Simplified Ganglioside Composition of Photoreceptors Compared to Other Retinal Neurons

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Purpose. The quantitative and qualitative ganglioside composition of retinal photoreceptor cells is unknown. The aim of this study was to analyze the lipid, especially ganglioside, make-up of photoreceptors compared to other retinal cells.

Methods. Retinas from adult normal rats were mechanically separated into outer (photoreceptors) and inner (other retinal neurons and glia) halves by planar vibratome sectioning. Total lipids were extracted, and each fraction (neutral, phospholipids, and glycosphingolipids) was eluted sequentially by column chromatography and quantitated through high-performance thin layer chromatogram analysis. Similar analyses were performed on entire retinas from adult normal rats, adult dystrophic rats lacking photoreceptors (RCS-rdy-p+ strain), and isolated photoreceptor outer segments.

Results. Whereas phospholipids were distributed equally between the two halves, inner retina contained significantly more cholesterol (68% total) and gangliosides (74% total) than outer retina on a unit protein basis. The distribution on a percent molar basis of specific gangliosides also was significantly different between the two halves: Outer retina was dominated by GD3 (45% total ganglioside) and contained only trace amounts (<4%) of complex species (GT1b and GQ1b); inner retina was more typical of mature brain tissue, exhibiting substantial amounts (~25%) of more complex species. These data were supported by lipid compositional analyses of mutant photoreceptor-less retina. However, isolated outer segments resembled whole retina in containing higher levels of complex gangliosides.

Conclusions. These data indicate that, compared to other central nervous system-derived neurons, photoreceptor cell body membranes exhibit a highly unusual simplified ganglioside composition. Such an unusual neuronal lipid composition may reflect structural adaptations to their specialized function. Invest Ophthalmol Vis Sci. 1996; 37:574-585.

The vertebrate retina has long been studied as a model system not only for central nervous system (CNS) cell biology but also for plasma membrane structure and function. For example, the ability to isolate to relative purity retinal photoreceptor outer segments (OS) has led to their use in analyzing many aspects of membrane structure and function.1 The presence of various lipid groups, including neutral lipids (NL) such as cholesterol (CHOL), phospholipids (PL), and fatty acids, has been examined within these organelles, where they have distinct roles such as maintaining membrane structure, modulating membrane receptor function, and providing sources of second messengers.2-7 The presence and distribution of gangliosides, a structurally diverse group of sialic acid-containing glycosphingolipids, has been documented within the retina.8-14 Although the precise roles of gangliosides are still unknown, the facts that they are found at relatively higher levels and in more complex forms in CNS tissue than elsewhere, and that they are differentially regulated during development and differentiation, strongly suggest they have important roles in CNS ontogeny and function.15

Relatively little, however, is known about quantitative variations in these lipid species within the entire photoreceptor cell and other retinal cell types. The mammalian retina exhibits a laminated arrangement of its constituent neural elements, with the outer nuclear layer (ONL) composed of cone and rod photore-
receptor only, the inner nuclear layer (INL) composed principally of horizontal, bipolar, and amacrine neurons as well as Müller glial cell bodies, and the ganglion cell layer (GCL) composed mainly of ganglion cells. These three cellular layers are separated from each other by two synaptic zones, the outer (OPL) and inner (IPL) plexiform layers, respectively. This laminated organization has enabled the development of techniques capable of separating the tissue into its component layers. Such approaches permit the analysis of enriched cell populations with quantitative biochemical methods, and we show here that the photoreceptor differ from all other known CNS neurons in possessing only low levels of gangliosides present primarily as simple precursor forms. These data are strengthened by similar analyses of dystrophic photoreceptor-less retina in which ganglioside levels are relatively higher. On the other hand, isolated rat OS more resemble whole retina with higher levels of complex gangliosides, indicating the existence of distinct lipid domains within the photoreceptor. The unusual lipid composition of photoreceptor might represent an adaptation to their highly specialized function of phototransduction and might have important functional consequences for these cells.

