DIFFERENCES IN THE INCORPORATION OF *N*-[ACETYL-^{3}H]MANNOSAMINE INTO THE SIALIC ACID OF THE MAJOR RETINAL GANGLIOSIDES, STUDIED IN VIVO

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Received 8 July 1974

1. Introduction

The ganglioside GD3 constitutes approximately 50% of the mammalian retinal gangliosides [1] and differs from the other retinal gangliosides both in fatty acid [1] and sphingosine [2] pattern. GD3 was not present in detectable amounts in the bovine optic nerve [3], which contains the axons of the retinal ganglion cells, while the other retinal gangliosides GM1, GD1a, GD1b and GT1 were all present in the optic nerve. This study was undertaken to find out if the retinal GD3 differed from the other retinal gangliosides also in its incorporation of radioactivity from the sialic acid precursor *N*-[Acetyl-^{3}H] mannosamine. The precursor was injected into both eyes of 60 albino rabbits. The animals were killed 1.5, 3, 4.5, 6, 9 and 12 hr after the injection. The specific radioactivities of sialidase labile and stable sialic acid of the retinal GD3 in one batch and of the retinal GM1, GD1a, GD1b and GT1 in another batch were determined. The incorporation curve of the sialic acid in GD3 differed from that of both sialidase labile and stable sialic acid in the other retinal gangliosides, in shape as well as in level of radioactivity. The specific activities of sialidase stable sialic acid, in the mixture of GM1, GD1a, GD1b and GT1, were at all points lower than those of sialidase labile sialic acid, confirming the results from previous studies on the brain gangliosides [4,5].

* Abbreviations used: C, chloroform; M, methanol; W, water; NeuNAc, *N*-acetyl-neuraminic acid.

2. Experimental

2.1. *Materials, precursor injection and ganglioside isolation*

N-[Acetyl-^{3}H] mannosamine (40μCi), 276 mCi/mmole (Radiochemical Centre, Amersham, England), in 25 μl of sterile isotone saline, was injected into the vitreous body, in both eyes of 60 albino rabbits. The animals weighed 1.5–2 kg and were anaesthetized by mebumal before the injection [6]. The animals were killed 1.5, 3, 4.5, 6, 9 and 12 hr after the injection, in groups of 10 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 Sephadex G-25 column [7]. The gangliosides in the lipid eluate were separated from the other lipids by column chromatography on 5 g of Silica Gel H (Fluka AG, Buchs, Switzerland). The column was first eluted with 11 vol of C–M–W*65:25:4 (by vol) which was discarded. GD3 was eluted with another 14 vol of C–M–W 65:25:4 (by vol) and GM1, GD1a, GD1b and GT1 together in 15 vol of C–M–W 60:35:8 (by vol). The ganglioside fractions were quantified by the determination of sialic acid with the resorcinol method [8].

2.2. *Isolation of sialic acids*

GD3. Approximately 150 nmoles of GD3 was

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hydrolysed with sialidase from *V.cholera* and the liberated sialic acid was isolated from the remaining lipid on a 1 g Sephadex G-25 column [5].

2.2.1. GM1, GD1a, GD1b and GT1.

An aliquot of the ganglioside mixture, corresponding to 250–400 nmoles of 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 columns [5].

2.3. *Radioactivity determination*

The samples of sialic acid were evaporated and dissolved in 2 ml of water. They were quantified by the resorcinol method [8] on duplicate samples. The radioactivities were determined by scintillation spectrophotometry on single samples containing 40–130 nmoles of sialic acid, counted 2 × 1 min. [5].

3. Results and discussion

In fig. 1 the specific activities of the sialic acid in GD3 are compared with the specific activities of sialidase labile and stable sialic acids in the ganglioside mixture of GM1, GD1a, GD1b and

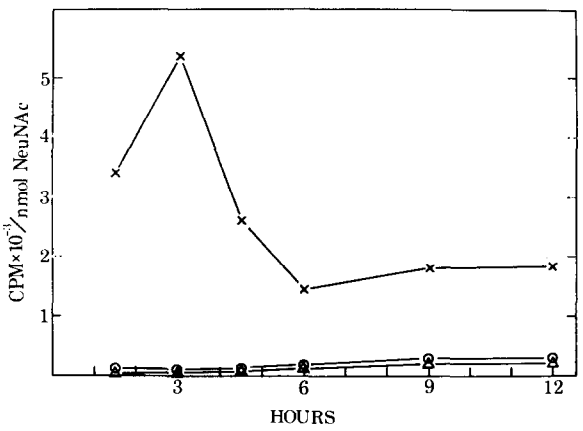

Fig. 1. The specific activities of the sialic acids of the rabbit retinal gangliosides, after an intraoccular injection of 40 μCi of N-[Acetyl-^{3}H] mannosamine. GD3, X- - - -X. Mixture of GM1, GD1a, GD1b and GT1: sialidase labile sialic acid, o- - - -o. sialidase stable sialic acid △- - - -△.

