas chromatography/mass spectroscopy analysis of system-A-purified *Cyanea capillata* sterol revealed a single sterol with the same relative molecular mass as cholesterol but a faster migration rate than cholesterol, stigmasterol or campesterol. The chemical properties of the major *Cyanea capillata* sterol are compared with those of other cholesterol-like sterols in Table 2.

**Phospholipids**

Samples from fraction 1 (TLE), examined with TLC using system C, formed three Molybdenum-Blue-positive spots (Fig. 4 and Table 3). Like (lyso)phosphatidylcholine, (L)PC, spot 1 turned orange when sprayed with Dragendorff’s reagent and blue when sprayed with Molybdenum Blue reagent (Dittmer and Lester, 1964) but, unlike (L)PC, spot 1 turned red/purple when sprayed with ninhydrin and had a migration rate different from that of (L)PC. Spray reagents revealed that spot 2 (R2) contained a phosphorus group and a choline group, but the migration rate of this spot was greater than that of PC and closer to that of PI (Table 3).
To exclude the possibility that the unusual chemical properties of the phosphorus-containing lipids in *Cyanea capillata* might be due to a mixture of unresolved phospholipids, fraction 1 was further resolved by two-dimensional TLC. This revealed five phosphorus-containing lipids in the Folch extract of *Cyanea capillata* tissue. Three of these had different migration rates in the first dimension, and all five had migration rates in the second dimension that were consistently higher than those of PS, PC, PI, (L)PS, (L)PC, (L)PI and (L)PE (Table 4 and Fig. 5).

The two furthest-migrating *Cyanea capillata* lipids had migration rates comparable to that of PE in system D, but the presence of PE in these samples had previously been excluded by the migration patterns of the endogenous phospholipids in system C. The absence of PE was confirmed by adding a sample of PE to the *Cyanea capillata* lipid fraction. TLC (system C) of this supplemented sample resulted in four phosphorus-containing spots, rather than the three previously detected in the absence of exogenous PE.

**Table 3. Phospholipid TLC of lipids from Cyanea using system C**

<table>
<thead>
<tr>
<th>TLC number</th>
<th>Spot number</th>
<th>Ninhydrin colour</th>
<th>Molybdenum Blue colour</th>
<th>Dragendorff’s reagent colour</th>
<th>$R_t$ (std)</th>
</tr>
</thead>
<tbody>
<tr>
<td>51.1</td>
<td>1</td>
<td>Red</td>
<td>Blue</td>
<td>Orange</td>
<td>R1([L]PC) 108</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>NS</td>
<td>Blue</td>
<td>Orange</td>
<td>R2 (PI) 117</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Red</td>
<td>Blue</td>
<td>NS</td>
<td>R2 (PC) 128</td>
</tr>
</tbody>
</table>

NS, no spots; $R_t$, relative migration rate of spot [where spot 1, denoted as R1, is closest to the origin. The closest phospholipid standard to which a sample spot was compared in terms of $R_t$ is denoted by (std)].

(L)PC, (lyso)phosphatidylcholine; PI, phosphatidylinositol; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine.

**Table 4. Phospholipid TLC of lipids from Cyanea using system D reagents**

<table>
<thead>
<tr>
<th>TLC number</th>
<th>Spot number</th>
<th>Ninhydrin colour</th>
<th>Molybdenum blue colour</th>
<th>Dragendorff’s first reagent colour</th>
<th>$R_t$ (std) first dimension</th>
<th>Dragendorff’s second reagent colour</th>
<th>$R_t$ (std) second dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folch extract</td>
<td>1.1</td>
<td>Red</td>
<td>Blue</td>
<td>NS</td>
<td>R1.1(PC) 127</td>
<td>R1.1(PC) 52</td>
<td>R1.1(PC) 97</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>NS</td>
<td>Blue</td>
<td>NS</td>
<td>R1.2(PC) 128</td>
<td>R1.2(PC) 90</td>
<td>R1.2(PC) 97</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>Red</td>
<td>Blue</td>
<td>NS</td>
<td>R1.3(PC) 136</td>
<td>R1.3(PC) 97</td>
<td>R1.3(PC) 97</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>NS</td>
<td>Blue</td>
<td>Orange</td>
<td>R2(PE) 95</td>
<td>R2(PE) 107</td>
<td>R2(PE) 107</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Red</td>
<td>Blue</td>
<td>Orange</td>
<td>R3(PE) 116</td>
<td>R3(PE) 104</td>
<td>R3(PE) 112</td>
</tr>
</tbody>
</table>

NS, no spots; $R_t$, relative migration rate of spot [where spot 1, denoted as R1.1, is closest to the origin. The closest phospholipid standard to which a sample spot was compared in terms of $R_t$ is denoted by (std)].