MATERIALS AND METHODS

Materials

All solvents and reagents were of analytical grade. Silicic acid (230 to 400 mesh) and high-performance thin layer chromatogram (HPTLC) plates were obtained from Merck and Whatman; goat anti-mouse immunoglobulin G conjugated with fluorescein isothiocyanate (gam-FITC) and poly D-lysine were obtained from Sigma (St. Louis, MO); rho-4D2 opsin monoclonal antibody was generated from a mouse hybridoma line raised using rat OS as immunogen. 18

Tissue Collection

All animals used in these studies were cared for and handled according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Three-month-old Wistar rats (n = 8) were anesthetized by CO2 inhalation, rapidly killed by cervical dislocation, and enucleated. Retinas were dissected free from other ocular tissue and processed using a technique originally developed for retinal transplanting studies.17 Briefly, isolated retinas were flat-mounted on a gelatin block with the GCL uppermost and sectioned along a horizontal plane using a vibratome (Fig. 1). Initial studies allowed the reproducible matching of a given section depth to a particular layer (Fig. 1). For lipid analysis, retinas were cut along the OPL, separating them into an inner retina (IR) (composed of the GCL, IPL, and INL) and an outer retina (OR) (remaining 100 µm composed entirely of photoreceptor). Samples were dry frozen at −20°C until ready for lipid extraction. For comparative purposes, whole retinas from five 3-month-old Wistar rats (WR) and thirteen 3-month-old RCS-ryd-p+ (retinal dystrophic) rats (DR), as well as whole brains from 3-month-old Wistar rats, were also removed for lipid analysis. Some WR and DR were examined histologically to assure the maintenance of tissue integrity in WR and the loss of photoreceptor within DR. Retinas were fixed in 4% paraformaldehyde immediately after dissection, rinsed, and processed for paraffin embedding. Sections were counterstained with haematoxylin–eosin and examined by light microscopy.

Outer Segment Preparation

Outer segments were isolated from retinas dissected from 2- to 3-month-old Wistar rats by flotation on continuous sucrose density gradients according to modifications of previously published methods.1 Retinas were homogenized gently by three passages in a handheld loose-fitting glass homogenizer in 5 ml 20 mM Tris-acetate buffer, pH 7.2, containing 0.25 mM MgCl2, 5 mM taurine, 10 mM glucose, and 20% sucrose, at 4°C, overlayed onto 27% to 50% continuous sucrose density gradients made in the same buffer in 35-ml polyethylene tubes and centrifuged at 100,000g for 2 hours at 4°C in a Beckman L5-65 ultracentrifuge using a Beckman SW28 swinging bucket rotor (Beckman Instruments France, Gagny, France). The uppermost two orange layers were collected and pooled together, diluted twofold with 20 mM Tris-acetate buffer, and pelleted at 20,000g for 20 minutes. The lipid composition of OS samples was analyzed in four independent experiments.

Protein Determination

Protein levels in homogenates of WR, DR, IR, OR, OS, and brain samples were determined using the Lowry method.19

Immunocytochemistry

To ensure purity of the OR, samples were dissociated into individual cells by papain digestion,20 fixed in 4% paraformaldehyde for 15 minutes, and sedimented at low speed onto poly D-lysine-treated coverslips. Cells were processed for immunocytochemical labeling using rho-4D2 anti-opsin monoclonal antibody as previously described.21 Labeling was detected with gam IgG–FITC (10 µg/ml for 30 minutes). Samples were washed extensively and observed with a Nikon Optiphot-2 microscope (Nikon Europe, Bodhoevedorp, Netherlands) equipped with fluorescence and differential interference contrast optics.
Extraction, Purification, and Separation of Lipid Classes

Retinal lipids were extracted as recently described by us, based on the original method of Folch-Pi et al. with the modifications reported by Suzuki. The interest of the technique resides in the sequential elution of the different lipid classes from a single sample: Separation and isolation of the different lipid fractions were achieved by silicic acid column chromatography (diameter 0.6 cm, height 2.5 cm) by adapting a combination of solvents used by previous laboratories for different purposes.

