BIODEGRADATION OF THE MAJOR RABBIT RETINAL GANGLIOSIDES, STUDIED IN VIVO

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Recieved 11 March 1977

1. Introduction

The major retinal ganglioside of mammals has now been conclusively identified as GD3, by mass spectrometry analysis [1] and by enzymatic analysis [2]. The retinal GD3 differs from the other retinal gangliosides in both fatty acid and sphingosine patterns [1,3,4]. It is also more intensively labelled after an intraocular injection of *N*-[*acetyl*-^{3}H]mannosamine [5]. The aim of this study was to analyse the degradation of the rabbit retinal GD3, in comparison to the other retinal gangliosides, in a long-term in vivo experiment. The gangliosides were labelled by an intraocular injection of *N*-[*acetyl*-^{3}H]mannosamine, and the specific activities of the ganglioside sialic acids were determined 2, 4, 8 and 12 weeks after the injection.

The sialic acid of the GD3 ganglioside was more intensively labelled than the sialic acids of the other gangliosides and was degraded in two phases. The first phase, between 2 and 4 weeks after the precursor injection showed a half-life of approximately 2 weeks, while the half-life of the second phase, from 4 weeks on, was 8–10 weeks. The sialic acids of the other gangliosides, GM1, GD1a, GD1b and GT1, did not show any decrease in specific activity until 4 weeks after the precursor injection. The half-life of their degradation was 8–10 weeks, equalling the second degradation phase of the GD3 sialic acid.

*Ganglioside nomenclature according to Svennerholm [8].

2. Experimental

2.1. *Materials, precursor injection and ganglioside isolation*

N-[*Acetyl*-^{3}H] Mannosamine (50 μCi) 500 mCi/mmol (The Radiochemical Centre, Amersham) in 25 μl sterile isotone saline, was injected into the vitreous body, in both eyes of 28 albino rabbits. The animals weighed 1.5–2.0 kg and were anaesthetized by mebumal before the precursor injection [6]. The animals were killed 2, 4, 8 and 12 weeks after the injection, in groups of 7 rabbits, and the retinae were immediately taken out, pooled and stored at −20°C until lipid extraction. The retinal lipids were extracted by chloroform–methanol 1:2(v/v). The lipid extract was saponified and, after neutralization, desalted and freed from possible precursor contamination on a 5 g Sephadex G-25 fine column [7]. The gangliosides in the lipid eluate were separated from the other lipids on a 5 g silica-gel H (Fluka AG, Buchs, Switzerland) column [5]. Two batches of gangliosides were collected, one containing the GD3 ganglioside, the other containing the remaining retinal gangliosides GM1, GD1a, GD1b and GT1. The gangliosides were quantified by the determination of sialic acid with the resorcinol method [8].

2.2. *Isolation of sialic acids**

2.2.1. GD3

Approximately 100 nmol GD3 was hydrolysed with sialidase from *Vibrio Cholera* (Neuraminidase, Behringwerke) [9] and the liberated sialic acid was isolated from the remaining lipid on a 1 g Sephadex G-25 fine column [9].

2.2.2. GM1, GD1a, GD1b and GT1

An aliquot of the ganglioside mixture, corresponding to 100–200 nmol sialic acid, was hydrolysed with sialidase and later formic acid, and the liberated sialidase labile and stable sialic acids were isolated from the remaining lipids on 1 g Sephadex G-25 fine columns [9].

2.3. *Radioactivity determination*

The samples of sialic acid were evaporated and dissolved in 2 ml water. They were quantified by the resorcinol method [8] on duplicate samples. The radioactivities were determined by scintillation spectrophotometry on single samples containing 30–100 nmol sialic acid 0.5 ml water mixed with 10 ml Instagel (Packard), counted 3 X 1 min.

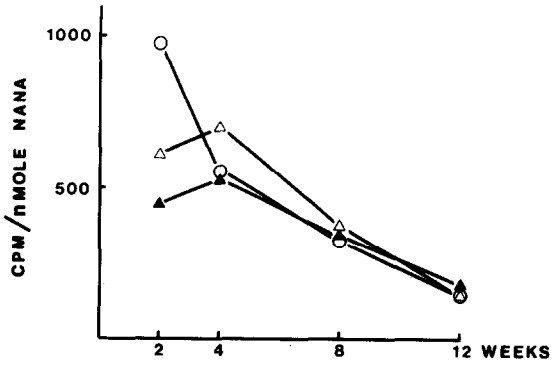

Fig.1. The specific activities of the sialic acids of the rabbit retinal gangliosides, after an intraocular injection of 50 μCi of *N*-[*acetyl*-^{3}H] mannosamine. GD3 ○; Mixture of GM1, GD1a, GD1b and GT1: sialidase labile sialic acid △; sialidase stable sialic acid ▲.

3. Results and discussion

The figure shows the specific activities of the sialic acid in the GD3 gangliosides and of the sialidase labile and stable sialic acids in the ganglioside mixture of GM1, GD1a, GD1b and GT1. The specific activity of the GD3 sialic acid was approximately two-fold higher than the specific activities of the sialidase labile and stable sialic acids of the other gangliosides, 2 weeks after the precursor injection. This is in agreement with the differences in specific activities for these gangliosides, previously found 12 h after the injection of the same precursor, in the short-term experiment [5]. The degradation-phase, in terms of decreasing specific activities, had started for the GD3 sialic acid already 2 weeks after the injection. The ganglioside mixture of GM1, GD1a, GD1b and GT1, however, still showed an increase in specific activities in both sialidase labile and stable sialic acids, between 2 and 4 weeks after the injection. Such a long incorporation phase was also found for these gangliosides in previous experiments on the optic pathway [6] and the brain [10]. The sialidase labile sialic acid of the ganglioside mixture GM1, GD1a, GD1b and GT1 had a higher specific activity than the sialidase stable sialic acid, up to 4 weeks after the precursor injection. This is in agreement with the results of the previous short-term experiments on retina [5] and brain [9,10].

The degradation of the GD3 seemed to take place in two phases. During the first period, between 2 and 4 weeks after the precursor injection, the specific activity of the GD3 sialic acid decreased rapidly, with a half-life of approximately 2 weeks, in contrast to the still increasing specific activity of the sialic acids of the other gangliosides. After 4 weeks, the specific activities of the sialic acids of both the GD3 and the ganglioside mixture of GM1, GD1a, GD1b and GT1, were approximately the same and decreased equally, with a half-life around 8–10 weeks. Such a long half-life was also found for the gangliosides in the optic pathway [6] and in the brain [10].

The differences between GD3 and the other gangliosides, in fatty acid and sphingosine patterns, and in the incorporation of the precursor *N*-(*acetyl*)mannosamine, was suggested to indicate a difference in cellular or subcellular localization (see e.g. [5]). The results of this study, showing a two-phase degradation curve for the GD3, with a rapid degradation of the GD3 between 2 and 4 weeks after the precursor injection, the other gangliosides still remaining in the incorporation phase, makes it probable that a considerable fraction of the retinal GD3 is to be found in a pool with a cellular or subcellular localization which is different from the localization of the other retinal gangliosides.

Acknowledgements

This work was supported by a grant from the Swedish Medical Research Council (project No. B76-03X-4744-01).

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