Other abbreviations are defined in Table 3.
Gangliosides

Although no gangliosides were detected in fraction 1 using system G, a diffuse resorcinol-positive ring was evident in many TLC runs. The possibility that this ring was due to gangliosides was studied by TLC of further-purified fractions using systems H and I. Resorcinol reaction products were detected in system H TLC of fraction 1.2 and fraction 4. The single spot formed in fraction 4 consisted of a large, diffuse, greenish-blue ring surrounding a large yellow spot that, in turn, contained a small purple spot. Samples of crude and purified ganglioside standards treated in the same manner produced violet/blue spots upon exposure to resorcinol reagent, while cholesterol present in crude ganglioside standards produced a greenish-blue colour. Subsequent work showed that the large yellow spot that ran near or at the solvent front in fraction 4 was due to the tetrahydrofuran solvent used in its extraction. No resorcinol-positive staining was evident in fraction 4 run on system G, although ganglioside standards stained normally. Although the fraction 4 spot run on system H yielded a light blue colour when stained with resorcinol, its sulpho-phospho-vanillin reaction colour was not like that of the ganglioside standards (Table 5). This fraction 4 spot is not free sialic acid, since sialic acid has a much lower migration rate in system G (data not shown), nor is it a small sialic-acid-containing protein since acetone (Fig. 1, extraction 3) extracts free sugars and protein contaminants, but not gangliosides. Furthermore, no detectable levels of protein were found in fraction 4, using either the Bradford microassay or absorbance at 280nm with a bovine serum albumin standard (data not shown).
In system H, fraction 1.2 formed a resorcinol-positive spot, but this spot was also antimony-pentachloride-positive. Sterols, but not gangliosides, are antimony-pentachloride-positive.

### Discussion

The sodium (Na\(^+\)) current that underlies the action potential in neurones of the jellyfish *Cyanea capillata* is unusual inasmuch as it is a Na\(^+\) current physiologically, but a calcium (Ca\(^{2+}\)) current pharmacologically (Anderson, 1987\textit{a,b}). While these pharmacological differences may reflect structural differences between the Na\(^+\) channel in *Cyanea capillata* and those of higher animals, one cannot exclude the possibility that some of these abnormalities may be a consequence of the lipid environment of the *Cyanea capillata* Na\(^+\) channels.

The lipid composition of excitable membranes can affect the functional properties of channel proteins responsible for gating the transmembrane movement of ions. Certain lipids, such as cholesterol and negatively charged phospholipids, affect channel conductances directly (Fong and McNamee, 1986, 1987) by altering membrane fluidity (Coronado \textit{et al.} 1984) and kinetics (Boilotina \textit{et al.} 1989). Glycosphingolipids (Sharom and Grant, 1975), gangliosides (Sharom and Grant, 1978) and (L)PE (Tilcock \textit{et al.} 1986) can also alter membrane fluidity and, therefore, may affect channel gating properties. Such lipid-mediated changes in membrane fluidity are physiologically relevant (Hennessey, 1992) and distinct from the effects of detergents on excitable membranes (Kishimoto and Adelman, 1964).

Other lipids affect channel desensitization rates (Liles \textit{et al.} 1986) and open states (DeRiemer \textit{et al.} 1985; Strong \textit{et al.} 1986) indirectly, through their actions on protein kinase C (PKC), which phosphorylates many channel proteins (Browning \textit{et al.} 1985; Catterall, 1988). PKC is inhibited by gangliosides, glycosphingolipids and sphingosine (Bazzi and Nelsestuen, 1987; Hannun and Bell, 1987; Kreutter \textit{et al.} 1987), but is

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### Table 5. Ganglioside TLC with various detection reagents

<table>
<thead>
<tr>
<th>Applied sample</th>
<th>TLC system</th>
<th>Resorcinol reaction colour</th>
<th>SbCl(_5) reaction colour</th>
<th>Sulpho-phospho-vanillin reaction colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr4</td>
<td>F</td>
<td>Purple</td>
<td>NS</td>
<td>Brown*</td>
</tr>
<tr>
<td>Fr1.2</td>
<td>F</td>
<td>Purple</td>
<td>Red</td>
<td>Purple</td>
</tr>
<tr>
<td>Ganglioside</td>
<td>F</td>
<td>Purple</td>
<td>NS</td>
<td>Rosy red</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>F</td>
<td>Greenish blue</td>
<td>Blue</td>
<td>Purple</td>
</tr>
<tr>
<td>Fr4</td>
<td>G</td>
<td>Reddish brown</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Fr1.2</td>
<td>G</td>
<td>Light blue</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Ganglioside</td>
<td>G</td>
<td>Bluish purple</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>G</td>
<td>Greenish blue</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

*Turned brown immediately, while ganglioside and sterol colour development with sulpho-phospho-vanillin requires 5–10 min of mild heating.

