Cardiac Gangliosides in Sphingolipidoses* (33565)

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Ganglioside is the trivial name for a heterogeneous group of sphingoglycolipids that contain *N*-acetyl-neuraminic acid (NANA or sialic acid). At least ten different types of ganglioside have been identified in mammalian brain, where they may normally constitute up to 0.3% of the wet weight of brain. They have been also isolated from nonneural tissue, e.g., liver, spleen, and blood cells (1,2), but have not, to our knowledge been reported in heart.

The present study demonstrated the ganglioside patterns obtained from normal heart and the hearts of three patients with sphingolipidosis. The sphingolipidosis group contained a case of infantile amaurotic idiocy [Tay–Sachs disease (TSD)], an atypical variant of systemic late infantile amaurotic idiocy (SLIAI) (3), and an infantile form of Niemann–Pick disease (NPD). Biochemically, the infantile and the systemic late infantile types both had abnormally high concentrations of G_5 (GM_2) ganglioside in brain tissue (3), while the case of NPD had elevated concentrations of sphingomyelin. Gangliosides were found in all four hearts. The predominant ganglioside fractions in normal and Niemann–Pick hearts were G_4 and G_6. In contrast, the G_5 fraction was elevated in the two types of ganglioside storage disease.

Methods. Reagent grade and redistilled solvents were used. Duplicate samples of each heart were diced, homogenized, and extracted according to Folch *et al.* (4). The extracts were dried in a flash evaporator connected to a Virtis freeze drying apparatus, and applied to a cellulose column and eluted according to Rouser *et al.* (5). The ganglioside and water soluble nonlipid eluates were dried as above and the gangliosides were separated from the water-soluble nonlipids on a Sephadex column according to Rouser *et al.* (5). The ganglioside eluates were dried and made up to 5 ml in chloroform–methanol 2:1. Aliquots were taken for sialic acid analysis according to the method of Svennerholm (6). Aliquots were spotted on thin-layer plates prepared with Adsorbosil-3 (silica gel with 10% $MgSO_4$), using the wedge technique for greater resolution. The TLC was carried out using chloroform–methanol–2.5 *N* ammonium hydroxide, 65:35:5, followed by TLC in propanol–water, 7:3. Both runs were ascending for 7 hr each. The plates were developed using the resorcinol spray technique of Svennerholm (6). Sialic acid containing compound produces a blue color with the resorcinol spray. The plates were photographed with a Kodak Wratten no. 25 (red) filter (7).

Results. Table I demonstrates that compared to normal heart the concentration of NANA per gram of wet weight was highest in the heart of the patient with TSD. It was also elevated in the heart of the child with SLIAI.

Figures 1 and 2 illustrate the ganglioside patterns found. In the normal and the NPD heart the major ganglioside fractions are G_6 and G_4. Both the TSD heart and the SLIAI heart are characterized by increased concentrations of G_5 ganglioside. However, the concentration of G_5 in TSD is greater than that seen in the atypical systemic late infantile form. These results on the two ganglioside storage diseases parallel the ganglioside concentrations and distribution in the brain.

Discussion. Sialic acid containing glycolipids have been found in various tissues of the body. Normally in liver, spleen, and red cells, the dominant ganglioside type is G_6 (1, 2) while in brain, G_4, G_3, and G_2 are the major fractions. These lipids are intimately

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TABLE I. Heart Sialic Acid (mean).

Sample	Total NANA (mg)	Wet wt. (g)	NANA (mg/g of wet wt.)
Normal	0.271	4.29	0.063
Tay–Sachs disease	0.540	4.40	0.123
Systemic late infantile amaurotic idiocy	0.375	4.37	0.086
Niemann–Pick disease	0.240	3.60	0.066

associated with membranes (8) and appear to play an important role in membrane transport phenomena (9).

In TSD there is a marked increase of G_5 ganglioside in brain and a parallel increase of this ganglioside in liver (10). While the classical form of systemic late infantile amaurotic idiocy has been described as a GM_1 (G_4) gangliosidosis, the case studied here was shown to be a GM_2 (G_5) disorder (3).

The present study indicates that heart contains ganglioside. The increased total concentrations of cardiac sialic acid, and the relative predominance of G_5 in both clinical types of G_5 ganglioside storage disease are similar to the changes seen in brain. Total sialic acid and thin-layer chromatograms of exsanguinated 4-week-old rat hearts were similar to those found in normal heart. This suggests that the patterns reflect cardiac rather than blood ganglioside. Gas-liquid chromatographic analysis of the ceramide moiety of G_5 ganglioside in Tay–Sachs liver has indicated that at least part of this ganglioside was synthesized *in situ* (10). Similar analytical studies on G_5 in TSD heart would provide additional evidence for the systemic nature of the disorder.

