FURTHER IN VIVO STUDIES ON THE BIOSYNTHETIC RELATIONSHIP BETWEEN THE MAJOR BRAIN GANGLIOSIDES IN RAT

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1. Introduction

The problem of a possible precursor–product relationship between the major brain gangliosides has earlier been studied in vivo by Suzuki and Korey [1], Maccioni and colleagues [2] and ourselves [3]. Labelled acetate and glucosamine were injected intracerebrally and the specific activities of sialidase labile and stable sialic acid of the individual gangliosides were determined at different intervals after precursor injection. To obtain more accurate data on the in vivo relationship, an immediate precursor of sialic acid, *N*-acetylmannosamine [4, 5], was injected intracerebrally in young rats. The sialidase labile and stable acids from the gangliosides G_{M1}, G_{D1a}, G_{D1b} and G_{T1} (nomenclature of L. Svennerholm, [6]) were isolated by Sephadex G-25 column chromatography. The specific activity of sialidase stable sialic acid was lower than that of sialidase labile sialic acid. No significant differences between the activities of the sialic acid from different gangliosides were obtained.

2. Experimental

2.1. *Materials, precursor injection and ganglioside isolation*

N-[Acetyl-^{3}H] mannosamine (75 μCi), 500 mCi/mmole (Radiochemical Centre, Amersham, England), in 100 μl of sterile isotone saline, was injected intracerebrally in each of 48 8-day-old rats of the Sprague–Dawley strain. The animals were killed 1.5, 3, 6 and 12 hr after the injection in groups of 12 rats, and the cerebra were taken out, pooled and stored at −20°C until lipid extraction. The gangliosides were extracted by chloroform–methanol (1:1, v/v), and separated from the predominant portion of other lipids by column chromatography on Silica Gel H (Fluka AG, Buchs, Switzerland) [3]. The crude ganglioside fraction was further purified by alkaline hydrolysis. The hydrolysate was neutralized with HCl and desalted on a Sephadex G-25 column (Pharmacia Fine Chemicals, Uppsala, Sweden) [7]. The major gangliosides, G_{M1}, G_{D1a}, G_{D1b} and G_{T1} were isolated by preparative thin-layer chromatography [3].

2.2. *Isolation of sialidase labile sialic acid*

Thirty nmoles of each ganglioside was evaporated in a small Kimax tube. Fifty μl of water, 5 μl of 1 M Tris–maleate buffer, pH 7.3, and 5 μl of sialidase, 500 units/ml (Behringwerke, Marburg/Lahn) were added. The mixture was incubated for 16 hr at 37°C. The incubate was evaporated to dryness and dissolved in 5 ml of chloroform–methanol–water (60:30:4.5, by vol) and applied to a 1 g Sephadex G-25 column. The gangliosides stable to the sialidase treatment were eluted by 5 ml of chloroform–methanol–water (60:30:4.5, by vol) and 5 ml of chloroform–methanol (2:1, v/v). The liberated sialic acid was eluted from the column by 8 ml of methanol–water (1:1, v/v).

2.3. *Isolation of sialidase stable sialic acid*

The sialidase treated gangliosides were hydrolysed in 1 ml of 1 M formic acid in a Kimax tube for 1 hr in a boiling water bath. After evaporation the liberated sialic acid was isolated on a 1 g Sephadex G-25 column as described above.

2.4. *Radioactivity determination*

The samples of sialic acid were evaporated and dissolved in 2 ml of water. The amount of sialic acid was determined by the resorcinol method [8] on duplicate samples of 0.5 ml each. For radioactivity determination 0.8 ml of the sialic acid solution was added to 10 ml of Instagel (Packard). Scintillation counting was performed for 10 min in a Packard Tri-Carb Scintillation Spectrophotometer, with a 50–1000 window and 48% gain. Quenching was checked by a channel ratio method.

2.5. *Statistics*

The S.D. of the specific activity as percentage of the mean was determined by duplicate analysis of sialidase labile and stable sialic acid from G_{D1a} and G_{T1} 6 and 12 hr after precursor injection [9].

3. Results

3.1. The S.D. obtained for the specific activity of sialic acids from G_{D1a} and G_{T1} 6 and 12 hr after precursor injection was 7% of the mean. The yield of sialic acid obtained by sialidase treatment was in all cases more than 95% of the theoretical value.

