Deficiency of Steroid β-glucosidase in Gaucher Disease

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Received September 25, 1975

Summary

A deficiency in the activity of steroid: β-glucosidase has been observed in the particulate fraction of Gauchers tissues. There was no diminution of the "soluble" form of this enzyme in adult tissue samples. In contrast, there was a marked reduction in the soluble steroid β-glucoside hydrolytic activity in the brain and spleen, and not liver from the infantile form of the disease.

Introduction

Gaucher's disease has as its clinical hallmark splenomegaly with attendant anemia, leukopenia, and thrombocytopenia. A lipid-laden foam cell present in bone marrow aspirates and in the circulation has been regarded as being pathonemonic. There are 2 clinically distinct forms of the disease. The adult form is reasonably benign and devoid of central nervous system involvement. The infantile form is invariably fatal due to the extensive central nervous system damage (1). Biochemically there is storage of glucosylceramide (2) in concert with a decreased detectable glucosylceramide β-glucosidase activity (3) in tissues of affected individuals with both forms of the disease.

We wish to report the reduced capacity of tissue samples from patients with Gaucher's disease for the hydrolysis of 17α-[6,7-^{3}H]-estradiol-3-β-D-glucoside.

Materials and Methods

[^{14}C]-glucosyl-ceramide (4), 17α-[6,7-^{3}H] estradiol-3-β-D-glucoside (6) and 17α-[6,7-^{3}H]estradiol-17-β-D-glucoside (8) were prepared as des-

cribed. Sodium taurocholate (crude) was purchased from Pfanstiehl (Waukegan, Ill.) and the 4-methylumbelliferyl (4MU) glycosides from Koch-Light Ltd. (Colnbrook, U.K.).

Homogenates were prepared in 10 volumes of 0.25M sucrose - 68 mM EDTA pH 7.5, an aliquot saved, and the remainder centrifuged at 100,000 x g for 1 hour. The supernate solution was removed. The pellet was homogenized in the original volume of a solution containing 0.2% Cutscum-1% sodium taurocholate, in 10 mM phosphate buffer pH 6.0, recentrifuged at 100,000 x g for 1 hr. and the supernates retained. This supernate was employed as the particulate enzyme preparation in these studies. The hydrolysis of glucosyl ceramide, the 4MU glycosides, the estradiol-3-β-glucoside and the estradiol-17-β-glucoside were assayed according to described methods (4,6,9).

Results

A comparison of the hydrolysis of the two labelled steroid β-D-glucosides, as well as 4 MU-β-D-glucoside, β-galactoside and N-acetyl-β-D-glucosaminide by whole homogenates of Gaucher, control, metachromatic leukodystrophy (MLD) and Nieman-Picks spleen tissue samples is shown in Table 1. It is evident that hydrolytic activity toward 17α-[6,7-^{3}H]-estradiol-17-β-D-glucoside and 17α [6,7-^{3}H]-estradiol-3-β-D-glucoside are reduced to 13% and 32% respectively in the Gaucher spleens as compared to the other 4 tissue samples. The hydrolysis of the galactoside and glucosaminide was not reduced in the Gaucher tissues. Mixing aliquots of the homogenates from the Gaucher spleens with controls gave the predicted values, suggesting that the decreased hydrolysis was not due to the presence of inhibitors. The low level of 4MU-β-D-glucoside cleavage is usual for Gaucher tissues.

Previous work suggests that there may be both a soluble and a particle bound form of the steroid-β-D-glucosidase, the relative amounts of which may vary between tissues (10). It was therefore of interest to examine the hydrolytic activity of the soluble and particulate fractions of tissue homogenates from the adult and infantile forms of the disease, and these

Table I: 17α-Estradiol-17β-D-glucoside: β-glucosidase; 17α-estradiol-3-β-D-glucoside: β-glucosidase 4-MU-β-D-glucosidase, β-galactosidase and β-N Acetyl hexosaminidase activities in human spleen samples

Sample	17α-Estradiol 17β-glucoside	17α-Estradiol 3β-glucoside	4-MU-β-glucoside	4MU-β-galactoside	4MUβ-N-Acetyl-glucosaminide
Gaucher	0.015	0.086	0.94	126.3	27,950
Gaucher	0.010	0.046	0.66	74.8	12,360
Normal	0.079	0.278	30.7	53.7	4,300
Normal	0.087	0.18	33.1	132.8	6,490
MLD	0.119	0.194	57.2	95.2	7,200
Nieman Pick	0.074	0.164	28.3	101.3	8,300

Activities expressed as nmoles of substrate hydrolyzed per h per mgm protein except 17α-[^{3}H]-Estradiol-3-17β-glucoside which were incubated 17 h.

