DEMONSTRATION OF AN ALTERATION OF GANGLIOSIDE METABOLISM IN TAY-SACHS DISEASE

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Summary

It has been shown through the use of specifically labeled Tay-Sachs ganglioside that normal human muscle tissue contains enzymes which catalyze the hydrolysis of the N-acetylneuraminyl and N-acetylgalactosaminyl moieties of this ganglioside. The hydrolysis of the N-acetylneuraminyl moiety in muscle preparations from patients with Tay-Sachs disease was similar to that of the controls. The enzyme which catalyzes the hydrolysis of the N-acetylgalactosaminyl moiety was completely absent in preparations from patients with Tay-Sachs disease.

Tay-Sachs disease is an inherited disorder in which there is an accumulation of the ganglioside N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosylceramide (G_{M2}) in brain and to a lesser extent in peripheral tissues of patients afflicted with this disease. These patients are severely retarded and eventually become blind. They generally have a cherry-red spot in the macula of the eye and they die before the 3rd year of life. The nature of the metabolic defects in all of the other major sphingolipid storage diseases has been demonstrated within the past few years and progress in this field has recently been summarized (1). In all of these diseases the abnormality

has been shown to be to a missing catabolic enzyme and a defect of this nature was anticipated in patients with Tay-Sachs disease (2). There seemed to be 2 alternative sites for such a metabolic lesion in this condition. The first was a deficiency of the enzyme which catalyzes the hydrolysis of the N-acetylneuraminyl moiety of Tay-Sachs ganglioside. This aspect was investigated through the preparation of Tay-Sachs ganglioside specifically labeled in the sialic acid portion of the molecule (3). No abnormality of this enzyme was found. This information suggested that investigations should be undertaken with Tay-Sachs ganglioside labeled in the N-acetylgalactosaminyl moiety as substrate. In the course of these experiments, Okada and O'Brien were able to demonstrate a missing hexosaminidase component in tissues of patients with Tay-Sachs disease through the use of p-nitrophenyl-β-D-N-acetylgalactosaminide and 4-methylumbelliferyl-β-D-N-acetylgalactosaminide (4). The revelance of this finding to the etiology of Tay-Sachs disease has now been demonstrated through the use of specifically labeled Tay-Sachs ganglioside and these data are presented in this report.

Materials and Methods

Tay-Sachs ganglioside labeled in the N-acetylgalactosaminyl and N-acetylneuraminyl moieties was prepared through a combination of biosynthesis and specific enzymatic degradation of mixed radioactive rat brain gangliosides. The details of the procedure and evidence for the localization of label have been submitted for publication (5). Essentially the method consists of the intracranial injection of N-acetyl-[^{3}H]-D-galactosamine or N-acetyl-[^{3}H]-D-mannosamine in 8 day old rats followed by the isolation of the labeled brain gangliosides. The radioactive polysialogangliosides are converted to [^{3}H]-monosialoceramidetetrahexoside (G_{M1}) through the action of V. cholera neuraminidase. [^{3}H]-G_{M1} is then converted to [^{3}H]-G_{M2} by the action of rat liver lysosomal β-galactosidase. The purity of the labeled G_{M2} was established by co-chromatography with authentic Tay-Sachs ganglioside in 4 solvent systems and by quantitative analysis of the components of the molecule. The locali-

zation of radioactivity in the [^{3}H]-G_{M2} was determined by mild acid hydrolysis using conditions which precluded the hydrolysis of the N-acetyl groups. When N-acetyl-[^{3}H]-galactosamine was used as the precursor, the label in the purified [^{3}H]-G_{M2} was equally distributed between the N-acetylgalactosaminyl and N-acetylneuraminyl moieties. When N-acetyl-[^{3}H]-mannosamine was the precursor, the radioactivity in [^{3}H]-G_{M2} was exclusively localized in the sialic acid moiety.

Human muscle tissue was used as the source of enzyme in these investigations because this tissue was found to exhibit good gangliosidase activity (3). Fresh specimens were obtained by biopsy of the vastus lateralis muscle. The tissue was finely minced and homogenized in 20 volumes (w/v) of 0.25 M sucrose-0.001 M EDTA solution. The suspension was centrifuged at 700 x g for 15 minutes.

