FIBROBLAST PHOSPHODIESTERASE DEFICIENCY IN NIEMANN-PICK DISEASE

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SUMMARY

Cultured skin fibroblasts from patients with Niemann-Pick disease types A and B were found to have diminished activity towards the synthetic substrates bis(4-methylumbelliferyl) pyrophosphate diester and bis(4-methylumbelliferyl) phosphate. Fibroblasts from a patient with Niemann-Pick disease type C exhibited less diminished activity. No reduction in activity was found towards bis(p-nitrophenyl) phosphate in types A, B or C fibroblasts. Maximum deficiency in types A and B fibroblasts was towards bis(4-methylumbelliferyl) pyrophosphate diester at pH 5.0, no deficiency being found at pH 7.2, either in the presence or absence of Mg^{++} and cysteine.

INTRODUCTION

Niemann-Pick disease is a heterogenous group of disorders designated types A-E (1,2) and characterised by excessive tissue storage of sphingomyelin (3) and an autosomal recessive mode of inheritance.

Sphingomyelinase activity is reduced in tissues of patients with types A (4) and B (5) and has been reported absent in isoenzyme II (6,7) in type C. No enzyme defect has yet been established for types D and E. Deficient activity towards sphingomyelin (8,9) and an analogue (10) has been reported in cultured fibroblasts from homozygous and heterozygous type A mutants and against bis(p-nitrophenyl) phosphate (Bis(pNP)P) in liver and brain from patients with types A and C (11).

Sphingomyelinase cleaves the phosphodiester bond which links C-1 of N-acylsphingosine to phosphorylcholine of sphingomyelin and is therefore a phosphodiesterase. Synthetic methylumbelliferyl glycosides and esters have been widely used for identification of genetic defects of lysosomal enzymes. However, reduced phosphodiesterase activity towards methylumbelliferyl diesters has not yet been reported for detection of Niemann-Pick disease. We now report deficient phosphodiesterase activity against the synthetic fluorogenic substrates bis(4-methylumbelliferyl) pyrophosphate diester (Bis(4MU)PP) and bis(4-methylumbelliferyl) phosphate (Bis(4MU)P) in fibroblasts from patients with Niemann-Pick disease types A and B and less marked deficiency in type C. Normal phosphodiesterase activity was found against (Bis(pNP)P).

METHODS

Fibroblasts were grown in Dulbecco's modified MEM containing 10% fetal bovine serum, penicillin (200 i.u./ml) and streptomycin (200ug/ml). Cells were harvested from confluent cultures with 0.02% EDTA in phosphate-buffered saline, washed in normal saline and disrupted by sonication. For measurement of phosphodiesterase activity the assay mixture contained 5mM Bis(4MU)PP, Bis(4MU)P or Bis(pNP)P, 100mM sodium acetate (pH 5.0) or 100mM tris-HCl (pH 7.2) buffer and 20-50µg protein in a final volume of 100µl. $MgCl_2$ (5mM) and cysteine (10mM) were added to the reaction mixture where indicated. After incubation for 30 min. at 37° reactions were stopped with 0.5M glycine-NaOH buffer, pH 10.3 (1.5ml) for determination of 4-methylumbelliferone or with 0.05M - NaOH (1ml) for determination of p-nitrophenol. The former was measured from the fluorescence produced at 448nm with an excitation wavelength of 360nm, and the latter from its absorbance at 400nm.

Sphingomyelinase was assayed by the method of Harzer and Benz (13). The substrate (^{3}H)-sphingomyelin was prepared by the Radiochemical Centre, Amersham, by reduction of sphingomyelin (Koch-Light Laboratories Ltd.) with tritium gas and was purified in this laboratory by preparative layer chromatography on silica gel plates using the solvent system chloroform : methanol : water (65:24:4, v/v).

All cultured fibroblasts used in this study are stored in the cell bank of human mutant cells of the Prince Philip Research Laboratories.