The various specimens were homogenized extensively in 0.5-ml dd.H2O, and total lipids were extracted with a tenfold excess of chloroform (C):methanol (M), 1:1 vol/vol to obtain a solvent mixture of C:M:H2O (5:5:1, vol/vol/vol). The crude suspension was centrifuged, the organic fraction was removed, and the pellet was reextracted in 5 ml C:M:H2O (5:5:1, vol/vol/vol). The suspension was recentrifuged, the organic fraction was removed, and the pellet was reextracted in 5 ml C:M (1:1, vol/vol). The suspension was centrifuged once more, the organic fraction was combined with the previous volumes, and the pellet was reextracted a third time in 3 ml C:M:H2O (50:40:10, vol/vol/vol). After a final centrifugation, the organic fractions were pooled and evaporated under nitrogen, redissolved in 2.5 ml C:M:H2O (60:30:4.5, vol/vol/vol), and loaded onto a Sephadex G25 column (diameter 0.9 cm, height 3.5 cm) equilibrated in the same solvent. After elution in 5 ml of the same solvent, 2.5 ml C:M (2:1 vol/vol) and 2.5 ml
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RESULTS

Histology of Fractionated Retina

Preparations of retinal tissue for ganglioside isolation were performed according to previously published techniques. 

**FIGURE 1.** Diagram representing vibratome isolation of retinal compartments, and morphologic, histologic, and immunocytochemical characterization of different retinal fractions. Panes a to d demonstrate the aspect of the corresponding section indicated on the central schematic illustration. (a) Ganglion cell layer (GCL): Ganglion cell bodies (G), nerve fiber bundles (NF), and large blood vessels (BV) are contained within the initial 20 μm. (b) Inner nuclear layer–inner plexiform layer (INL–IPL): Neuronal cell bodies of different sizes are visible (arrows) within the following 60 to 80 μm; (c) Outer plexiform layer (OPL): The retina is separated into external and internal halves along this boundary, visible as cell bodies interspersed with synaptic material and small blood vessels (BV). (d) Outer nuclear layer (ONL): This layer is uniquely constituted by regularly sized small neurons (arrows). (e) Cells dissociated from the ONL isolated from a young (8-day-old) rat retina and immobilized on glass coverslips. (f) Immunocytochemical labeling using anti-opsin antibody of the normal intact Wistar rat retina, showing the appearance of tissue after dissection. Note the well-preserved aspect of the different layers, especially the presence of numerous inner and outer segments (IS and OS, respectively). (h) Similarly prepared section of 3-month-old dystrophic rat retina. Whereas the ONL has degenerated completely, a broad band of membranous debris (MD) is observed along the scleral surface. Compared to the normal retina, the INL and IPL are wider, perhaps because of the presence of vestigial cone photoreceptor and hypertrophy of the Müller glia. Scale bars = 10 μm (a to d), 20 μm (e,f), and 45 μm (g,h).
TABLE 1. Quantitative Estimates of Protein and Lipid Class Contents of Various Retinal and Brain Fractions*