TABLE I

Metabolism of [^{3}H]-Tay-Sachs Ganglioside Specifically Labeled in the N-acetylneuraminyl Moiety

Patient	Age	Condition	Sialic acid hydrolyzed
	yrs		picomoles per mg of protein per hr.
V. P.	2	Normal	388
G. D.	50	Cushing's syndrome	434
J. K.	44	Polymyositis	397
L. B.	2	Normal	295
M. C.	2	Tay-Sachs disease	404
G. T.	2	" " "	236
A. M.	2	" " "	450
S. G.	1	" " "	313

The reaction mixtures contained 22 nanomoles of [^{3}H]-G_{M2} (745 cpm/nanomole), 20 µmoles of potassium acetate buffer (pH 5.0), 170 µl aliquots of the respective enzyme preparations (270 to 440 µg of protein) and water in a final volume of 0.2 ml. The incubation time was 4 hours at 37°. The reaction was terminated by the addition of 0.8 ml of water, 10 mg of human serum albumin, and 0.1 ml of a 100% solution of CCl_3COOH. The suspension was centrifuged and the supernatant solution was decanted. The precipitate was washed with 1 ml of 10% CCl_3COOH and centrifuged. The supernatant solutions were combined and an aliquot was taken for radioactivity determination by liquid scintillation spectrometry.

The supernatant solution was decanted and was used as the source of enzyme.

Results

When [^{3}H]-G_{M2} labeled in the sialic acid portion of the molecule was used as substrate, the enzyme which catalyzes the hydrolysis of the N-acetylneuraminyl moiety of this molecule was found to be equally active in control human muscle preparations and those from patients with Tay-Sachs disease (Table I). This information prompted an investigation of the metabolism of

TABLE II

Enzymatic Hydrolysis of Tay-Sachs Ganglioside-[^{3}H] Labeled in the N-Acetylgalactosaminyl Moiety

Patient	Age	Condition	N-acetylgalactosamine hydrolyzed
			picomoles per mg of protein per hr
R.I.	14 yrs.	Neuromuscular disease	221
P.J.	33 yrs.	Myotonic dystrophy	339
M.I.	3 yrs.	Normal	293
J.D.	37 yrs.	Normal	362
C.S.	2 yrs.	Tay-Sachs disease	0
D.H.	1 yr. 9 mo.	Tay-Sachs disease	0
D.L.	2 yrs. 5 mo.	Tay-Sachs disease	0
J.D. + C.S.		Mixed Experiment*	201
J.D. + D.H.		Mixed Experiment	200
J.D. + D.L.		Mixed Experiment	81

The reaction mixtures contained 20 nanomoles of [^{3}H]-G_{M2} labeled in the sialic and N-acetylgalactosaminyl moieties. The specific activity of the aminosugar portion was 450 cpm per nanomole. The incubation conditions and procedure for work up of the product were the same as in Table I with the following modification. In order to remove free [^{3}H]-N-acetylneuraminic acid, the pH of the combined aqueous solutions was adjusted to 9.0 with 2.5 N NH_4OH and they were then passed over a 5 x 13 mm column of Dowex-1 (OH^-). The columns were washed with 0.5 ml of water and aliquots of the combined effluent solutions were assayed for radioactivity. The radioactive product from the control muscle preparations was passed over a Dowex-50 (H^+) column and the effluent solutions were lyophilized. The residue was taken up in 25 µl of 50% ethanol and applied to a thin-layer chromatogram (6). All of the radioactivity was confined to the region of the chromatogram which corresponded to the migration of N-acetylgalactosamine standard.

* 85 µl aliquots of the respective muscle preparations were mixed and incubated with the labeled substrate.

[^{3}H]-G_{M2} also labeled in the N-acetylgalactosaminyl moiety. The enzyme which catalyzes the hydrolysis of this aminosugar was present in normal human muscle preparations and in the various other control muscle biopsy specimens. However, this hexosaminidase activity is completely absent in muscle preparations obtained from patients with Tay-Sachs disease (Table II). In experiments in which equal amounts of control enzyme preparations were mixed with similar aliquots from the Tay-Sachs preparations, there was no evidence of inhibition of hexosaminidase activity with 2 of the Tay-Sachs preparations although some interference was caused by the third. The reason for this discrepancy is not known at this time.

Discussion

The present experiments conclusively demonstrate a deficiency of the enzyme which catalyzes the hydrolysis of the N-acetylgalactosaminyl moiety of ganglioside G_{M2} in muscle tissue obtained from patients with Tay-Sachs disease. Since enzymatic defects are ubiquitous in tissues of patients with lipid storage diseases (1, 2, 4), it may be concluded that the deficiency of this particular hexosaminidase is generalized in patients with Tay-Sachs disease. Further experiments are required and they will be undertaken to determine if the N-acetylneuraminyl moiety of G_{M2} must be cleaved prior to the hydrolysis of N-acetylgalactosamine. Nevertheless, the data presented clearly indicate an abnormality of ganglioside catabolism localized at the site of hydrolytic cleavage of the N-acetylgalactosaminyl moiety of G_{M2} in patients with Tay-Sachs disease.

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