ENZYMATIC DEFICIENCY IN NEUROLOGICAL MUTANTS. BRAIN URIDINE DIPHOSPHATE GALACTOSE:CERAMIDE GALACTOSYL TRANSFERASE IN JIMPY MOUSE

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1. Introduction

There is a substantial amount of data supporting the view that the myelination defect in the Jimpy mutation is accompanied by a disturbance of glycolipid biosynthesis in the central nervous system (CNS). Cerebrosides and sulphatides, important glycolipid components of the myelin sheath, are significantly reduced in Jimpy brain [1–3]. The incorporation of galactose-^{14}C *in vivo* into cerebrosides and sulphatides is much lower in Jimpy brain than in normal controls [4–6]. Further, there is a marked decrease of a galactosyltransferase catalyzing the transfer of galactose from UDP-galactose to sphingosine to give galactosyl-sphingosine (psychosine) [5, 7]. The activity of this enzyme was also decreased in Quaking mouse, another mutant with defective myelination [8]. This finding was of particular interest since psychosine is considered as an intermediate in the postulated pathway of cerebroside biosynthesis [9, 10].

Ceramide was active as the acceptor of both glucose and galactose from their nucleotide derivatives in the presence of glycosyltransferases from embryonic chicken brain [11]. The presence of a galactosylceramide transferase (UDP-galactose:ceramide galactosyl transferase) has also been reported in mouse brain [12]. The enzyme was active only with ceramide containing hydroxy fatty acids. The following reaction catalyzed by this enzyme was proposed as a possible route of cerebroside synthesis in brain [12]:

HFA-ceramide* + UDP-galactose → HFA-cerebroside + UDP

In the present work experiments with 15- to 19-day old Jimpy and control mice have demonstrated a very pronounced decrease of the galactosyl-ceramide transferase activity in mutant brain.

2. Materials and methods

Ceramides were prepared from beef brain cerebrosides [13] and purified by the silicic acid column chromatography and crystallization from ethyl acetate. HFA-Ceramides were separated from NFA-ceramides by TLC on silica gel G with chloroform-acetone-ethanol-ammonia-water (70:40:6:1:1, v/v) as developing solvent. Only sphingosine and hydroxy fatty acids were detected by TLC in the hydrolysis products after treatment of this material with methanolic KOH [13]. The origins of standard lipids, labelled sugar nucleotide and other reagents were as described in previously published papers [5, 7].

For experiments with subcellular fractions, a 10% brain homogenate of 19-day old mice in 0.32 M sucrose containing 1 mM EDTA was centrifuged at 800 *g* for 10 min to remove nuclei and cell debris.

* *Abbreviations:*
HFA, hydroxy fatty acids
NFA, non-hydroxy fatty acids
TLC, thin-layer chromatography.

The crude mitochondrial fraction was then sedimented by centrifuging at 13,500 *g* for 15 min. The supernatant suspension from this step was centrifuged at 100,000 *g* for 60 min. The pellet obtained (crude microsomal fraction) was resuspended in 0.32 M sucrose and centrifuged at 15,000 *g* for 15 min. The pellet was discarded and the supernatant centrifuged at 100,000 *g* for 60 min. The pellet from this step (purified microsomal fraction) was resuspended in 0.32 M sucrose containing 1 mM EDTA to give a final concentration of about 5 mg of protein per ml. The crude mitochondrial fraction was resuspended in 0.32 M sucrose, layered over a discontinuous gradient (1.2, 1.0 and 0.75 M sucrose) and centrifuged at 100,000 *g* for 60 min. The fractions M_1 (between 0.32 and 0.75 M), M_2 (between 0.75 and 1.0 M) and M_3 (between 1.0 and 1.2 M) were collected and resuspended in 0.32 M sucrose containing 1 mM EDTA to give a concentration of about 5 mg of protein per ml. Protein in subcellular fractions was determined by the method of Lowry et al. [14].

The appropriate amounts of ceramide and detergent solutions in chloroform-methanol (2:1) were mixed and dried in tubes before addition of other components of the incubation mixture. The reaction was stopped with chloroform-methanol (2:1 v/v, 10 vol.) and carrier glycolipids were added. The solution was partitioned by the addition of water (1 vol.) and the lower phase washed twice with chloroform-methanol-water (3:48:47, v/v). For determination of the total radioactivity, an aliquot of the lower phase was dried in the counting vial before the addition of the scintillation fluid. For the radioactivity measurement of individual glycolipids, an aliquot of the lower phase was chromatographed on thin-layer plates as described previously [5, 7] and the corresponding zones of adsorbant were scraped directly into counting vials. Radioactivity was determined by a liquid scintillation spectrometer (ABAC SL 40, Intertechnique, France).

3. Results and discussion

After incubation of UDP-galactose-^{14}C and HFA-ceramide with control and Jimpy brain homogenates (table 1), most of the lipid radioactivity was found in the fraction corresponding to galactocerebrosides. The incorporation of galactose-^{14}C was much lower with Jimpy brain homogenates.