<table>
<thead>
<tr>
<th>Sample</th>
<th>WR</th>
<th>DR</th>
<th>IR</th>
<th>OR</th>
<th>OS</th>
<th>Br</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (mg/tissue)†</td>
<td>1.02 ± 0.10 (5)</td>
<td>0.57 ± 0.11 (5)</td>
<td>0.34 ± 0.10 (9)</td>
<td>0.48 ± 0.11 (7)</td>
<td>0.05 ± 0.01 (4)</td>
<td>~200</td>
</tr>
<tr>
<td>Cholesterol (µg/retina)</td>
<td>29.58 ± 2.47 (4)</td>
<td>23.20 ± 2.88 (4)</td>
<td>13.60 ± 1.12 (3)</td>
<td>8.31 ± 0.94 (3)</td>
<td>1.63 ± 0.02 (4)</td>
<td>NA</td>
</tr>
<tr>
<td>Cholesterol (nmol/mg protein)</td>
<td>72.4 ± 6.7 (4)</td>
<td>105.2 ± 8.1 (4)</td>
<td>102.4 ± 9.4 (3)</td>
<td>48.2 ± 5.7 (3)</td>
<td>84.0 ± 9.0 (4)</td>
<td>395.3 ± 72 (3)</td>
</tr>
<tr>
<td>% cholesterol total</td>
<td>NA</td>
<td>NA</td>
<td>68 (57.8)</td>
<td>32 (35.3)</td>
<td>94.6 (40.9)</td>
<td>NA</td>
</tr>
<tr>
<td>PL (µg/retina)</td>
<td>214.5 ± 29 (5)</td>
<td>130.8 ± 30 (6)</td>
<td>84.0 ± 19 (5)</td>
<td>110.8 ± 12 (5)</td>
<td>34.5 ± 2.0 (4)</td>
<td>NA</td>
</tr>
<tr>
<td>PL (nmol/mg protein)</td>
<td>270.0 ± 30 (5)</td>
<td>296.0 ± 42 (6)</td>
<td>292.2 ± 53 (5)</td>
<td>292.2 ± 36 (5)</td>
<td>884.8 ± 49</td>
<td>463.8 ± 86 (5)</td>
</tr>
<tr>
<td>% PL total</td>
<td>NA</td>
<td>NA</td>
<td>50 (36.6)</td>
<td>50 (48.3)</td>
<td>50 (15.1)</td>
<td>NA</td>
</tr>
<tr>
<td>NeuNAC (µg/retina)</td>
<td>2.57 ± 0.27 (5)</td>
<td>2.10 ± 0.50 (5)</td>
<td>1.42 ± 0.69 (9)</td>
<td>0.59 ± 0.07 (7)</td>
<td>0.15 ± 0.02 (4)</td>
<td>NA</td>
</tr>
<tr>
<td>NeuNAC (nmol/mg protein)</td>
<td>2.52 ± 0.23 (5)</td>
<td>3.69 ± 0.48 (5)</td>
<td>3.66 ± 0.39 (8)</td>
<td>1.30 ± 0.11 (7)</td>
<td>2.95 ± 0.3 (4)</td>
<td>5.47 ± 0.7 (5)</td>
</tr>
<tr>
<td>% NeuNAC total</td>
<td>NA</td>
<td>NA</td>
<td>73.7 (65.7)</td>
<td>26.3 (27.3)</td>
<td>26.3 (27.3)</td>
<td>(69)</td>
</tr>
<tr>
<td>GG (nmol/retina)</td>
<td>4.68 ± 0.10 (5)</td>
<td>3.75 ± 0.16 (5)</td>
<td>2.47 ± 0.22 (8)</td>
<td>0.92 ± 0.10 (8)</td>
<td>0.25 ± 0.03 (4)</td>
<td>NA</td>
</tr>
<tr>
<td>GG (nmol/mg protein)</td>
<td>4.59 ± 0.10 (5)</td>
<td>6.58 ± 0.28 (5)</td>
<td>5.90 ± 0.08 (8)</td>
<td>2.43 ± 0.05 (8)</td>
<td>4.97 ± 0.51 (4)</td>
<td>9.60 ± 1.2 (5)</td>
</tr>
<tr>
<td>% GG total</td>
<td>NA</td>
<td>NA</td>
<td>73.5 (67.9)</td>
<td>26.5 (25.2)</td>
<td>26.5 (25.2)</td>
<td>(69)</td>
</tr>
</tbody>
</table>

* Values are given as mean ± SD (number of samples in parentheses). Abbreviations for different samples as in Figs. 2, 3, and 6.
† OS protein values were estimated per retina, and values for cholesterol, PL, NeuNAC, and GG/per retina are calculated relative to this amount.
‡ Protein levels are averaged for single tissue samples; the percent loss from vibratome sectioning can be calculated from the difference between WR and IR + OR.
§ Estimates are given of the percent contribution in a particular lipid class (cholesterol, PL, NeuNAC, or GG) for IR, OR, and OS per retina. Values in parentheses take into account the projected contribution from OS.
|| P < 0.0001, statistical comparisons between WR and DR, IR and OR, and OS and OR.
NA = not applicable.

a given depth: In a total retinal thickness of 0.2 mm, major blood vessels, ganglion cell bodies, and their axons were present in the initial 40 µm, the IPL and INL were encompassed in the second 60 µm, and the remaining 100 µm was composed uniquely of photoreceptor cell bodies and the apical portion of Müller glia (Figs. 1a to 1d). Immunocytochemical labeling of cells dissociated from such isolated OR showed that >95% were positive for anti-opsin staining (Figs. 1e, 1f). Figures 1g and 1h show histologic sections of representative freshly dissected WR and DR, respectively, illustrating the integrity of the different retinal layers including OS in the former and the loss of the ONL and presence of membranous debris in the latter.