NS means no spots detected; NC means that these spray reagents are not compatible with the TLC system employed.
stimulated by PS, (L)PC (Oishi et al. 1988) and arachidonic acid (Hansson et al. 1986). Finally, the lipid composition of membranes can affect specific aspects of ion channel pharmacology, while leaving other channel properties intact (Feller et al. 1985). The contribution of lipids to channel function may be further compounded by the ability of gangliosides to compartmentalize (cluster) other lipids, as well as glycosylated proteins, within the plasma membrane (Sharom and Grant, 1978). These lipid-mediated effects on membrane fluidity, channel phosphorylation state and channel pharmacology may arise from alterations in ion channel structure or reflect alterations in the accessibility of specific binding sites. Interestingly, tetrodotoxin (TTX) alters the surface tension of cholesterol films but not of films of cholesterol-like sterols (Villegas et al. 1970), and does so in a dose-dependent manner, with a half-maximal effect at 260nmoll⁻¹. The TTX-insensitivity of *Cyanea capillata* Na⁺ currents may be, in part, because cholesterol is not the major sterol in these membranes.

**Sterols**

The complex colour reaction of the antimony-pentachloride-positive spot (Fig. 2), and the results of the TLC with acetylated fractions of this spot (Fig. 3), indicate that multiple sterol components are present in the lipid extract. The minor spot (R1) that ran closest to the origin in TLC of the acetylated fraction had a relative migration rate sufficiently close to that of fucosterol to suggest that fucosterol, which is present in sea water (Gagosian, 1976) and is available to *Cyanea capillata* by way of its diet, is a minor constituent of these membranes.

The second minor spot (R2) migrated with acetylated cholesterol, indicating that minor amounts of cholesterol are probably present. The major sterol cannot, however, be cholesterol since its migration rate in gas chromatography and that of its acetylated derivative in TLC (system B) were both slightly faster than those of cholesterol. Since *Cyanea capillata* sterol(s) does not have a characteristically selective absorption spectrum (data not shown), further purification by HPLC is not possible.

This finding that neurone-rich tissue from *Cyanea capillata* does not have cholesterol as the major sterol component conflicts with a study using whole specimens of another scyphozoan jellyfish, *Rhizostoma* (Von Aarem et al. 1964). Von Aarem’s conclusion that whole jellyfish contain cholesterol was based on his finding that an extensively recrystallized sterol from *Rhizostoma* had a melting point close (1˚C lower) to that of pure cholesterol. One possible explanation for the discrepancy between our finding and that reported by Von Aarem et al. (1964) is that the cholesterol detected in *Rhizostoma* could have come from residues of its prey rather than from its own membranes, since whole jellyfish were used in that analysis. The lower than expected melting point could be attributed to residual contamination. Alternatively, the lower than expected melting point might argue, as we do here, that the major sterol in question is a cholesterol isomer. In this respect, our findings concur with those of Sipos and Ackman (1968), who reported that the major sterol in *Cyanea capillata* collected in the vicinity of Nova Scotia had an *R*ₐ value on TLC that was similar, but not identical, to that of cholesterol.

The major *Cyanea capillata* sterol has many features of cholesterol (a 3-β hydroxyl
group, no marked absorption spectra and a relative molecular mass of 386) but its absence as the major sterol in these membranes suggests that cholesterol obtained either from the environment (Gagosian, 1976) or in the diet may have undergone an enzymatic repositioning of a double bond in the sterol ring core or enantiomerization of a side group.

The lack of cholesterol as the dominant sterol in *Cyanea capillata* nerve membranes is very unusual since it is the dominant sterol in the membranes of most multicellular organisms. The importance of cholesterol in the operation of ion channels in the membranes of jellyfish neurones is unclear. Some bacteria also lack cholesterol but, like *Cyanea capillata*, possess functional ion channels (Kung, 1989). Similarly, *Paramecium* lacks cholesterol, but has functional ion channels whose activity is affected by the type of sterol present (Weglar et al. 1989). Interestingly, altering cholesterol levels in higher organisms does affect ion channel gating kinetics (Boilotina et al. 1989).