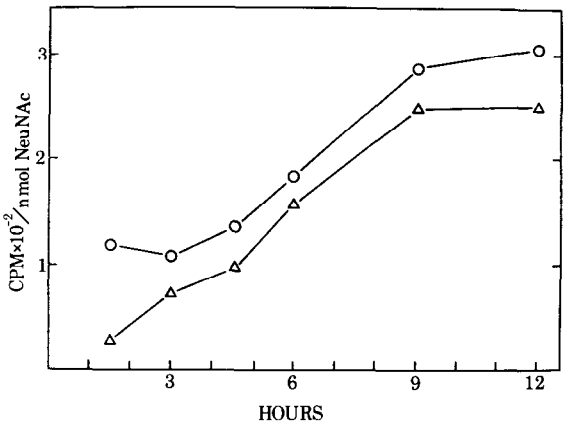

Fig. 2. The specific activities of the sialic acids of the rabbit retinal gangliosides GM1, GD1a, GD1b and GT1, in mixture, after an intraoccular injection of 40 μCi of N-[Acetyl-^{3}H]-mannosamine. Sialidase labile sialic acid, o- - - -o, sialidase stable sialic acid △- - - -△.

GT1. In fig. 2 the specific activities of the sialic acids of the GM1, GD1a, GD1b and GT1 mixture are again presented, now with another scale, to make possible a more detailed comparison.

The aim of this study was to compare GD3 with the other retinal gangliosides. Furthermore, in previous studies on retinal [6] and brain [4,5] gangliosides, no differences in incorporation of sialic acid precursors was found between the gangliosides GM1, GD1a, GD1b and GT1. Therefore, no attempts were made, in this study, to analyse the sialic acids of the GM1, GD1a, GD1b and GT1 individually.

The specific activities of the GD3 sialic acid were at all points higher, 125 to 6 times, than the specific activities of the sialic acids of the other gangliosides (fig.1). The possibility of an unspecific contamination with any radioactive low molecular precursor seems in our opinion not probable, since the column chromatographies on both Sephadex G-25 and Silica Gel H should give an adequate decontamination. One possible explanation is that the biosynthetic rate of GD3 is much higher than that of the other retinal gangliosides. This may be physiological, but may also be a stimulated biosynthesis as a result of the exogenous addition of the precursor. Another explanation would be that the precursor pool for GD3 produc-

tion was much higher labelled by the exogenous precursor, than the precursor pool(s) for GM1, GD1a, GD1b and GT1.

The incorporation curves also differed in shape (fig. 1 and 2), between the GD3 and the other retinal gangliosides. Thus both the sialidase labile and stable sialic acids of the GM1, GD1a, GD1b and GT1 mixture increased steadily in specific activity up to 12 hr after the injection, in the same manner as was shown for the same gangliosides in brain [4,5]. The sialic acid of GD3, however, followed another curve with two phases of increasing activities and one phase of decreasing activities in between. Such a double phase incorporation curve was also found for the GM1, GD1a, GD1b and GT1 gangliosides in rabbit optic pathway [6] and rat brain [4], but for these gangliosides the second phase of increasing activities did not start until 1–2 days after the injection of precursor.

The differences in incorporation curves between the retinal GD3 and the other retinal gangliosides give a further support for the suggestion, from the fatty acid [1] and sphingosine [2] analysis, that GD3 is localised at another site than the retinal GM1, GD1a, GD1b and GT1. Furthermore, from the fact that GD3 was not detectable in the bovine optic nerve [3], which contains the axons of the retinal ganglion cells, it seems most probable that GD3 is localised in any of several other cell types present in retina.

Acknowledgement

We thank Anders Glansholm, PhD, for drawing the figures. This work was supported by a grant from the Swedish Medical Research Council (project no. 13-627)

References

[1] Holm, M., Månsson, J.-E., Vanier, M.-T. and Svennerholm, L. (1972) Biochim. Biophys. Acta 280, 356–364.
[2] Holm, M. and Månsson, J.-E. (1974) FEBS Letters 38, 261–262.
[3] Holm, M. and Månsson, J.-E. (1974) FEBS Letters, in press.
[4] Holm, M. and Svennerholm, L. (1972) J. Neurochem. 19, 609–622.
[5] Holm, M., Månsson, J.-E. and Svennerholm, L. (1974) FEBS Letters 38, 271–273.
[6] Holm, M. (1972) J. Neurochem. 19, 623–629.
[7] Wells, M. A. and Dittmer, J. C. (1963) Biochemistry 2, 1259–1263.
[8] Svennerholm, L. (1957) Biochim. Biophys. Acta 24, 604–611.