Neutral Lipid and Phospholipid Analysis of Retina

Protein contents of the different samples are indicated in Table 1. Based on these values, OR represented 55% and IR represented 39% of the protein content of WR, although it was clear that, compared to intact WR, there was some loss of material during vibratome sectioning because the combined IR–OR protein values were only 75% to 85% those measured for WR. Dystrophic rats show breakdown of the ONL (Fig. 1h), whereas the other layers remain relatively unaltered apart from the formation of extensive glial overgrowth. As observed in Table 1, total protein levels for DR were lower, consistent with cell loss. Outer segments represented approximately 6% of retinal protein.

CHOL levels in single adult WR averaged 29 µg, but this was not distributed evenly between the two halves when calculated on a unit protein basis: 68% was present in the IR and 32% in the OR (Table 1) (P < 0.0001). Examination of HPTLC plates of NL revealed that all fractions were similar, with large quantities of CHOL (approximately 50% total NL) and triglyceride (TG) (approximately 30% total NL) (Fig. 2). Outer segments contained similar CHOL levels to WR and were significantly different from OR. In addition, this fraction contained abundant diglycerides (DG) (Fig. 2). On a unit protein basis, DR contained higher amounts of CHOL (42% more) compared to WR (P < 0.0001). The distribution of NL in DR resembled that observed for the other retinal samples (data not shown).

Phospholipid concentrations were similar for all retinal samples except OS, the distribution was equivalent between IR and OR (228 µg PL/mg protein for both) (Table 1). Examination of individual PL species showed that WR contained a majority of phosphatidylcholine (Ptd-Chol) (48%) and phosphatidylethanolamine (Ptd-Et) (27%). The relative amounts of Ptd-Chol and Ptd-Et were different for IR and OR: whereas IR contained greater amounts of Ptd-Chol (49%) compared to Ptd-Et (24%), OR contained more Ptd-Et (30%) and consequently less Ptd-Chol (45%) (Table 2). All the minor species were present in approximately equal amounts between the different fractions (Table 2). The distribution of individual PL species was also largely similar between WR and DR except...
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FIGURE 2. High-performance thin layer chromatogram plate of neutral lipids (NL) isolated from different retinal samples. CHOL levels were assayed in the different samples, and 25 μg CHOL was spotted onto each lane: NL standards (St) (first lane), outer segments (OS) (second lane), whole retina (WR) (third lane), inner retina (IR) (fourth lane), and outer retina (OR) (fifth lane). NL were essentially present as CHOL and triglyceride (TG), together with a preponderance of diglycerides (DG) visible in the OS and OR.

for a reduction in the quantity of Ptd-Et in the latter (16.5% versus 28.7%) (Table 2). Outer segments were relatively enriched in PL (threefold greater concentrations than observed in the other samples) (Table 1). Regarding individual PL species, OS contained somewhat less Ptd-Cho (37%) and more Ptd-Et (40%) than other fractions. On a quantitative scale, OS contained large excesses of all PL species, especially Ptd-Et (fivefold more), compared to all other fractions (data not shown).

Ganglioside Analysis of Retina

Comparison of the lipid-associated NeuNAc and ganglioside content for the different retinal fractions revealed highly significant differences in the levels of total amounts and individual species. WR contained an average of 2.5 μg NeuNAc (4.6 nmol ganglioside/mg protein) (Table 1). However, as for CHOL, the distribution of these components was unequal between the two halves; 74% was in the IR, and only 26% was in the OR (Table 1). Determinations of lipid-bound NeuNAc and ganglioside contents for the various samples revealed that IR contained 3.7 μg NeuNAc/mg protein (5.9 nmol ganglioside/mg protein) compared to the OR, which contained only 1.3 μg NeuNAc/mg protein (2.4 nmol ganglioside/mg protein) (P < 0.0001) (Table 1). The ganglioside content of OS resembled WR, with 3.0 μg NeuNAc (5.0 nmol ganglioside/mg protein). On a unit protein basis, DR contained significantly more lipid-bound NeuNAc than WR (46% more, P < 0.0001).