Torres stated that the EKG abnormalities found in patients with TSD (11), are indica-

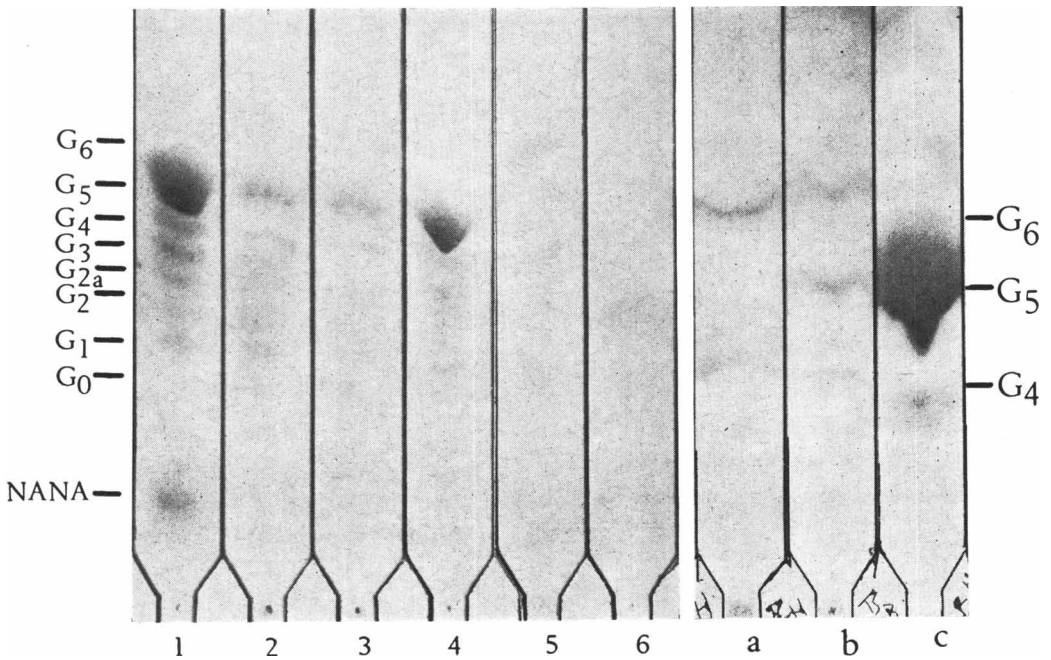

FIG. 1. (Left) Thin-layer chromatograms of cardiac ganglioside: 1. Tay–Sachs disease (brain); 2. atypical systemic late infantile amaurotic idiocy (heart); 3. atypical systemic late infantile amaurotic idiocy (heart); 4. Tay–Sachs disease (brain); 5. Niemann-Pick disease (heart); and 6. galactose.

FIG. 2. (Right) TL chromatograms of cardiac ganglioside: a. normal (heart); b. Tay–Sachs disease (heart); and c. Tay–Sachs disease (brain).

tive of primary myocardial damage. These changes, may in part, be due to the accumulation of ganglioside in the heart. The abnormal but characteristic ganglioside pattern in heart muscle may be duplicated in striated muscle. If this is proven to be the case, then biopsied striated muscle can be used instead of brain biopsy for biochemical identification of certain ganglioside disorders.

Summary. The finding of ganglioside in heart is further evidence of the widespread distribution of these lipids. The increase of GM_2 (G_5) ganglioside in the heart of two patients with GM_2 gangliosidosis suggests that the metabolic defect is not limited to the nervous system.

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Stimulation of Thyrotropin (TSH) Secretion by TSH-Releasing Factor (TRF) in Organ Cultures of Anterior Pituitary* (33566)

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Guillemin *et al.* (1) reported purification of a thyrotropin releasing factor from ovine hypothalami. Schally *et al.* (2, 3) isolated porcine TRF by procedures including gel filtration on Sephadex, phenol extraction, carboxymethylcellulose chromatography, countercurrent distribution, free-flow electrophoresis, and partition chromatography. These porcine preparations stimulated the release of TSH in codeine–thyroxine treated mice at a dose of 1 ng and depleted pituitary TSH in the same animals at a dose of 4 ng (2–5). These preparations also raised plasma TSH levels in rats (6) and stimulated TSH release *in vitro* (7).

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McKenzie (8) recently demonstrated that crude hypothalamic extracts caused increased incorporation of radioactive amino acids into TSH *in vitro*. We utilized our porcine TRF preparation, which was considered essentially homogeneous (2, 3), to undertake studies on the question of whether or not TRF can cause the stimulation of synthesis as well as release of TSH.

Materials and Methods. Tissue cultures of male rat anterior pituitaries were carried out by the method of Nicoll and Meites (9) with slight modifications.

Adult male rats of the Sprague-Dawley strain (Cheek-Jones Co., Houston, Texas) were used as donors for cultured tissue. The animals were decapitated and each posterior pituitary was removed and discarded. The anterior pituitary was removed and cut into