TABLE 1

Hydrolysis of Bis(4MU)PP and (^{3}H)-sphingomyelin by extracts of cultured skin fibroblasts

	Bis(4MU)PP (nmole 4-methylumbelliferone released/h/mg protein)	(^{3}H)-sphingomyelin (nmole (^{3}H)-ceramide released/h/mg protein)
Normal individuals	83-272 (n=8); mean 147	49-136 (n=4); mean 82
Niemann-Pick disease type A	20-33 (n=4); mean 28	1.8-4.5 (n=4); mean 2.6
Niemann-Pick disease type B	28*	2.9
Niemann-Pick disease type C	63	46
Mother of type B	76	21
Father of type B	98	43
Fabry's disease	106	109
Gaucher's disease type I	300	73
GM_1-gangliosidosis type I	337	66
GM_1-gangliosidosis type II	292	74
Krabbe's disease	187	82
Juvenile metachromatic leucodystrophy	167	-

* Range 23-34 in separate determinations on four cultures.

RESULTS AND DISCUSSION

Fibroblast phosphodiesterase activities against Bis(4MU)PP at pH 5.0, together with sphingomyelinase activities are shown in Table 1.

TABLE 2

Hydrolysis of bis(4-methylumbelliferyl) phosphate and bis(p-nitrophenyl) phosphate at pH 5.0 and of bis(4-methylumbelliferyl) pyrophosphate at pH 7.2 by extracts of cultured skin fibroblasts

Diagnosis (no. of cultures)	4-methylumbelliferone or p-nitrophenol released (nmole/h/mg protein)			
	Substrate			
	Bis(4MU)P pH 5.0	Bis(pNP)P pH 5.0	Bis(4MU)PP pH 7.2	Bis(4MU)PP pH 7.2 + $MgCl_2$ + cysteine
Normal controls (n=3)	78-85 mean 80	178-199 mean 187	5.3-9.0 mean 6.6	7.9-12.2 mean 9.7
Niemann-Pick disease, A (n=3)	43-69 mean 57	131-185 mean 169	3.1-16.8 mean 9.2	6.3-19.2 mean 12.1
Niemann-Pick disease, B (n=1)	41	161	7.7	10.3
Niemann-Pick disease, C (n=1)	57	182	8.4	11.6
Parents of type B patient (n=2)	M 80 and F 86	F 191 and M 214	M 5.7 and F 7.0	M 8.6 and F 8.9

Mean phosphodiesterase activities in fibroblasts from four patients with Niemann-Pick disease type A and in one with type B were reduced to 19% of control mean values. Activities in fibroblasts from two obligatory type B heterozygotes (who were parents of one of the patients) were 67% (father) and 52% (mother) of the control mean, the value for the father being within the control range. Activity in type C fibroblasts was 43% of the control mean and just below the control range. In contrast, no deficiency was detected in fibroblasts from patients with other lipidoses. Thus activities in fibroblasts from patients with Fabry's and Krabbe's diseases and juvenile metachromatic leucodystrophy were within the control range, while activities were elevated in GM_1-gangliosidosis types 1 and 2, and in Gaucher's disease type 1 (Table 1).

Reduction of phosphodiesterase activities in type A at pH 5.0 was less marked against Bis(4MU)P than against Bis(4MU)PP (Tables 1 and 2); nevertheless, all values were below the control range. Activities against Bis(4MU)P were also below the normal range in type B (52% of control mean) and in type C (71%) fibroblasts. Type B heterozygotes, however, showed no reduction in activity against Bis(4MU)P. No deficiency of phosphodiesterase activity was detected against the third synthetic substrate, Bis(pNP)P, in homozygous mutant (types A, B & C) or heterozygous (type B) cells (Table 2).

Callahan et al., 1974 (11) found that hydrolysis of Bis(pNP)P was most decreased in brains and livers from patients with Niemann-Pick disease type A or C at pH 7.2 in the presence of magnesium and cysteine. In contrast, we found maximum deficiency in fibroblasts at pH 5.0 (over the range pH 3.5 - 8.0) and no deficiency when activities were measured at pH 7.2

(tris-HCl), irrespective of the presence of magnesium and cysteine in the assay mixture (Table 2).

The reason for the concomittent decrease of sphingomyelinase and phosphodiesterase activities in Niemann-Pick fibroblasts is unknown. However, the data are in accord with the explanation that sphingomyelinase has phosphodiesterase activity.

Our results suggest that assay of cultured fibroblasts Bis(4MU)PP phosphodiesterase will be useful for diagnosis and genetic study of Niemann-Pick homozygotes.

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