Table II: The hydrolysis of 4MU-β-D-glucoside, glucosyl ceramide and 17α-estradiol-3-β-D-glucoside by the soluble and particulate fraction of infantile and adult Gaucher tissues.*

	4-MU-β-Glucoside		Glucocerbroside		Steroid β glucoside	
	Soluble	Particulate	Soluble	Particulate	Soluble	Particulate
INF. Control Spleen (1)**	16	27.5	6.6	215.2	9.4	13.1
INF. Gaucher Spleen (2)	1.5	0.3	4.4	18.2	NDH	NDH
INF. Gaucher Spleen (3)	NDH	0.4	2.6	0.9	1.6	0.8
INF. Control Liver (1)	72.6	32.5	5.5	204.7	11.8	12.1
INF. Gaucher Liver (2)	51.6	2.6	13.2	27.1	29.7	1.7
INF. Gaucher Liver (3)	77.6	1.2	3.8	10.9	21.5	.6
INF. Gaucher Liver (4)	NDH	1.0	4.9	17.3	13.8	.6
INF. Control Brain (1)	2.26	93.3	0	773.1	7.2	30.55
INF. Control Brain (11)	3.8	27.9	0.7	679.4	7.5	27.1
INF. Gaucher Brain (2)	0	7.2	2.0	96.9	0.2	3.5
INF. Gaucher Brain (3)	0.7	2.1	2.5	24.1	2.1	1.2
Control Adult Spleen (5)	5.5	44.3	61.2	260.0	4.7	6.7
Control Adult Spleen (6)	32.5	43.4	9.5	248.1	9.3	25.5
Adult Gaucher Spleen (7)	2.2	1.0	3.8	12.1	2.5	NDH
Adult Gaucher Spleen (8)	5.5	3.3	9.2	33.7	9.5	1.6
Adult Gaucher Spleen (9)	8.8	3.0	9.1	27.5	7.4	1.4
Adult Gaucher Spleen (10)	12.9	5.2	6.3	51.8	15.3	3.1

*Activities expressed as nmoles cleaved/mg protein/unit time.
**Numbers in parenthesis designate tissues from different individuals

NDH = no detectable hydrolysis

data, along with control values, are presented in Table II. It is apparent that with the particulate fraction of all Gaucher's patient tissues examined there was a significant reduction in the hydrolysis of estradiol-3-β-D-glucoside as well as of 4 MU-β-D-glucoside and glucosyl ceramide. The activities in the soluble portions of the adult Gaucher spleen and infantile Gaucher liver were not reduced. There was an appreciable reduction in the hydrolysis of the steroid β-D-glucoside and the 4 MU-β-D-glucoside by both the soluble and the particulate fraction of the infantile Gaucher spleen and brain.

Discussion

This laboratory recently reported both a decreased hydrolysis of glucosyl sphingosine (4) and its isolation from (5) Gaucher's tissue samples. This was the first indication of biochemical abnormalities in this disease in addition to the classical alterations of glucosylceramide.

We previously reported that the purified enzyme from calf spleen (6) and brain (7) hydrolyzes at least 5 compounds. These were 4 MU-β-D-glucoside, pNP-β-glucoside, glucosylsphingosine, glucosyl ceramide and deoxycorticosterone-β-D-glucoside. We speculated from these results that there may be deficiency of steroid β-D-glucoside hydrolysis in Gaucher's tissue. The current results support this hypothesis. As shown in Table I there is a decreased hydrolysis of both 17α-[6,7-^{3}H]-estradiol-3- and 17-β-D-glucosides in homogenates of Gaucher's spleen. There is no decrease in activity towards these 2 substrates in homogenates from two other sphingolipidoses, namely Niemann-Picks disease and metachromatic leukodystrophy.

It appears that there are two distinct steroid β-D-glucosidases in mammalian tissues, one soluble and the other particulate. A soluble rabbit liver enzyme has been highly purified (9) and its properties studied. It was found incapable of hydrolyzing glucosyl ceramide. This laboratory has recently purified a similar enzyme from porcine kidney (12), and showed that the preparation effectively hydrolyzes 4 MU-β-D-glucoside and 17α-estradiol-3-β-D-glucoside but does not cleave glucosyl ceramide. In contrast,

the highly purified particulate calf brain (7) and spleen (6) enzyme hydrolyzes all three substrates. The relative ratios of the amount of soluble and particulate enzyme varies in different tissues (9).

It appears that the particulate form of the enzyme is affected in both the adult and infantile Gaucher tissue. The soluble activity is not diminished (Table II) in adult Gaucher spleen tissue while it is reduced in infantile Gaucher brain and spleen, but not liver. This suggests that these two forms of the enzyme may be products of different genes and in these particular tissues the soluble activity is unaffected in the adult form. However, in the infantile Gaucher spleen and brain both the soluble and the particulate β-glucosidase activities are decreased. This may provide the biochemical basis distinguishing the adult and infantile forms of Gaucher's disease. Prior attempts to explain the clinical differences between these two types have been unsuccessful. It is possible that altered steroid β-D-glucoside metabolism may adversely affect normal brain development resulting in the characteristic central nervous system abnormality found in infantile Gaucher's disease.

Acknowledgements

Supported in part from U.S.P.H.S. Grants NS10330, HD05515, HD04147 and MRC of Canada MT 3287.

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