High-performance thin layer chromatogram separation of the different preparations revealed several differences between the samples. WR contained a wide spectrum of ganglioside species, ranging from the simplest (GM3) to the most complex (GQ1b), with particularly prominent bands corresponding to GD3, GD1a, GD1b, and GT1b (Fig. 3). Many of these gangliosides were present as doublets, indicating the existence of differentially glycosylated isoforms. The ganglioside distribution profile of DR appeared slightly more complex than their normal counterparts, with an increased number of minor bands such as GM2 and GT1L (Fig. 3). Comparison of HPTLC plates of IR and OR demonstrated major differences in several gangliosides: IR contained only trace amounts of GM3 but abundant polysialo-ganglioside (GT1b, GQ1b) (Fig. 4). Outer retina samples contained similar levels of GM3 to those seen in WR, but also exhibited an especially prominent band of GD3, whereas the bands corresponding to the polysialylated forms GT1b and GQ1b were very faint (Fig. 4). Outer segment profiles were similar to WR, with a wide range of ganglioside species visible (data not shown).

Densitometric scanning of HPTLC plates permitted quantification of the respective ganglioside profiles (Fig. 5). When relative lipid-bound NeuNAc levels were expressed on a percent basis within the different samples, WR exhibited a complicated profile containing substantial amounts of mono-sialoGG, di-sialoGG, and tri-sialoGG. The distribution was dominated by GD3, with moderate amounts of GM3, GM1, and GD1a and substantial levels of GD1b and GT1b (Fig. 6). Analysis of the relative proportions of gangliosides in DR revealed essentially similar profiles to WR. Inner retina showed a reduction in GM3 and GM1, roughly similar levels of di-sialoGG, and higher amounts of tri-sialoGG and tetra-sialoGG. Compared to the IR, OR showed excesses of GM3 and GD3 and especially low levels of the more complex gangliosides, GT1b and GQ1b (below the limits of detection) (P < 0.0001) (Fig. 6). The ganglioside profile of isolated
OS was different from that observed for OR: Its distribution was still dominated by GD3, but it contained in addition large amounts of GD1b and GT1b (Fig. 6).

Compared to the ganglioside profile of whole brain, many of these relative percentages were very different: Brain expressed excesses of GM1, GD1a, and GT1b, and very little GD3 (Fig. 6). When these same values were expressed on a simple quantitative scale (μg NeuNAc/mg protein), the values obtained for IR and OR were all highly significantly different (P < 0.0001) except for GM3. Especially large excesses of GM1 and GD1b (3-fold), GD1a (4-fold), GT1b (14-fold) and GQ1b (≈20-fold) were observed for IR compared to OR (Fig. 7).

DISCUSSION

The retina, because of its laminated architecture in which different cell types are restricted to different layers, is amenable to mechanical fractionation permitting the analysis of different CNS populations. In the case of gangliosides and other lipids, this provides an opportunity to study qualitative and quantitative changes in such populations and to correlate lipid levels with known functional differences in these cells. In the current work, we have addressed this by mechanically isolating different layers, and we present data indicating that photoreceptor cells have very different lipid profiles from other retinal or brain neurons. The total amount of gangliosides is reduced in OR compared to IR, and there is a great decrease in the more complex gangliosides (GD1a, GD1b, GT1b, GQ1b) with a consequent enrichment of simple gangliosides (GD3) within the OR. CHOL levels also are reduced relative to the IR.

