GANGLIOSIDES OF BOVINE OPTIC NERVE

M. HOLM and J.-E. MÅNSSON
Department of Neurochemistry, Psychiatric Research Centre, University of Göteborg, Medicinaregatan 9, Fack, S-400 33, Göteborg, Sweden

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1. Introduction

A ganglioside transport through the optic pathway was proposed by Ledeen et al. [1,2], and Rösner et al. [3], from the results of their short term in vivo studies in fish. Holm [4], however, was not able to demonstrate any ganglioside transport along the rabbit optic pathway in short and long term in vivo experiments. None of these studies included any identification of the gangliosides in any part of the optic pathway, a fact which in part invalidated the interpretation of the results. Therefore, we previously analysed the major gangliosides of the mammalian retina [5], and now present the results of the characterization of the gangliosides of the mammalian optic nerve. Total gangliosides were isolated from 40 g of bovine optic nerve and the major gangliosides were analysed for chromatographic behaviour, carbohydrate composition and fatty acid and sphingosine patterns. Four major gangliosides were found, GM1, GD1a, GD1b and GT1, contributing more than 90% of the total gangliosides of the bovine optic nerve. Both fatty acid and sphingosine patterns of the gangliosides of the optic nerve differed from those of the retinal gangliosides.

2. Materials and methods

Bovine eye bulbs including the optic nerve were frozen immediately after slaughter. After thawing, the optic nerves were cut from the bulbs and freed from the surrounding connective tissue.

Abbreviations used: C, chloroform: M, methanol; W, water; P, propanol; TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

2.1. *Isolation of gangliosides*

Tissue from the optic nerve, 40 g wet weight, was extracted for lipids by chloroform–methanol (1:1, v/v) After alkaline hydrolysis the lipid extract was desalted on a Sephadex G-25 column [6]. The major gangliosides were separated from the predominant portion of other lipids by column chromatography on Silica Gel H [7]. All lipids except gangliosides were obtained by elution with 15 vol of C–M–W, 65:25:4 (by vol). The major gangliosides were eluted with 15 vol of C–M–W 60:35:8 (by vol). Less than 5% of the total lipid-sialic acid were obtained in the first eluate. The individual gangliosides of the C–M–W, 60:35:8 eluate were isolated by preparative TLC [7]. The individual gangliosides were tested for purity by analytical TLC [5].

2.2. *Characterization of the major gangliosides*

2.2.1. Chromatographic behaviour

The isolated gangliosides were tested by TLC in P–W 3:1 (v/v) and C–M–W 60:30:6 (by vol). A mixture of gangliosides prepared from human brains was used as reference.

2.2.2. Sialidase treatment

Approximately 10 nmoles of each ganglioside was incubated with sialidase [5]. The incubate was purified on a column of 0.2 g of Sephadex G-25 [8] and the residual lipid analysed by TLC [5].

2.2.3. Determination of Carbohydrate composition

Sialic acid was determined by the resorcinol

method [9], and glucose, galactose, glucosamine and galactosamine by GLC as alditol acetates [5].

2.2.4. Analysis of ceramide portion

Fatty acid and sphingosine patterns were analysed by GLC [10].

3. Results

The concentration of gangliosides in the bovine optic nerve was approximately 500 nmoles of lipid-sialic acid/g fresh weight. The hexosamine was found to be exclusively galactosamine by GLC.

3.1. *Monosialosyltetraglycosylceramide GM1*

The least polar of the major gangliosides migrated at the same rate as the reference of brain GM1, on TLC developed in P–W, 3:1 (v/v). No extra band was revealed when the TLC was developed in C–M–W, 60:30:6 (by vol). Sialidase treatment did not change the migration rate of the single resorcinol-positive band in any of the solvents and did not produce any product moving like the authentic reference sample of lactosylceramide. The intact ganglioside contained glucose, galactose, galactosamine and sialic acid in the molar proportion 1:2:1 (table 1).