The subcellular distribution of the enzyme activity is shown in table 2. The highest activity was found in the microsomal fraction. In both normal and mutant mice almost all of the radioactivity incorporated into cerebrosides was recovered in the HFA-containing fraction. With the exception of the supernatant fraction in which galactosyl-ceramide transferase activity was virtually absent, significantly lowered values were obtained with subcellular fractions of Jimpy brain. This observation was confirmed when galactosyl-ceramide transferase activity was determined as a function of the enzyme concentrations using a purified microsomal fraction of control and mutant brain (fig. 1).

The present results coupled with previously estab-

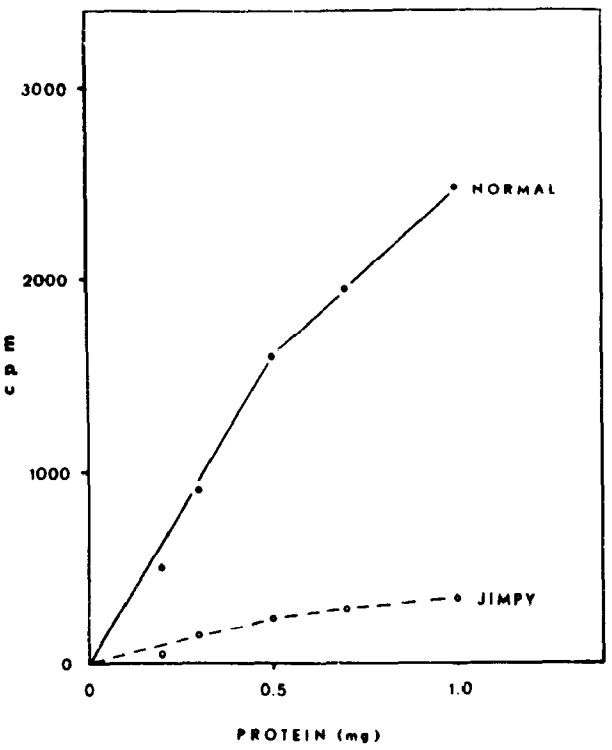

Fig. 1. Effect of the microsomal protein concentration on the galactosyl-ceramide transferase activity in normal and Jimpy mice. Incubation mixture contained (in 0.25 ml final volume): UDP-galactose-^{14}C, 10 nmoles (5 μCi/μmole); $MgCl_2$, 1 μmole; HFA-ceramide, 0.25 μmole; Triton X-100, 0.65 mg; tris-HCl buffer, 10 μmoles; purified microsomal fraction as indicated. Incubation was for 15 min at 37°. Total lipid radioactivity was determined. Further experimental details as given in the text.

Table 1
Biosynthesis of glycolipids from UDP-galactose-^{14}C and ceramide containing hydroxy fatty acids with normal and Jimpy brain homogenates.

Homogenate (mg of protein)	Radioactivity incorporated (cpm)							
	Glucocerebrosides		Galactocerebrosides		Sulfatides		Psychosine	
	N	Jp	N	Jp	N	Jp	N	Jp
2	0	0	1356	241	114	14	9	0
3	0	0	2155	414	191	24	18	0

Abbreviations: N, normal mice; Jp, Jimpy mice.

The reaction mixture contained (in 0.5 ml final volume): UDP-galactose-^{14}C, 20 nmoles (5 μCi/μmole); $MgCl_2$, 2 μmoles; HFA-ceramide, 0.75 μmole; Triton X-100, 1.5 mg; tris-HCl buffer, 20 μmoles (pH 7.3); 10% brain homogenate of 15-day old mice in 0.05 M tris-HCl (pH 7.3) as indicated. Incubation was for 90 min at 37°. Glycolipids were separated by TLC on the borate impregnated plates [5]. Further incubation conditions and the enzyme assay were as described in the text.

lished deficiency of psychosine synthesis [7, 7] may lead to a conclusion that two different galactosyltransferase activities are involved in the metabolic defect of Jimpy mutation. These findings, in addition to other enzymatic defects reported in Jimpy brain [15, 16], would be in accordance with the hypothesis of a multi-enzymatic deficiency in mutant CNS that we proposed earlier [8]. However, a more complete characterization of two galactosyltransferase activities which bring out *in vitro* synthesis of psychosine and cerebroside, respectively, will be needed to confirm the existence of two different enzymes.

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Table 2
Distribution of galactosyl-ceramide transferase activity in subcellular fractions of normal and Jimpy mice.

Source of enzyme	Radioactivity incorporated (cpm/mg protein)			
	HFA-galactocerebrosides		NFA-galactocerebrosides	
	N	Jp	N	Jp
Homogenate	205	38	4	2
M_1	726	104	5	26
M_2	211	58	3	0
M_3	161	85	4	7
Microsomes	1688	198	12	6
Supernatant	0	10	5	0

Abbreviations: N, normal mice; Jp, Jimpy mice.

The incubation mixture contained (in 0.5 ml final volume): UDP-galactose-^{14}C, 10 nmoles (5 μCi/μmole); $MgCl_2$, 2 μmoles; HFA-ceramide, 0.50 μmole; Triton X-100, 1.3 mg; tris-HCl buffer, 20 μmoles (pH 7.3); enzyme source, 1.0 mg of protein. Incubation was for 120 min at 37°. Before the radioactivity determination glycolipids were separated by TLC [7]. Further experimental details as given in the text.

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