The ganglioside composition of retinal cells has been studied through quantitative measures of gangliosides in intact retina, metabolic labeling, or qualitative descriptions of anti-ganglioside antibody binding to different cell types. We have used a column chromatography-based method that, although leading to some loss of material (estimated at 10% to 15% for PL), permits direct comparisons of ganglioside profiles and amounts approach more closely typical composition of such populations as amacrine and ganglion cells. These observations are consistent with previous data indicating that other "typical" neuronal markers, such as tetanus toxin receptor and Thy-1 antigen, are limited to the IR. These data are supported further by A2B5 labeling of retinal neurons in vitro in the current study. Some in vivo and in vitro studies have associated the more complex gangliosides with neurites and synapses, which may be one reason for the higher amounts of di-sialoGG and tri-sialoGG in IR samples that contain the majority of gangliosides; Ptdlns = phosphatidylinositol; PtdOH = phosphatidic acid; PtdSer = phosphatidylserine.
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FIGURE 3. High-performance thin layer chromatogram plate of gangliosides isolated from whole retina (left lane) and dystrophic retina (DR) (right lane). Standards (St) obtained from bovine brain were supplemented with purified GM3 and GQlb (middle lane). NeuNAc was assayed in each different sample, and 4 to 6 μg were spotted onto the plate. The overall similarity of the two retinal samples and the large spread of different ganglioside species are visible. Ganglioside nomenclature is according to Svennerholm, and abbreviations are as in Figure 2.

Complex gangliosides (GT1b, GQ1b) were reduced particularly in adult rat OR, which, in contrast, were dominated by GD3 (accounting for more than 45% total gangliosides). In contrast, preparations of isolated OS possessed similar ganglioside contents and profiles to WR. Because OS are included in the OR fractions isolated by vibratome, it can be estimated that the ganglioside make-up of photoreceptor cell bodies is actually even simpler than calculated; for example, it contains very little GT1b. These lipid differences in photoreceptor cell compartments reveal the existence of distinct membrane domains, which have been suggested through earlier studies. The similarity of the ganglioside profile of rat OS to rat WR agrees well with previously published data for bovine OS, which were seen to resemble whole bovine retina. We cannot rule out that rat OS preparations are not contaminated by other retinal membranes, although the special NL (presence of DG) and PL profiles (threefold higher total PL levels, higher content of PtdEt) of this fraction indicate it is not similar to WR in other respects.

The possible reasons for the special ganglioside composition of photoreceptor are not clear. Interestingly, the cells that surround or interact directly with the photoreceptor, namely Müller glia and pigmented epithelium, also have simplified ganglioside profiles.

The relative enrichment of GD3 perhaps indicates a special function for this ganglioside. GD3 has been observed to be associated with cells undergoing intense metabolic activity, such as synaptic material. In addition, the low α:β ganglioside pathway ratio for IR is more typical of brain tissue, particularly visual cortex. With regard to the quantitative findings, it was not possible to assign particular ganglioside species to specific IR cell types because these tissue samples contain alternating cellular and synaptic regions. Further subdivision of IR fractions into INL and GCL compartments should provide more information.

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FIGURE 4. High-performance thin layer chromatogram plate of gangliosides isolated from inner retina (middle lane) and outer retina (right lane). Standards (St) obtained from bovine brain were supplemented with GM3 (left lane). Five micrograms NeuNAc were loaded onto each lane. The large differences in GM3, GD3, GT1b, and GQ1b content of the two retinal samples are easily visible. Abbreviations are as in Figure 2.
FIGURE 5. Densitometric scan profiles of gangliosides separated from the different retinal samples by high-performance thin layer chromatogram such as shown in Figures 3 and 4. Numbers refer to the identity of each peak with respect to standards (St) run in parallel. (upper trace) St = ganglioside standards from bovine brain supplemented with GM3 and GD3 show the different peaks corresponding to GM3 (1), GM1 (2), GD3 (3), GD1a (4), GD1b (5), GT1b (6), and GQ1 (7). The position of individual ganglioside peaks varies slightly between traces owing to small differences between individual experiments. Certain multiple peaks are visible, especially for DR: 1', possibly GM2; 5', possibly GT1L. Notice the absence of peaks 6 and 7 (lower trace) corresponding to OR. Abbreviations are as in Figures 2 and 3.