3.2. The specific activities of the sialidase labile and stable sialic acid are given in fig. 1. The specific activities for sialidase sialic acid of G_{D1a}, G_{D1b} and G_{T1} were lower than the specific activities of corresponding sialidase labile sialic acid. The specific activities of the sialidase labile sialic acids were linear up to 6 hr after precursor injection. The activity curves for sialidase stable sialic acid were lower in G_{D1b} and G_{T1} than in G_{D1a} and G_{M1}.

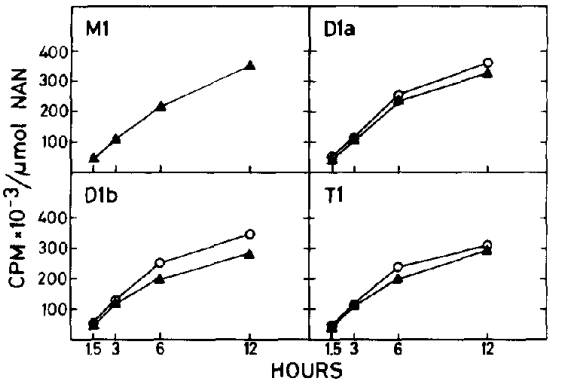

Fig. 1. Specific activities of sialidase labile (○) and stable (▲) sialic acid in cerebral G_{M1}, G_{D1a}, G_{D1b} and G_{T1} after an intracerebral injection of 75 μCi of *N*-[acetyl-^{3}H]-mannosamine into 8-day-old rats. Duplicate analysis for 6 and 12 hr G_{M1}, G_{D1a} and G_{T1}; otherwise single analysis.

4. Discussion

4.1. The specific activities of the sialidase stable sialic acid were lower, in all cases, than those of the labile sialic acid. These results confirm those obtained previously by us [3] but they are in contradiction to those of Suzuki and Korey [1] and Maccioni et al. [2]. The reliability of our results has, however, been increased by the fact that the standard deviation of the specific activity determinations was only 7%. The most probable explanation to our results is that there is a small fraction of previously synthetised G_{M1} with unlabelled sialic acid, which will serve as precursor for the formation of di- and tri sialoganglioside. Another possibility is that the stable sialic acid is incorporated into the ganliosides from a sialic acid pool different from the pool which furnishes the labile sialic acid.

4.2. The increase of the specific activities of the sialidase labile sialic acids was linear up to 6 hr after the precursor injection. Thus the specific activity of the precursor sialic acid pool was constant during these 6 hr, presuming a steady biosynthetic rate. This could only occur by a feed-back inhibition of the synthesis of sialic acid. Such a mechanism has in fact already been suggested by Kornfeld and co-workers [10]. They proposed that CMP-*N*-acetylneuraminic acid,

activated sialic acid, acted as an inhibitor for the formation of *N*-acetylmannosamine from *N*-acetylglucosamine. The injected *N*-acetylmannosamine in our experiment may initially have given such a large pool of sialic acid that the biosynthesis of *N*-acetylmannosamine was temporarily inhibited.

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References

[1] Suzuki, K. and Korey, S.R. (1964) J. Neurochem. 11, 647–653.
[2] Maccioni, H.J., Arce, A. and Caputto, R. (1971) Biochem. J. 125, 1131–1137.
[3] Holm, M. and Svennerholm, L. (1972) J. Neurochem. 19, 609–622.
[4] Jourdian, G.W. and Roseman, S. (1963) Ann. N.Y. Acad. Sci. 106, 202–217.
[5] Warren, L., Blacklow, R.S. and Spearing, C.W. (1963) Ann. N.Y. Acad, Sci. 106, 191–201.
[6] Svennerholm, L. (1963) J. Neurochem. 10, 613–623.
[7] Wells, M.A. and Dittmer, J.C. (1963) Biochemistry 2, 1259–1263.
[8] Svennerholm, L. (1957) Biochim. Biophys. Acta 24, 604–611.
[9] Dahlberg, G. (1948) in: Statistical Methods for Medical and Biological Students p. 125, Allen and Unwin, London.
[10] Kornfeld, S., Kornfeld, R., Neufeld, E.F. and O'Brien, P.J. (1964) Proc. Natl. Acad. Sci. U.S. 52, 371–379.