3.2. *Disialosyltetraglycosylceramide GD1a and GD1b*

These migrated with the same rate as the brain reference gangliosides GD1a and GD1b in P–W, 3:1 (v/v) and C–M–W, 60:30:6 (by vol). Sialidase treatment gave one, resorcinol positive, band with the same R_F-value as brain GM1, and no other bands, tested with both solvents. The two gangliosides contained glucose, galactose, galactosamine and sialic acid in the molar proportion 1:2:1:2 (table 1).

Table 1
Carbohydrate composition of the major gangliosides of the bovine optic nerve, in molar ratios

	Sialic acid	Gal	Glc	GalNAc
GM1	1.0	1.8	1.0	0.7
GD1a	2.0	2.3	1.3	0.6
GD1b	2.0	2.1	1.1	0.7
GT1	3.0	2.3	1.3	1.0

Table 2
Fatty acid composition of the major gangliosides of the bovine optic nerve, in mole-%

	GM1	GD1a	GD1b	GT1
16:0	5	3	2	2
18:0	62	71	70	58
18:1	1	1	1	1
20:0	8	10	11	11
22:0	4	6	5	8
23:0	2	2	3	6
24:0	5	2	2	4
24:1	9	4	5	8
Σ 22–24	20	14	15	26

The following fatty acids were detected in less than 1%: 16:1, 20:1 and 22:1.

3.3. *Trisialosyltetraglycosylceramide GT1*

The fourth major ganglioside of bovine optic nerve had the same R_F-value as the brain reference GT1 in both P–W, 3:1 (v/v) and C–M–W, 60:30:6 (by vol). Sialidase treatment gave one, resorcinol positive band with the same migration rate as brain GM1 in both solvents. The molar composition of glucose, galactose, galactosamine and sialic acid was 1:2:1:3 (table 1).

3.4. *Fatty acid and sphingosine patterns*

These are shown in table 2 and table 3 respectively.

3.5. *Ganglioside composition of the optic nerve*

The four major gangliosides characterised constituted together more than 90% of total lipid-sialic acid. From a visual judgement of the analytical plates stained with resorcinol, each ganglioside fraction contained 15–25% of the total lipid-sialic acid.

Table 3
Sphingosine composition of the major gangliosides of the bovine optic nerve, in mole %

	GM1	GD1a	GD1b	GT1
dl8:0	2	0	0	0
dl8:1	48	40	46	49
d20:0	2	2	2	2
d20:1	48	58	52	49

4. Discussion

The optic nerve contains the myelinated axons of the retinal ganglion cells. We found the optic nerve to contain mainly GM1, GD1a, GD1b and GT1. No GD3 could be traced. This is in marked contrast to the ganglioside distribution in retina, where GD3 was shown to constitute approximately 50% of the gangliosides [5]. These results indicate that GD3 in retina is localised in cells other than the retinal ganglion cells. This has also been suggested from previous studies on fatty acid and sphingosine patterns of the retinal gangliosides [5,11]. It seems therefore not probable that the major retinal ganglioside GD3 can be transported through the optic pathway.

The major gangliosides of the bovine optic nerve, GM1, GD1a, GD1b and GT1, characterised in this study, were all present in the bovine retina [5]. However, the retinal gangliosides did not have the same fatty acid and sphingosine patterns as the same gangliosides of the optic nerve ([5,11], tables 2 and 3). All gangliosides of the optic nerve contained a larger portion of long-chain fatty acids, C_{20-24}, than the retinal gangliosides GD1a, GD1b and GT1. Besides, all gangliosides of the optic nerve had a larger fraction of d20:1 sphingosine than the retinal gangliosides. The sphingosine pattern of gangliosides vary with age [12], but this can not explain the described differences since the optic nerves extracted in this study were taken from the same eye bulbs that were used previously for study of the retinal gangliosides [5]. Instead, the results of both fatty acid and sphingosine analysis contradict the possibility of ganglioside transport from the retina through the optic nerve. However, we find the results in agreement with the suggestion by Holm [4], that the gangliosides of the optic pathway are synthetised in loco.

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