formed tumorigenic lines and reactive glia, where it is speculated that its lipophilic structure would favor permeability to ions and metabolites. Photoreceptors represent perhaps the most metabolically active neuron known, with a high energetic consumption devoted to the maintenance of ionic currents, membrane synthesis, and turnover; hence, GD3 may be important in this respect. High levels of GD3 are observed during the early stages of embryonic retinal and brain development, where it is thought to be involved in cell proliferation and migration. Because adult photoreceptors retain a high degree of plasticity, as witnessed by the continual turnover of their OS and their ability to survive and regenerate neurites in vitro, GD3 may be implicated. As exogenously added gangliosides exhibit neuroprotective effects against different insults, particularly when used in conjunction with soluble growth factors, their relative paucity in photoreceptor may contribute to the observed fragility of these cells in the face of diverse trauma, such as phototoxicity. Recently, we have demonstrated that systemic ganglioside injections exhibit protective effects against retinal ischemic damage, and they may prove to be useful in retarding photoreceptor degeneration.

There is a certain degree of heterogeneity in the retinal ganglioside profiles observed by HPTLC in the current study. Several gangliosides, such as GM3, GD3, GD1b, and GQ1b, migrated as closely spaced doublets. At least for GD3, the presence of distinct acetylated isoforms within the retina has been documented, and the existence of differentially glycosylated forms of other gangliosides also is known and provides an explanation for this micro-heterogeneity.

Additional supporting evidence for the unique ganglioside composition of photoreceptor comes from the analysis of mutant retina. The RCS-rdy-p+ strain of rat exhibits inherited progressive photoreceptor degeneration, resulting in total photoreceptor loss by 2 months of age, with the accumulation of abundant membranous debris within the subretinal space. The other retinal layers remain intact, although there is reactionary glial hypertrophy. Data showing increased CHOL and ganglioside levels, and decreased Ptd-Et amounts, are consistent with results indicating the differential distribution of these molecules between the IR and the OR. Previous investigators also have signaled the higher ganglioside content of mutant retina, although the explanation proposed concerned the increased glial content. It is probable that the glial cells do modify the ganglioside content because the ganglioside distribution in these retinas do not correspond with an expected enrichment.
in the IR. Indeed, we have shown that cultured Müller cells express GM2 while lacking the more complex gangliosides.22

In conclusion, this is the first report indicating the unusual simplicity of the ganglioside profile of differentiated photoreceptors relative to other CNS-derived neurons. Because CNS tissue routinely is characterized by its high and complex ganglioside content,15 photoreceptors constitute a notable exception to this rule. Indeed, with respect to both their low ganglioside content and their simplified profile, photoreceptors resemble non-CNS tissue more,49 and this special ganglioside profile may constitute one of the fundamental characteristics of mature photoreceptor. As the experimental approach adopted results in viable cells, it will permit us to analyze lipid metabolism in distinct retinal populations as a function of aging or disease. For example, because these data are consistent with either the preferential maintenance of a high GD3 synthase activity and/or a diminution of GM2 and GD2 synthase activities13 in the OR compared to the IR, it will be of interest to see whether there are reductions of the relevant sialyltransferase activities in photoreceptor cells and to examine any possible maturational or pathologic changes in these activities in vivo.

Key Words
analytical biochemistry, gangliosides, lipids, photoreceptors, tissue culture

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FIGURE 6. Histogram showing relative percentages of lipid-bound NeuNAc between different retinal and brain (Br) fractions. Data (mean ± SD) are from four to nine separate experiments, each performed in duplicate. Abbreviations are as in Figures 2 and 3.

FIGURE 7. Histogram showing absolute amounts of lipid-bound NeuNAc per unit protein in inner retina and outer retina fractions. Data (mean ± SD) are from four separate experiments, each performed in duplicate. Abbreviations are as in Figure 2.

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FIGURE 8. Histogram showing relative percentages of lipid-bound NeuNAc between different retinal and brain (Br) fractions. Data (mean ± SD) are from four to nine separate experiments, each performed in duplicate. Abbreviations are as in Figures 2 and 3.

FIGURE 9. Histogram showing absolute amounts of lipid-bound NeuNAc per unit protein in inner retina and outer retina fractions. Data (mean ± SD) are from four separate experiments, each performed in duplicate. Abbreviations are as in Figure 2.
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