**Phospholipids**

Membranes from *Cyanea capillata* contain five distinct phosphorus-containing lipids, none of which co-migrates with any of the phospholipid (PS, PI, PC, PE) or lysophospholipid [(L)PS, (L)PI, (L)PC, (L)PE] standards tested (Figs 4 and 5). From these results we are forced to conclude that jellyfish neurones contain detectable levels of phosphorus-containing lipids, but that these are distinct from those typically present in other organisms. It is unlikely that differences in fatty acid side chain length could account for the rather large differences in TLC migration rates (Tables 3 and 4), especially since fatty acids synthesized by another scyphozoan jellyfish, *Rhizostoma*, do not differ much in their carbon chain lengths (14–20 carbons) and degree of saturation (one or no carbon–carbon double bonds) (Von Aarem et al. 1964). Although the amount and proportions of phospholipids in cellular membranes can vary quite drastically between different animals, the apparent lack of all the typical phospholipids in the neuronal membranes of *Cyanea capillata* is surprising. Although lipids containing phosphorus and primary amines are undoubtedly present in *Cyanea capillata*, PE and PS are clearly absent (Figs 4 and 5), an absence that could account for the inability of *Leiurus quinquestriatus* toxin to affect the gating of *Cyanea capillata* sodium currents (Anderson, 1987a).

**Gangliosides**

Our finding that nerve-rich tissue from *Cyanea capillata* contains no detectable gangliosides is open to two potential criticisms. First, that significant amounts of ganglioside may have been lost during purification, resulting in undetectable levels, and second, that the gangliosides may be sufficiently novel as to be overlooked by the purification protocols employed in this study. It is unlikely that our isolation procedure resulted in the loss of any ganglioside, since the method of Harth et al. (1978) for isolating microgram amounts of ganglioside from as little as 1–15mg of wet tissue, revealed no detectable levels of ganglioside. The amounts of tissue used here were considerably greater. Furthermore, multiple, exhaustive extracts of the tissue pellet either in sequence, as described in the Materials and methods section, or separately, did not extract detectable gangliosides. With respect to the possible presence of novel
gangliosides, all known gangliosides are readily solubilized by one or more of the following extraction solvents: chloroform:methanol (2:1 v/v and 1:1 v/v) (Folch et al. 1957; Suzuki, 1965), diisopropyl ether:1-butanol (3:2 v/v) (Ladisch and Gillard, 1985) and tetrahydrofuran:potassium phosphate (0.01mol l1) (6:1 v/v) (Tettamanti et al. 1973). All of these extraction solvent systems were employed in our procedures, and any ganglioside components present in the jellyfish would have been evident in at least one of these extracts. Indeed, even the structurally novel gangliosides present in the starfish Asterina pectinifera are readily solubilized by a conventional chloroform:methanol extraction (Sugita, 1979).

Our conclusion that jellyfish neuronal membranes contain no gangliosides is supported by the finding that another cnidarian, the sea anemone Metridium senile (Hurley et al. 1977), contains no lipid-bound sialic acid which, by definition, means that it cannot contain gangliosides. The absence of gangliosides in Cyanea capillata cannot be attributed to the previously-held view that sialic acids are found only in deuterostomes (Warren, 1963), since sialic acid has recently been discovered in Drosophila melanogaster (Roth et al. 1992), and it is present in some bacteria (McGuire and Binkley, 1964) and platyhelminths (Warren, 1963) and perhaps also Aplysia californica (Ewald and Eckert, 1983). The lack of gangliosides in Cyanea capillata may have physiological consequences since they are known binding sites for toxins (Heyningen, 1963; Cuatrecasas, 1973a,b) and peptide hormones (Ledley et al. 1976) and can modulate the functions of a variety of receptors and enzymes (Mullin et al. 1976; Hollman and Seifert, 1986; Spiegel et al. 1986; Karpiak et al. 1984).

In conclusion, membranes from nerve-rich tissue in Cyanea capillata are unusual in that they contain no detectable levels of gangliosides or typical phospholipids (PC, PS, PE, PI and their lysoderivatives) and have a cholesterol isomer rather than cholesterol as their major sterol component. Although it is too early to attribute the unusual pharmacological properties of Na+ currents in Cyanea capillata to the lipid environment of the Na+ channels, this possibility should be borne in mind when interpreting these and similar data from related animals.

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